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THE EVOLUTION OF ANORECTAL MANOMETRY

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The history of physiology testing

The history of anorectal research can be dated back to 1200 BC to the Chester Beatty Medical Papyrus [1] which described anal diseases and contained scanty descriptions of the anal canal. This knowledge was further enhanced by the descriptions of Galen and illustrations of Versalius and John Calcar. It was many centuries later that the physiology of the anal sphincter control was elucidated. In 1867, Masius identified the center of the lower spinal cord as being responsible for anal sphincter tone and reflex contractions in the dog and rabbit. During the same period, manometry studies were first carried out to study anorectal function. The first phenomenon to be described was the recto-anal inhibitory reflex reported by Gowers in 1877. This was followed by the observations of Langley and Anderson where they demonstrated that in the cat, the stimulation of lumbar sympathetic nerves causes relaxation of the rectum and the contraction of the internal anal sphincter.

Anorectal manometry

Joltrain et al first described a method for measuring colorectal pressures in 1919. He used a rectal tube after rectal infusion to measure pressures of the lower gastro intestinal tract. This method was refined in 1940 by White et al [2] who developed the colonometrogram which was based on cystometry. This primitive device contained of a vertical glass tube manometer connected in one side to an intravenous drip line and at the other end to a rectal tube (Figure 1). He used this to assess the colonic tone in patients with injuries in the brain, spinal cord, cauda equina or sacral nerves and noted that the compliance of the colon and rectum depended on the level of the lesion.

Based on these initial maneuvers, Anorectal manometry (ARM) was developed. The procedure involves insertion of a catheter into the anorectum and connecting it to a pressure recorder to measure the intraluminal pressure. It had first been used in assessing patients in the 1980s , although more complex procedures had been attempted several decades previously [3]. Transducers have often been developed first for oesophageal manometry and subsequently the same technology used to create devices for anorectal manometry. The initial devices had an intraluminal balloon. Subsequently water perfused and solid-state manometers had been used. Conventional manometry probes contained a few sensors that were spaced at 3-5 cm and incapable of acquiring the pressures the entire anal canal simultaneously. Therefore, they required pull-through manoeuvres or rotation to sample the entire area of interest. This prevented a continuous measurement of pressures throughout the entire anal canal. Moreover, radial sensors required a pull through procedure that introduced motion artefacts.

With the advancement of electronics and miniaturisation of sensors, more and more sensors could be fitted into the probes, and this resulted in the development of high-resolution anorectal manometry (HRARM) in 2007 [4]. In HRARM, the space between 2 adjacent sensors is less than 1 cm. Most systems have circumferential sensors, each with 12 pressure sensitive segments arranged radially. Ten of these sensors are fitted within 6 cm on the probe. The 12 sector pressures are averaged to obtain a single mean pressure value for each level.

Three dimensional (3D) high definition anorectal manometry (3DHDM) was introduced in 2010 [5]. This uses 16 sensors, each with 16 radial pressure sensitive sectors, arranged over a space of 6.4 cm. This sensor arrangement for the first time provided sufficient radial resolution to allow accurate, simultaneous circumferential assessment of the anal ASC. It is also a static test and therefore minimises motion artefacts and other confounders.

Both these modern techniques are heavily dependent computer hardware and software for recording and interpolation of the data. There are several advantages and disadvantages in HRARM / 3DHDM when compared to conventional manometry [6] (see Table 1).

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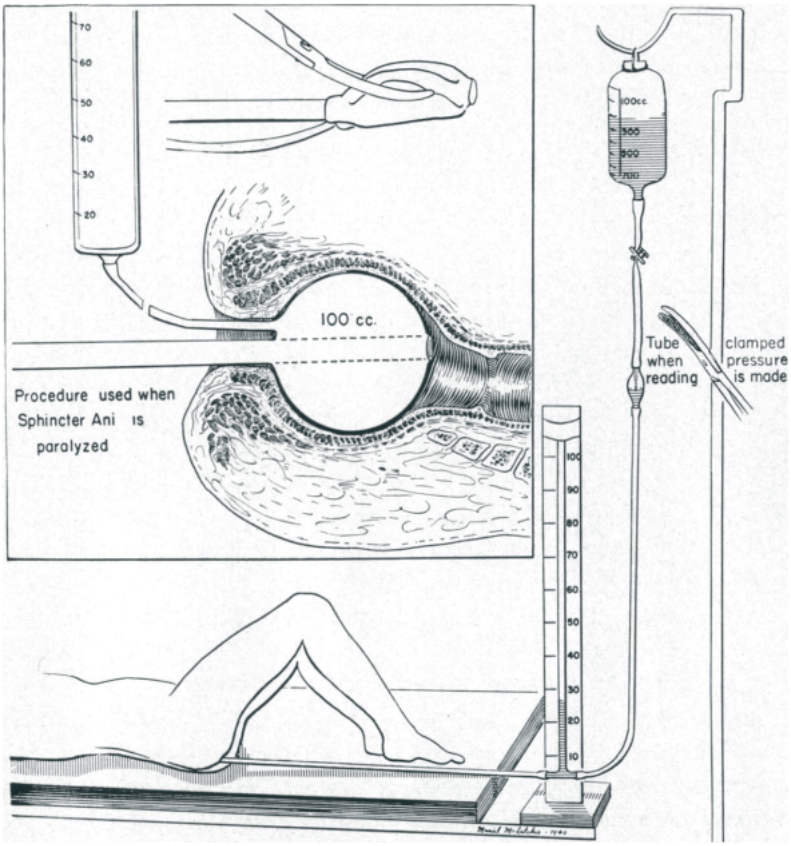

Fig. 1. Colonometrogram, developed by White et al [2].

Table 1. Comparison of conventional manometry and HRARM / 3DHDM, From Lee et al [6]

HRM / 3DHDM	Conventional manometry
More sensors at close intervals (continuum in space and time) E-sleeve for high-pressure zone	Few sensors at wider intervals Dent sleeve for high-pressure zone
Stationary examination, less discomfort	Pull through, can be uncomfortable
Color topographic display, better resolution allowing easier interpretation with less time	Lines display, poor anatomical resolution, less easy to interpret and time-consuming
High resolution allows radial bedside pressure measurement	Only circular pressure measurement
More fragile, shorter life span, greater maintenance required	Less susceptible to wear and tear, little maintenance and seldom malfunctions

ARM provides information about the resting pressure (RP), squeeze pressure (SP) and length of the anal canal (anal high pressure zone length – HPZL) by direct measurement. A balloon attached to the tip of the catheter allows additional measurements such as rectal sensory thresholds and rectoanal inhibitory reflex to be elicited.

The normal pressure values for a given age and gender varies significantly, depending on the technique and the type of catheter used and thus it is recommended that every laboratory establish its own normal values for every technique. Presently, there is a classification system for anal incontinence based on anorectal manometry findings [7].

A group of patients who are incontinent will demonstrate normal anorectal manometry findings under static conditions. They require monitoring of anorectal motor events over a prolonged period and in the fully ambulatory state. The methods used utilise micro pressure transducers with or without simultaneous EMG recordings of the EAS. Ambulatory ARM was first used in patient evaluation in the last decade of the 20th century [8]. The study by Kumar et al [8] identified that spontaneous transient relaxations of the IAS were more frequent and of longer duration in patients with idiopathic anal incontinence. Furthermore, the motility index of the rectum and colon were lower in patients with slow transit constipation. Despite the promising results, the clinical role of ambulatory ARM has not yet been established.

Traditional manometry assesses the pressure in the anal canal at each level as a single value and ignores the possibility of radial asymmetry. Vector manometry assesses the radial and longitudinal pressure profile along the entire length of the anal sphincter. Radial asymmetry, which is expressed as a percentage calculates the degree to which the integrated cross sections deviate from a perfect circle. The Vector Symmetry Index (VSI) on the other hand, is expressed as a value from 0 to 1 [9] with values closer to 0 indicate greater asymmetry. Despite being shown to have an accuracy comparable to endoanal ultrasound and needle EMG in some studies, Yang and Wexner found that the localisation of sphincter injuries with vector manometry is poor.

The diagnostic utility of anorectal manometry

Anorectal manometry is useful in objectively evaluating a multitude of disorders. In patients with chronic constipation, ARM helps identify patients with defaecatory disorders [10]. However, there can be significant overlap between the subtypes. Manometry can help in distinguishing weaknesses in the internal and external anal sphincters in patients with anal incontinence [10]. The response to treatment can also be serially monitored using ARM. ARM is also useful in excluding dyssynergic defaecation in patients with proctalgia.

ЕВОЛЮЦІЯ АНОРЕКТАЛЬНОЇ МАНОМЕТРІЇ

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У статті розглянуто основні віхи розвитку методу аноректальної манометрії, який використовується для об'єктивної оцінки тонусу аноректальних м'язів і скоординованості скорочень прямої кишки та сфінктерів ануса шляхом прямого вимірювання.

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DN4 QUESTIONNAIRE IN FAMILY PRACTICE FOR EVALUATION OF CLINICAL MANIFESTATIONS OF NEUROPATHIC PAIN IN TYPE 2 DIABETES PATIENTS TREATED BY LIGHT THERAPY

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Background. *Peripheral diabetic polyneuropathy (DPN) is one of the most frequent neurological complications of diabetes mellitus (DM). Despite the large number of pharmacological agents, its treatment is not sufficiently effective, which necessitates the search for new therapies.*

Objective. *The aim of the study was to increase the effectiveness of treatment of neuropathic pain in the patients with diabetic polyneuropathy by incorporating procedures using polarizing polychromatic non-coherent light (Bioptron light therapy) into the complex therapy of this disease.*

Methods. *We examined 67 patients with type 2 diabetes complicated with diabetic polyneuropathy. Patients were divided into two groups: group 1 consisted of 32 patients, who received standard treatment; group 2 comprised 35 patients, who additionally underwent 12 light therapy treatments by means of the Bioptron Physiotherapy Unit. The evaluation of neuropathic pain intensity was performed using a modified questionnaire DN4.*

Results. *A positive clinical effect of treatment was evidenced in both groups in 12 days of treatment. In 3 months, the intensity of complaints was significantly lower ($p<0.05$) only in the group with additional use of polarizing light. In 6 months, the positive effect of the therapy was leveled in the patients of both groups.*

Conclusions. *The use of the DN4 questionnaire with a modified scale for assessing the parameters of neuropathic pain can optimize its diagnosis. The light therapy procedures together with the standard complex therapy of diabetic polyneuropathy increase the clinical efficacy of neuropathic pain treatment and help to preserve the therapeutic effect within 3 months.*

KEY WORDS: **diabetic polyneuropathy; neuropathic pain; DN4 questionnaire; polarized polychromatic noncoherent light (Bioptron).**

Introduction

Peripheral diabetic polyneuropathy (DPN) is a typical early and most frequent complication of diabetes mellitus (DM) [1]. It develops due to affection of nerve fibers, caused by diabetes and occurs in more than 50 % of patients with this illness. Polyneuropathy is revealed in both young and elderly patients with type 1 and type 2 diabetes mellitus [2]. There is no single classification of peripheral diabetic neuropathy. Some authors recommend defining hypohyperglycemic, generalized, focal and multifocal types (Thomas P.K. 1997); whereas others insist on singling outing asymptomatic, symptomatic and marked symptoms (Dyck P. J. 1999) or mono, polyneuropathy and autonomic polyneuropathy (I. I. Dedov et al., 2002). According to the protocol of medical care [11], peripheral polyneuropathy is divided into somatic (motor, sensory and sensory-motor), vegetative and mononeuropathy.

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To diagnose pathological process in the nervous tissue Boulton et al (2005) suggested allocating three clinical forms of peripheral DPN (silent, acute pain and chronic pain). Chronic pain form of DPN is the most commonly reveled in the patients with diabetes [1].

The severity of this complication depends on its clinical consequences, in particular, trophic disorders and neuropathic pain, which adversely affect patients' quality of life. A number of questionnaires are used to diagnose neuropathic pain [3]. DN4 questionnaire, being one of the most practical, where a positive response to four or more questions out of ten substantiates 'neuropathic pain' diagnosis, is used as a screening to detect neuropathic pain syndromes [4].

Despite the large number of pharmacological agents, treatment of patients with diabetic polyneuropathy is not sufficiently effective [5, 6, 7], which necessitates the search for new methods of treatment. Since the mid-80s, there have been physiotherapeutic devices that emit visible (wavelength 80-3400 nm) linearly polarized (95 %) incoherent (desynchronized in time and space) low-energy (non-destructive,

with the energy flux intensity of 40 mW/cm^2) (Pyler) light, and the light stream, transformed by polarization, that lacks both ultraviolet and a significant part of the infrared rays. Studies conducted at the end of the last century proved a positive effect of physiotherapeutic procedures using this light for treatment of diseases with lesions of peripheral nerves [8, 9, 10]. The aim of the study is to increase the effectiveness of treatment of neuropathic pain in patients with diabetic polyneuropathy by including light therapy procedures into the complex treatment of this disease.

Methods

67 patients were examined (36 males (53.7 %) and 31 females (46.3 %)) with type 2 diabetes and DPN. The diagnosis of DPN was based on anamnesis and clinical examination data. the age of the examined persons ranged from 45 to 65 years old (mean age 57.0±5.2 years old). Duration of diabetes mellitus was from 4 to 19 years (average duration 9.4±3.7 years), and of DPN was from 1 to 12 years (which averaged 5.5±2.9 years).

For the convenience of systematization and objectification of data comparing, all examined patients were divided into two groups: the 1st – control group comprised 32 patients with type 2 DM and DPN, who received standard treatment according to a unified clinical protocol of primary and secondary (specialized) medical care (No.1118, dated December 21, 2012) [11]. The 2nd group involved patients undergoing standard treatment together with 12 light therapy treatments by means of the Bioptron Physiotherapy Unit [12]. The duration of the procedure was 10 minutes with a directed flow of light on the lower limbs. General characteristic features of patients with diabetes are presented in Table 1.

Clinical examination of patients was performed before the treatment and on the 12th day after the beginning of the treatment.

Neuropathic pain was diagnosed using the modifi questionnaire DN4 (2005) [13, 14]. The questionnaire structure included two blocks of questions: 7 questions of the fi block revealed sensory symptoms, including spontaneous pain (burning, painful cold, electric shocks), paresthesia and dysesthesia (tingling, pins and needles, numbness, itching); three conclusions of the physician, based on the clinical examination, which comprised the second block, give the physician the opportunity to identify the allodynia and negative sensory symptoms. Neuropathic pain was set at a score of 4 or more points.

For the details of each question, we modified the DN4 questionnaire by ranging the intensity (scale 1 to 10) of the sensations listed in the first question block.

Evaluation of the results was carried out at the admission of patients to the hospital and in 12 days after the start of diabetic polyneuropathy treatment. Long-term results of the therapy were administered in 3 and 6 months by performing the call-in poll among the patients using the first question block of the questionnaire.

The analysis and processing of statistical data of clinical examinations results were carried out on a personal computer using STATISTICA 10 and MS excel xP application packages. All data are presented as mean value and standard deviation (M±σ). Relations between continuous variables were examined by the Pearson correlation coefficient χ^2. Comparison of the rates between the groups was carried out using the Student T-test, and those within the group were compared using Wilcoxon matched paired test. The difference in rates was considered statistically significant at $p<0.05$.

Results

According to the DN4 questionnaire, 49 (73.1 %) of the surveyed patients suffered neuropathic pain before treatment, which is consistent with the literature [15]. no signifi differences between the groups were noticed before treatment ($p>0.05$) (Table 2).

Subjective symptoms in the general group of patients were presented as follows: 67.2 % of patients suffered burning sensation, while 31.34 % experienced painful cold. 74.6 % of people were disturbed by tingling. 40.29 % of patients with diabetes sensed electric shocks. Pins and needles sensation and that of numbness troubled 58.2 % and 59.7 % of patients respectively. 34.3 % of respondents had complains of itching. The

Table 1. General characteristic features of the patients (M±σ)

Characteristic features of the groups of patients	1st group, n=32	2nd group, n=35	p
Age, years	58.7±5.2	55.6±4.9	p>0.05
Duration of DM, years	8.6±3.3	10.1±4.0	p>0.05
Duration of DPN, years	4.9±2.8	6.0±3.1	p>0.05

Table 2. Follow-up of neuropathic pain severity in the examined groups of patients with diabetes mellitus according to the DN4 questionnaire before and after treatment (M±σ)

	1st group	2nd group	P
Before treatment	4.7±1.4	4.8±1.6	p>0.05
After treatment	3.4±1.5	2.9±1.2	p>0.05

*Notes: * – significant difference before and after treatment (p<0.05).*

objective examination of the patients' lower extremities proved that the pain was localized in the area with a reduced sensitivity to touching (in 70.2 %), pricking (in 37.3 %) and in the area of irritation with a brush (in 19.4 %), indicating a tactile and sensory sensitivity disturbance. The intensity of each of the following complaints before treatment in the examined groups of patients is presented in Fig. 1.

After the course of treatment, a decrease in the level of neuropathic pain was evidenced, together with a positive dynamics of the intensity of subjective complaints of the patients.

The analysis of data of the DN4 questionnaire proved a decrease in the signs of neuropathic pain by 41.7 % (χ^2=2.5; p>0.05) in the patients of control group, and by 64.0 % (χ^2=27.6; p<0.05) in the group with additional light therapy procedures. The rate of neuropathic pain presence after the course of treatment was much lower in the 2nd group.

The study of individual rates of neuropathic pain in each of the groups on the 12th day after the beginning of treatment proved that the patients of the 1st and the 2nd groups experienced burning sensation decrease by 15.6 % (χ^2=1.6; p>0.05) and 34.4 % (χ^2=6.9; p<0.05) respectively. Painful cold sensation insignificantly decreased by 6.3 % (χ^2=0.291; p>0.05) in group 1 and by 5.7 % (χ^2=0.3; p>0.05) in group 2. The sensation of electric shocks was reduced by 28.13 % (χ^2=6.5; p<0.05) in the patients of the 1st group and by 28.13 % (χ^2=5.9; p<0.05) in those of group 2. Tingling worried the patients with diabetes less by 15.6 % (χ^2=1.6; p>0.05) of group 1 and by 22.9 % (χ^2=4.2; p<0.05) of group 2. Pins and needles as well as numbness decreased by 21.88 % (χ^2=3.1; p<0.05) and 3.1 % (χ^2=3.1; p>0.05) in the patients of the 1st group, and by 25.7 % (χ^2=4.629; p<0.05) and 28.6 % (χ^2=5.7; p<0.05) in those of the 2nd group. Itching began to bother less the respondents of the 1st group by 3.1 % (χ^2=0.1; p>0.05) and by 14.3 % (χ^2=1.7; p>0.05) those in the 2nd group. The pain, which was localized in the area of reduced sensitivity to touching, decreased by 15.6 % (χ^2=1.9;

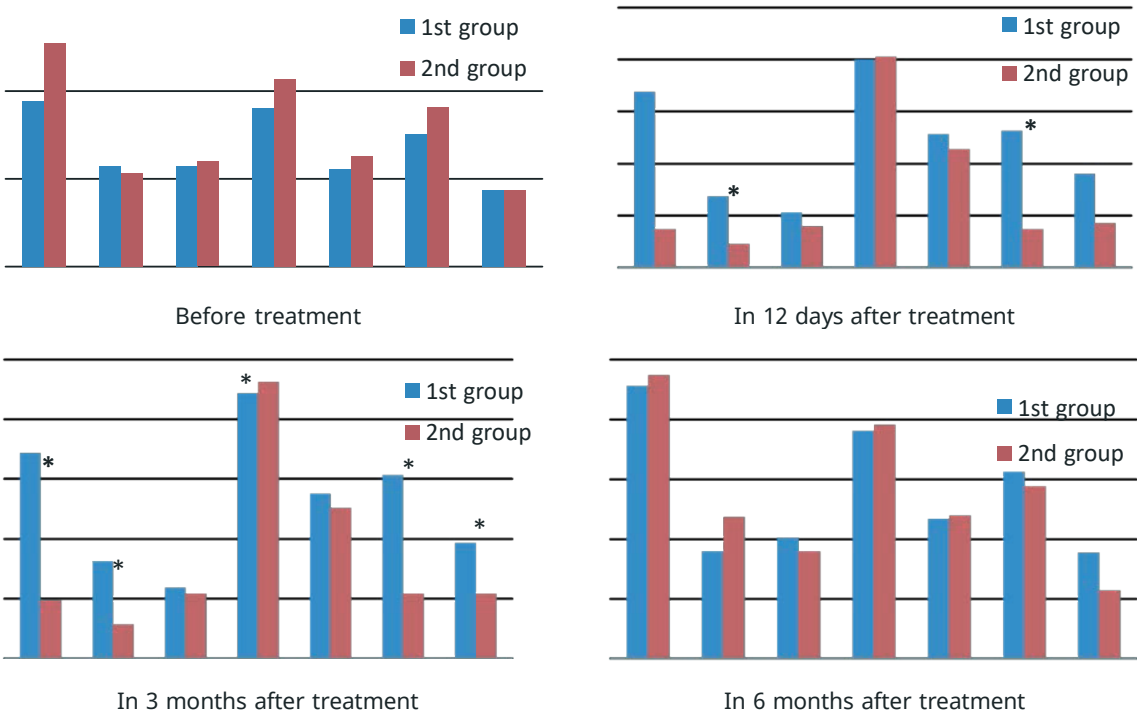

Fig. 1. Comparison of the intensity of complaints between the patient groups undergoing treatment.
*Notes: sensation of 1 – burning; 2 – painful cold; 3 – electric shocks; 4 – tingling; 5 – pins and needles; 6 – numbness; 7 – itching; * – p<0.05.*

p>0.05) and 14.3 % (χ^2=1.4; p>0.05), to pricking by 25 % (χ^2=4.4; p<0.05) and 22,9 % (χ^2=4.6; p<0.05), and to irritation with a brush by 6,5 % (χ^2=0.3; p>0.05) and 5.71 % (χ^2=0.7; p<0.05) in the examined groups 1 and 2, respectively (Table 3).

The survey of the patients in 3 months after the treatment, proved that in the 1st group such sensations as burning, painful cold, electric shocks, tingling, pinsand needles, numbness and itching were experienced by 17 (53.1 %), 10 (31.3 %), 12 (37.5 %), 20 (62.5 %), 10 (31.3 %), 18 (56.3 %) and 9 (28.1 %) patients, whereas in the 2nd group – by 7 (20 %), 8 (22.9 %), 6 (17.1 %), 23 (65.7 %), 14 (40 %), 11 (31.4 %) and 7 (20 %) patients respectively.

The analysis of survey of the patients with diabetes mellitus, conducted in 6 months after the treatment, proved that the patients with diabetes had sensations of burning, painful cold, electric shocks, tingling, pins and needles, numbness and itching in 22 (68.5 %), 12 (37.5 %), 14 (43.8 %), 22 (68.5 %), 15 (46.9 %), 21 (65.6 %) and 9 (28.1 %) cases in group 1, and in 23 (65.7 %), 10 (28.6 %), 13 (37.1 %), 26 (74.3 %), 20 (57.1 %), 14 (40 %) and 12 (34.3 %) cases in group 2 respectively. The comparison of complaints intensity between the patient groups is presented in Fig. 1.

Discussion

The results of our study are consistent with the recent literature [1]. The standard therapy (α-lipoic acid, actovegin and complex of vitamins of the group B) decreases the intensity of pain and neuropathic disorders [11]. The adding of light therapy procedures allows not only the achievement of this effect, but also its long-term preservation [10, 12]. The use of the questionnaire DN4 has long been practiced for the diagnosis of neuropathic pain [13, 14], but only its modification [9] allows evaluating the therapy effectiveness. The obtained results prove that significant improvement in the DN4 questionnaire's quantitative indicators occurred in 12 days after the beginning of treatment and persisted for three months after the treatment in both groups. At the same time, the intensity of the indicators of block 1 of the questionnaire in three months after the treatment was considerably less signifi in the patients who received additional light therapy. Six months later, quantitative and qualitative indicators of the presence and intensity of neuropathic pain resumed to the initial level.

Conclusions

Using a modified DN4 questionnaire in the patients with type 2 diabetes can improve the diagnosis of neuropathic pain.

Table 3. Follow-up of neuropathic pain intensity in the examined groups of patients under the influence of therapy (M±σ)

Index	Before treatment	After treatment in			p1	p2	p3	p4	p5
		12 days	3 months	6 months					
	1st group								
1	4.7±3.7	1.7±1.9	1.7±1.9	4.6±3.5	<0.05	>0.05	<0.05	<0.05	>0.05
2	2.3±3.3	0.7±1.3	0.8±1.3	2.4±3.4	<0.05	>0.05	<0.05	<0.05	>0.05
3	2.3±3.4	0.5±1.5	0.6±0.8	2.0±2.6	<0.05	>0.05	<0.05	<0.05	>0.05
4	3.6±3.5	2.0±2.6	2.2±2.1	3.8±3.2	<0.05	>0.05	<0.05	<0.05	>0.05
5	2.2±3.7	1.3±2.3	1.4±2.2	2.3±3.1	<0.05	>0.05	<0.05	<0.05	>0.05
6	3.0±3.3	1.3±2.2	1.5±1.7	3.1±2.9	<0.05	>0.05	<0.05	<0.05	>0.05
7	1.750±2.828	0.9±1.9	1.7±2.9	1.8±3.1	<0.05	>0.05	<0.05	<0.05	>0.05
2nd group									
1	5.1±3.8	0.2±0.4	0.3±0.6	4.7±3.9	<0.05	>0.05	<0.05	<0.05	>0.05
2	2.1±3.5	0.5±1.0	0.5±1.1	1.8±3.1	<0.05	>0.05	<0.05	<0.05	>0.05
3	2.4±3.2	0.4±1.0	0.5±1.3	1.8±2.6	<0.05	>0.05	<0.05	<0.05	>0.05
4	4.3±3.1	2.0±2.0	2.0±2.6	3.9±3.1	<0.05	>0.05	<0.05	<0.05	>0.05
5	2.5±2.5	1.1±1.8	1.3±1.8	2.4±2.4	<0.05	>0.05	<0.05	<0.05	>0.05
6	3.6±3.3	0.4±0.5	0.5±0.9	2.9±3.7	<0.05	>0.05	<0.05	<0.05	>0.05
7	1.7±2.8	0.4±1.0	0.5±1.3	1.1±2.3	<0.05	>0.05	<0.05	<0.05	>0.05

Notes: sensation of **1** *– burning;* **2** *– painful cold;* **3** *– electric shocks;* **4** *– tingling;* **5** *– pinsand needles;* **6** *– numbness;* **7** *– itching;*
p1 – significant differences of indexes before and in 12 days after treatment;
p2 – significant differences of indexes in 12 days and in 3 months after treatment;
p3 – significant differences of indexes before and in 3 months after treatment;
p4 – significant differences of indexes in 12 days and in 6 months after treatment;
p5 – significant differences of indexes before and in 6 months after treatment.

The presence of phototherapeutic procedures by the Bioptron together with the standard complex therapy of diabetic polyneuropathy has a pronounced clinical effect and contributes to a 3-month-long reduction of quantitative and qualitative indicators of neuropathic pain.

The temporary clinical effect of the use of polarizing light in the treatment of diabetic polyneuropathy proves the feasibility of studying new therapies that would influence the pathogenesis of neuropathic pain.

ОПИТУВАЛЬНИК DN4 У ПРАКТИЦІ СІМЕЙНОГО ЛІКАРЯ ДЛЯ ОЦІНКИ НЕЙРОПАТИЧНОГО БОЛЮ У ХВОРИХ З ЦУКРОВИМ ДІАБЕТОМ 2-ГО ТИПУ ПРИ ЛІКУВАННЯ СВІТЛОТЕРАПІЄЮ

Н. Р. Макарчук

ТЕРНОПІЛЬСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Периферична діабетична полінейропатія є одним з найбільш частих неврологічних ускладнень цукрового діабету. Незважаючи на широкий спектр існуючих лікарських засобів, її лікування є недостатньо ефективним, що зумовлює пошук нових статегій та засобів.*

Мета дослідження – *підвищити ефективність лікування нейропатичного болю у хворих на цукровий діабет шляхом включення процедур із використанням поляризаційного поліхромного некогерентного світла (біоптронна світлотерапія).*

Методи. *Обстежено 67 хворих на діабет 2 типу, який ускладнений діабетичною поліневропатією. Пацієнтів було розділено на дві групи: 1 група – 32 пацієнтів, які отримали стандартне лікування; 2 група 35 хворих, які додатково пройшли 12 процедур світлотерапії за допомогою світлотерапевтичного пристрою Біоптрон. Оцінку інтенсивності невропатичного болю проводили за допомогою модифікованої анкети DN4.*

Результати. *Через 12 днів лікування позитивний клінічний ефект лікування спостерігався в обох групах. Однак, через 3 місяці інтенсивність скарг була значно меншою ($p<0,05$) в 2-ій групі пацієнтів, які отримували додатково лікування поляризаційним світлом. Через 6 місяців ефект терапії був однаковим у пацієнтів обох груп.*

Висновки. *Використання опитувальника DN4 з модифікованою шкалою для оцінки параметрів нейропатичного болю може оптимізувати його діагностику та оцінку. Світлотерапевтичні процедури у поєднанні зі стандартною комплексною терапією діабетичної поліневропатії підвищують клінічну ефективність лікування невропатичного болю і допомагають зберегти терапевтичний ефект протягом 3 місяців.*

КЛЮЧОВІ СЛОВА: **діабетична поліневропатія; невропатичний біль; опитувальник DN4; поляризоване поліхроматичне некогерентне світло (Біоптрон).**

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THE LEVELS OF FUNCTIONAL-VEGETATIVE HOMEOSTASIS AS CRITERIA FOR MAGNETOTHERAPY EFFICACY

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Background. *Disorders of autonomic nervous system caused up to 80% of functional disorders. There is no information about the influence of magnetotherapy (MT) on the indicators of vegetative homeostasis, which disturbance is a cause of functional pathology.*

Objectives. *The aim of the study is to investigate vegetative rehabilitation trend of MT in various initial conditions of functional-vegetative disorders.*

Methods. *Functional-vegetative diagnostics method by V.G. Makats was chosen as a method of control of MT impact. The diagnostic complex BIOTEST-12M was a technical tool. 38 children of different age and gender treated in the Department of Physiotherapy of Vinnytsia Regional Children Clinical Hospital in 2016-2017 were involved in the research. The patients were divided into 7 groups according to the levels of functional vegetative homeostasis.*

Results. *MT had a different effect on vegetative activity as well as systemic and functional dependence, according to the coefficient of functional vegetative homeostasis. The most positive effect was evidenced in a group with a significant parasympathicotonia (group 1). In the group with severe sympathicotonia (group 6) it had negative effect. There were no gender and age-related characteristic features of the influence of MT on the dispersion of vegetative levels.*

Conclusions. *Rehabilitation expediency requires maintenance of functional vegetative homeostasis at the level of FcP-VB-FcS in conjunction with functional-vegetative diagnostics using the method of V.G. Makats. Magnetotherapy can be recommended to be used only for patients with significant and expressed parasympathicotonia.*

KEY WORDS: **magnetotherapy; functional-vegetative diagnostics; vegetative homeostasis; vegetative level; vegetative coefficient.**

Introduction

The disorders of autonomic nervous system (ANS) caused 25-80 % of functional disorders of reserves and mechanisms of adaptation to the changing environment, physical and psychosomatic efforts [1, 2]. Being of integrative importance at the central level, the ANS ensures regulation of vegetative homeostasis, systemic disorders of which determine the pathogenetic basis of functional and somatic diseases [1, 3, 4]. Their fundamental evaluation goes beyond certain parameters of vegetative regulation, have need of a systematic approach and study of a holistic organism as a multilevel interdependent system [5, 6]. A significant support for the problem of vegetative pathogenesis is the normalization of vegetative indices in the rehabilitation period. In this case, special attention should be paid to the use of traditional FAZ (functionally active skin zones) [7-9].

Nowadays, according to the WHO recommendations, 'Electro acupuncture diagnostics and reflexotherapy' should become the basis for rehabilitation medicine (the WHO International Council, Yerevan, 19.09.03). Its General Assembly (2014) herewith officially advises the States (the WHO participants) to include these areas into national health programs and draws attention to the development of biophysical methods of controlling the rehabilitation effectiveness of physiotherapeutic and preformed factors [5, 10, 11]. Therefore, much attention should be paid to the recently discovered 'Functional-vegetative system of a human' (FVS) [12], which has proved the biophysical reality of traditional 'acupuncture channels' as well as their direct relation to the vegetative homeostasis and, in addition, requires defining of its place in the classical physiological system [13, 14].

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Low-frequency magnetotherapy (MT) is widely used in modern physiotherapeutic practice using the magnetic component of low-frequency electromagnetic fields [15-17]. MT has an impressively wide range of indications (anti-inflammatory, anti-oedematous, trophic, hypocoagulant, vasoactive, anaesthetic, stimulating reparative processes and immuno-modelling) [18-20]. The official list of absolute and relative official contraindications is, concurrently, also impressive. The attention is drawn to complete absence of information on the influence of MT on the indicators of vegetative homeostasis, the disturbance of which is conditioned by the development of any functional pathology.

The aim of the study is to investigate autonomic rehabilitation of MT at different initial states of functional vegetative disorders.

Methods

The research is a fragment of the program 'Two-stage system of rehabilitation of vegetative disorders in children living in the zone of ecological (radiation) control of Ukraine' (is being carried out according to the assignment of the Cabinet of Ministers of Ukraine No. 12010/87). 'Functional-vegetative diagnostics' (FVD) by the method of V.G. Makats has been chosen as a method of control of functional and vegetative efficiency of MT [12-14]. This method has been admitted to be used in medical practice by the Academic Council of the Ministry of Health of Ukraine and the joint session of the Republican Problem Commissions (RPC) of Paediatrics, Obstetrics and Gynaecology, Quantum Medicine, Haematology and Transfusiology, New Medical Technology and New Diagnostic Tools (Minutes No. 1, 08-01 dated September 11, 1994).

The diagnostic complex BIOTEST-12M is a technical tool of FVD. The latter does not use traditional external power sources and the RPC 'New medical technology and new methods of diagnostics, prevention and rehabilitation' by the Ministry of Health of Ukraine (Minutes No. 5 dated December 25, 1991) has been approved for practical use. Statistical significance of the obtained data was estimated by means of parametric and nonparametric statistics. The analysis of the results was carried out by means of the computer programs 'Search' (developed by the European Centre for Postgraduate Education of the UNAP).

Functional autonomic systems ('acupuncture channels') are based on the international 'acupuncture nomenclature' (IAN) suggested by the WHO (Table 1). The following zones (levels) of functional-vegetative homeostasis are scientifically based on the coefficients of functional-vegetative homeostasis (k-V) [3]: PA-sig (k-V to 0.75 – a zone of significant parasympathetic activity); PA-exp (k 0,76-0,86 – a zone of expressed parasympathetic activity); FcP (k-V 0,87-0,94 – a functional compensation zone of parasympathicotonia); VB (k-V 0,95-1,05 – a zone of vegetative balance); FcS (k-V 1,06-1,14 – a zone of functional compensation of sympathicotonia); SA-exp (k-V 1,14-1,26 – a zone of clear sympathetic activity); SA-sig. (k-V≥1,26 – a zone of significant sympathetic activity). The planned research activities were conducted under the guidance of a high-level expert of the National Academy of Sciences of Ukraine, Doctor Habilitatus in Medicine, Professor V.G. Makats.

38 children (7-16 years old, 13 females and 25 males) were involved in the study; they underwent treatment at the Department of Physiotherapy of the Vinnytsia Regional Children Clinical Hospital in 2016-2017. The children were divided into 7 groups according to the levels of functional vegetative homeostasis: the 1st group – with significant parasympathetic activity (PA-sig); the 2nd – with expressed parasympathetic activity (PA-exp); the 3rd – with the zone of admissible functional vegetative norm (ZAN); the 4th – with expressed sympathetic activity (SA-exp); the 5th – with a significant sympathetic activity (SA-sig). They were also divided into 2 groups according to gender: a female group (FG) – 13 children and

Table 1. Functional autonomic systems 'acupuncture channels' (based on the international 'acupuncture nomenclature' (IAN) suggested by the WHO)

Traditional Channel	MAH	Traditional Channel	MAH
Lungs	LU	Bladder	BL
Large Intestine	LI	Kidneys	KI
Stomach	ST	Pericardium	PC
Spleen, Pancreas	SP	Triple heater	TE
Heart	HT	Gallbladder	GB
Small intestine	SI	Liver	LR

a male one (MG) – 25 children. FVD was twice held in the first half of the day (10:00-12:00) before and after the MT session. The bioelectric activity of 12 symmetrical pairs of functionally active skin zones (24 FAZs) was studied, 2016 tests were carried out. The attention was paid to the direction of dispersion of integral vegetative homeostasis levels. The following areas of influence were chosen for MT: parasternal, epigastric and anterior abdominal wall, intrascapular, lumbar-sacral regions and the region of pelvic (femoral), knee and ankle joints.

Results

The study of the influence of MT on vegetative activity as well as systemic and functional dependence in the initial significant parasympathicotonia (group 1) proved that, regardless of the topography of the effect, MT positively affects the vegetative homeostasis transforming its vegetative coefficients to a higher level of functional activity at the functional compensation zone of parasympathicotonia (FcP), as evidenced by the increase of functional-vegetative homeostasis coefficient (k-V) from 0.66 to 0.88 (Fig. 1, Table 2).

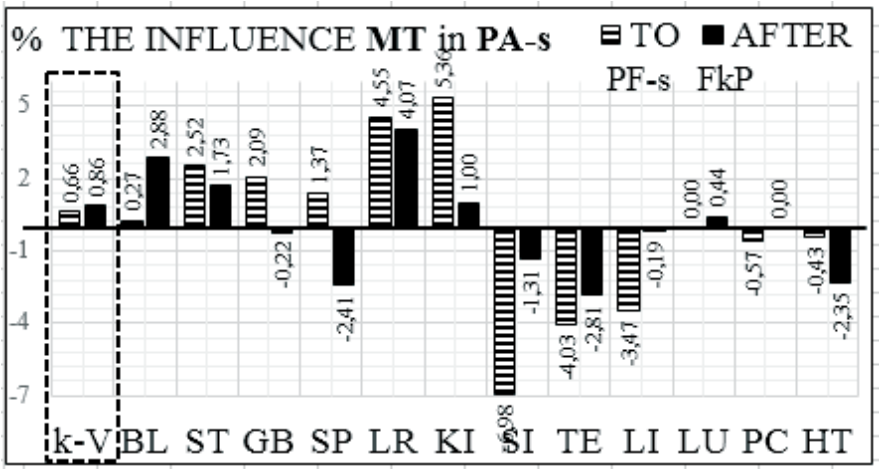

Fig. 1. The influence of MT at PA-sig.

Table 2. The effect of MT on systemic-functional dependence

MT	k-V	LU	PC	HT	SI	TE	LI	SP	LR	KI	BL	GB	ST
Initial significant parasympathicotonia (group 1)													
1*	**0.66**	8.7	7	7	3.8	2.8	3.8	12	12	13	11.1	8.4	9.8
2*	**0.88**	8.7	7.7	5.1	8.2	2.6	5.1	8.2	12	12	14.1	7.2	10.2
Initial expressed parasympathicotonia (group 2)													
1	**0.85**	11.2	9.4	10.2	12.6	2.7	8.3	8.8	8.6	6.7	9.4	5.3	7.8
2	**0.90**	8.0	8.4	10.5	8.8	6.3	4.2	8.0	11.3	6.3	9.2	10.5	8.4
Initial functional compensation of parasympathicotonia (group 3)													
1	**0.90**	7.4	9.7	9.5	11.4	5.9	6.7	8.6	9.0	8.0	9.9	7.6	5.9
2	**1.25**	6.8	6.5	6.8	13.5	9.5	7.2	9.0	7.2	9.0	11.3	7.9	7.2
Initial autonomic balance of VNS (group 4)													
1	**1.06**	11.0	9.3	8.2	11.4	3.0	7.6	11.0	4.0	4.9	18.3	5.3	5.5
2	**0.86**	6.7	8.3	8.8	10.2	5.4	10.0	14.0	8.0	7.9	10.6	4.8	5.4
Initial functional compensation of sympathicotonia (group 5)													
1	**1.07**	7.5	7.2	8.7	13.7	5.0	8.0	8.7	7.5	8.6	10.1	7.2	7.7
2	**0.86**	7.5	9.2	9.6	11.7	4.7	4.9	10.0	8.2	9.6	9.2	7.0	8.8
Initial significant and expressed sympathicotonia (groups 6,7)													
1	**1.26**	8.8	6.3	5.7	9.1	10.0	11.0	7.1	8.5	8.0	8.8	6.0	11.1
2	**2.28**	4.6	6.8	3.0	6.1	10.6	11.0	9.5	2.7	3.8	31.6	3.8	6.1

*Notes: 1 * – here and further. Before the exposure of magnetic radiation.*
*2 * – here and further. After the exposure of magnetic radiation.*

The study of the influence of MT on vegetative activity and systemic-functional dependence in the initial expressed parasympathicotonia (group 2) revealed that, regardless of the topography of the effect, MT affects the vegetative homeostasis relatively positively, translating its vegetative coefficients to higher levels of functional activity, as proved by the increase of the coefficient of functional vegetative homeostasis (kV) from 0.85 to 0.9 (Fig. 2, Table 2).

What is more, the dispersion of the levels of vegetative balance (LVB) under the influence of MT was present, which was accompanied by the transition of 28.57 % of children to the FcP zone and 14.28 % to the zone of VB and FcS (Table 3).

The system correlation of 'acupuncture channels LU-PC-HT, SI-TE-LI, ST-GB-KI-LR) was in a state of interrelated dynamic functional compensation (Fig. 2).

The study of the influence of MT on vegetative activity and systemic an functional dependence taking into account the initial functional compensation of parasympathicotonia (group 3), the initial autonomic balance of VNS (group 4) and the initial functional compensation of sympathicotonia (group 5) proved that the effect of magnetotherapy was relatively neutral concerning functional vegetative homeostasis, which in approximately 70 % of cases fluctuated within the functional compensation of sympathetic and parasympathetic activities (Table 3). The value of ve-

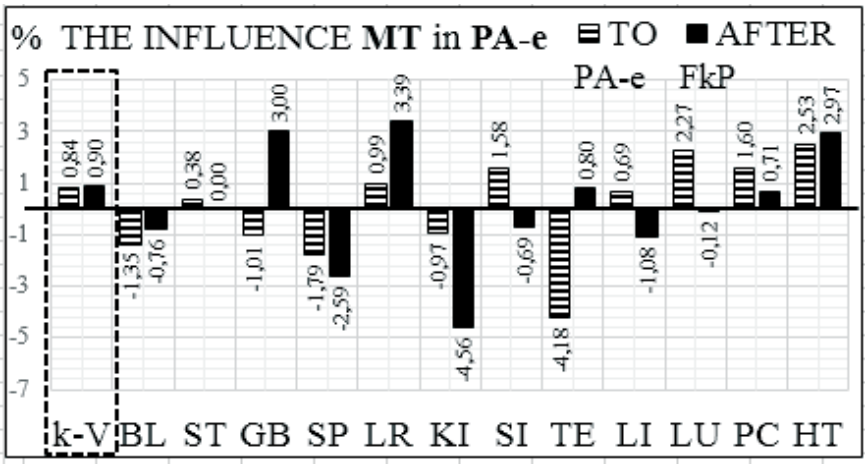

Fig. 2. The influence of MT at PA-exp.

Table 3. The influence of MT on the dispersion of the levels of vegetative balance (LVB), %

MT	PA-sig	PA-exp	FCP	VB	FcS	SA-exp	**SA-sig**
Initial significant parasympathicotonia (group 1)							
1	100						
2	33.33	33.33	33.33				
Initial expressed parasympathicotonia (group 2)							
1		100					
2	14.28	28.57	28.57	14.28	14.28		
Initial functional compensation of parasympathicotonia (group 3)							
1			100				
2			37.50	25.00		25.00	12.50
Initial autonomic balance of VNS (group 4)							
1				100			
2	20.00		20.00	30.00	20.00	10.00	
Initial functional compensation of sympathicotonia (group 5)							
1					100		
2		12.50	12.50	50.00	12.50		12.50
Initial significant and expressed sympathicotonia (groups 6,7)							
1						100	
2					25.00	50.00	25.00

getative coefficients (kV) pointed to the tendency to 'maintain stability' and the systemic ratio of acupuncture channels LU-PC-HT, SI-TE-LI, ST-GB-KI-LR, in its turn, was staying in a state of interdependent dynamically functional compensation (Table 2).

The study of the influence of MT on vegetative activity and systemic and functional dependence in the initial expressed and significant sympathicotonia (groups 6-7) revealed that MT, regardless of the topography of the effect, negatively affected the vegetative homeostasis increasing its level of significant neo-rewards (Fig. 3, Table 2), which was accompanied by a significant increase in the value of kV from 1.26 to 2.28. In case of MT only 25 % were transformed to the poor level of functional activity at the zone of functional compensation of sympathicotonia (FcS) (Table 3). The systemic ratio of acupuncture channels LU-PC-HT, SI-TE-LI, ST-GB-KI-LR), in this case, was in a state of interdependent dynamic functional compensation (Fig. 3).

Investigating the gender and age characteristic features of the influence of MT on the dispersion of the vegetative levels, the same type of dispersion of vegetative levels in the female and male study groups was revealed (Table 3).

The analysis proved that there was no gender and age-related influence of MT on the variance of vegetative levels. Under all conditions, this factor positively affected only the initial levels of the benefits of parasympathetic activity. Its usage in other vegetative disorders caused development of a higher sympathetic orientation. The conclusion was drawn according to the directed dispersion of vegetative levels at various states of initial vegetative disorders (Table 4).

Discussion

The data on the effect of magnetic radiation on the vegetative status of animals and humans can be found in the literature. The effect of weak (up to 3.5 mT) and low-frequency (up to 100 Hz) impulse magnetic field on the state of vegetative nervous system of animals has been studied by analyzing the variability of heart rate. The effect of magnetic field was evaluated by a individually designed complex for recording cardiac signals of animals. Several specially selected regimes of impulse magnetic fields were studied. It was proved that the impulse magnetic field was of a high biological activity at all regimes used, and the indices of vegetative nervous system after the exposure to impulse magnetic field were about to the values typical for normotonic animals. This made it possible to use magnetic fields at those regimes in magnetotherapy [21].

The study of the effect of transcranial magnetotherapy on 63 people with the 1st degree AG at the age of 38-50 years old proved that after the course of therapy the number of patients with hyper-sympathicotonia decreased from 24 (69 %) to 14 (40 %) and the number of patients with normal and asympathicotonic vegetative regulation were 39 % and 21 % respectively [22].

The effect of electromagnetic field of ultra-highfrequency range results in the optimization of autonomic balance of the body in the patients with autonomic nervous system disorders was also evidenced. The electromagnetic field (EMF) had sympatholytic, parasympatolytic and tonic action on a person [23].

We still believe that the data obtained from these studies cannot fully characterize the effect of magnetic and wave effect on the body due to the selected methods of vegetative state

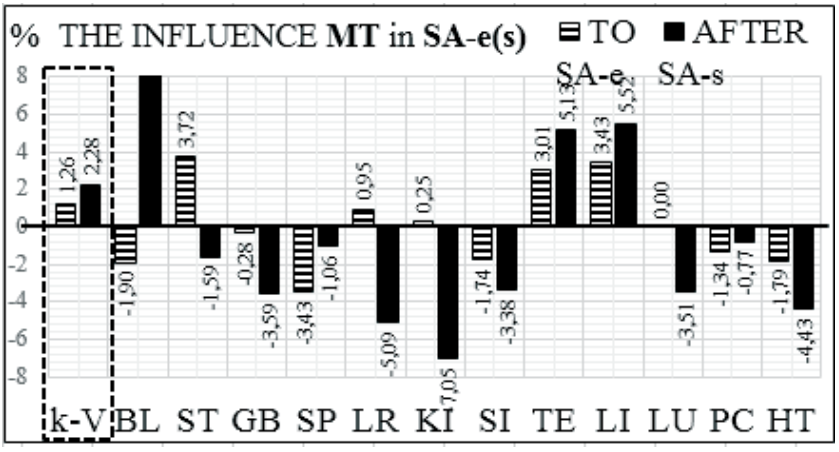

Fig. 3. The influence of MT at initial SA-exp (sig).

Table 4. The effect of magnetotherapy (MT) on the vegetative dispersion at various stages of initial vegetative disorders in female and male groups

№	Female group (in %)							Male group (in %)						
	PA-sig	PA-exp	FcP	VB	FcS	SA-exp	SA-sig	PA-sig	PA-exp	FcP	VB	FcS	SA-exp	SA-sig
Initial significant parasympathicotonia														
1	100							100						
2			100						50		50			
Initial expressed parasympathicotonia (group 2)														
1		100							100					
2			100					20	20	20	20	20		
Initial functional compensation of parasympathicotonia														
1			100							100				
2						100				42,8	42,8			14,3
Initial autonomic balance of VNS (group 4)														
1				100							100			
2			50	25	25			40			60			
Initial functional compensation of sympathicotonia (group 5)														
1					100							100		
2				66,6	16,6		16,6		50	50				
Initial significant and expressed sympathicotonia														
1						100							100	
2					10	60	30					25	50	25

diagnosing. In the first two cases rhythmocardiography was used. Diagnostic tables were used in the third case. These research methods of VNS record the functional state of individual VNS subsystems and separate mechanisms of vegetative regulation. The method of functional-vegetative diagnostics by V.G. Makats provides the results more complete, stable, and comparable in time periods.

Due to the fact that the organism is a multilevel hierarchical system the effect of magnetic fields manifests itself at different levels of organization and depends on many external and internal factors. The reactions of the organism to the action of magnetic fields are characterized by the diversity, instability and phase flow, during which an opposite change is often observed. It is determined by the differences in individual sensitivity of the organism, its systems, and their initial state as well as by the nonspecific character of the action of magnetic fields. The effect of magnetic fields in many cases is normalizing or corrective. To start with, it should be noted that the central parasympatolytic effect during the action of low-frequency magnetotherapy in all groups of observations was revealed. That happens possibly due to the improvement of the parameters of peripheral hemodynamics and sanogenesis processes. This effect, along with the sedative effect of low-frequency magnetotherapy, is realized by affecting the subcortical centres and the pituitary-hypothalamic system. It should also be noted that low-frequency magnetotherapy is capable of exerting an activating influence on the processes of sanogenesis which contributes to restoration of disturbed self-regulation of many functional systems and the organism as a whole. It forms effective protective reactions as well as compensatory and adaptive processes and, moreover, it expands the range of homeostatic response of the organism in conditions of disturbed mechanisms of self-regulation. The obtained data do not coincide with the literature, where the corrective effect of the magnetotherapeutic influence on the parasympathetic and sympathetic units of vegetative NS or pronounced sympatholytic activity is described. This is primarily due to the age characteristic features of the child's organism and its vegetative status. The second important aspect is the method of functional and vegetative diagnosis according to V.G. Makats that was chosen to determine the vegetative status of children, as more universal and complete one.

Conclusions

The results of the studies have proved that MT has a different effect on the vegetative activity as well as systemic and functional dependence in the study groups that depends on the coefficient of functional vegetative homeo-stasis. That is, in a group with a significant parasympathicotonia (group 1), MT has a positive effect; in the group with the expressed parasympathicotonia (group 2) it has a relatively positive effect; in the groups with functional compensation of parasympathicotonia (group 3), with the autonomic balance of VNS (group 4) and in the group with functional compensation of sympathicotonia (group 5) MT has a relatively neutral effect; in a group with severe sympathicotonia (group 6) it has negative effect and in a group with significant parasympathicotonia (group 7) it has negative effect as well.

According to the results of our research, the absence of gender and age-related influence of MT on the dispersion of vegetative levels has been revealed. Sympathetic vegetative orientation is typical for a low-frequency magnetotherapy in all study groups. Therefore, it can only be used to patients with significant and severe parasympathicotonia.

The rehabilitation expediency requires the maintenance of functional vegetative homeostasis at the level of FcP-VB-FcS in conjunction with functional-vegetative diagnostics using the method of V.G. Makats. The method of functional-vegetative diagnosis is easy to use; it provides repeated comparable results and can be applied in hospital and out-patient environment.

ФУНКЦІОНАЛЬНО-ВЕГЕТАТИВНІ РІВНІ ЯК КРИТЕРІЙ ЕФЕКТИВНОСТІ МАГНІТОТЕРАПІЇ

О. В. Єрмішев

ДОНЕЦЬКИЙ НАЦІОНАЛЬНИЙ УНІВЕРСИТЕТ ІМЕНІ ВАСИЛЯ СТУСА, ВІННИЦЯ, УКРАЇНА

Вступ. *Близько 80 % функціональних порушень зумовлено розладами вегетативної нервової системи. На сьогодні немає достатньо даних щодо впливу магнітотерапії (МТ) на показники вегетативного гомеостазу при функціональній патології.*

Мета дослідження – *дослідити тенденції реабілітації при МТ на початкових етапах різних функціонально-вегетативних розладів.*

Методи. *Як метод контролю впливу МТ була обрана «Функціонально-вегетативна діагностика» (ФВД) за В. Г. Макацом. Технічним засобом ФВД виступав діагностично-реабілітаційний комплекс БІОТЕСТ-12М. У дослідженні взяли участь 38 дітей різного віку та статі, які проходили лікування у відділенні фізіотерапії Вінницької обласної дитячої клінічної лікарні у 2016-2017 роках. Пацієнтів поділили на 7 груп відповідно до рівнів функціонального вегетативного гомеостазу.*

Результати. *За коефіцієнтом функціонального вегетативного гомеостазу МТ мала різний вплив як на вегетативну активність, так і на системну, і функціональну залежність. У групі зі значною парасимпатікотонією (група 1) виявлено найбільш позитивний ефект. У групі з вираженою симпатикотонією (група 6) – негативний ефект. Не виявлено гендерних та вікових характерних особливостей впливу МТ на вегетативні рівні.*

Висновки. *Доцільність здійснення реабілітаційних заходів вимагає підтримки функціонального вегетативного гомеостазу на рівні FcP-VB-FcS у поєднанні з функціонально-вегетативною діагностикою з використанням методу В. Г. Макац. Можна рекомендувати використовувати магнітотерапію лише для хворих зі значно вираженою парасимпатокотонією.*

КЛЮЧОВІ СЛОВА: **магнітотерапія; функціонально-вегетативна діагностика; вегетативний гомеостаз; вегетативний рівень; вегетативний коефіцієнт.**

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BONE MINERAL STATUS AND METABOLISM DISORDERS IN PATIENTS WITH CHRONIC SPINAL CORD INJURY

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Background. *The study of late complications, including osteoporosis, is urgent due to the increasing life expectancy of patients with complete spinal cord injury (SCI).*

Objective. *The aim of the study is to evaluate bone mineral status and bone turnover markers in the patients with chronic SCI.*

Methods. *73 SCI patients and 57 healthy persons were examined. Bone status was determined by ultrasound (US) densitometry. Bone turnover markers and vitamin D were evaluated by electrochemiluminescence method.*

Results. *In the SCI patients bone mineral status was significantly lower compared with the individuals of control group. The stiffness index (SI) was 51.4±11.8% vs. 98.5±16.6%, p<0.05 in men and 50.1±9.8% vs. 92.9±11.1%, p<0.05 in women. In the SCI patients the levels of bone turnover markers were significantly higher than the reference values. The bone mineral status of patients with SCI, which occurred before peak bone mass development, is significantly worse, compared with the individuals with the already developed peak bone mass,*

that makes them a high risk group for fracture.

Conclusions. *SCI leads to increased bone resorption with development of secondary osteoporosis (according to the ultrasound densitometry of calcaneal bone). In chronic SCI, bone resorption is higher than in the individuals with combined low levels of vitamin D, and the absence of axial load results in continued loss of bone mass.*

KEY WORDS: **spinal cord injury; osteoporosis; tetraplegia; paraplegia; bone turnover markers; ultrasound densitometry.**

Introduction

Improved social adaptation and increased life expectancy of persons with complete spinal cord injury pay attention to late complications of spinal cord injuries (SCI) including osteoporosis and fragility fractures. SCI-induced osteoporosis has been studied since the first half of the 20th century, but no consistent approach to its diagnosis and treatment has been developed. Currently, diagnostic criteria for osteoporosis in postmenopausal women and men over 50 years are established, but the vast majority of SCI patients are young men. Most of studies are devoted to bone changes in the first days, weeks and months after the injury, when intense bone resorption starts. during the fi years after complete spinal cord injury, rapid decrease in bone mass takes place, which reaches 20-50% of bone mineral density (BMD) of paralyzed limbs [1]. Demineralization of spongy bone occurs faster than that of cortical bone, due to its higher metabolic activity [1]. Also there is no consensus for increased resorption duration after SCI. Some studies have proved that bone loss slows down 3-5 years after the injury [2]. Other study findings evidenced continued bone loss in later stages of the post-traumatic period [3, 4]. The extent and duration of bone loss are caused by many factors, which depend on the characteristics of the injury as well as on many parameters of various systems of the patient. The significance of a degree of spinal cord injury and opportunities for verticalization and movement of bone loss has been proved, but the role of factors affecting bone tissue in the patients with complete spinal cord injury has not been defined so far.

Objective of the study is to evaluate the bone mineral status and parameters of bone metabolism in the patients with chronic spinal cord injuries.

Methods

73 people who suffered a spinal trauma with complete spinal cord injury with tetra- or paraplegia were examined: 38 men, average age 40.1±12.2 years old; 35 women, average age 41.3±11.3 years old; duration of the post-traumatic period was 15.7±10.6 years. Patient

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examination was conducted in cooperation with the Association of Disabled Persons with Spinal Cord injuries. the examined patients lead an active lifestyle that excluded the impact of prolonged bed rest. volunteers accompanying people with SCi were also examined as a comparison group. volunteers were usually represented by patients' relatives: parents, children, spouses, which could partially reduce the impact of other factors affecting bone tissue, including eating habits, living environment, and genetic factors.

Bone status was determined by ultrasound (US) densitometry of calcaneal bone using quantitative ultrasonometer Sahara (Hologic Inc., model 04874, 2008). Ultrasound densitometry evaluated: speed of sound (SoS, m/s); broadband ultrasound attenuation (BUA, dB/MHz); stiffness index (SI, %), calculated by computer on the basis of SOS and BUA parameters; extrapolated mineral density of calcaneal bone; T-score (patient's SI difference from the index of conventionally healthy adults aged 20 years old was presented in standard deviations); Z-index (patient's SI difference from the index of conventionally healthy adults of the same age was presented in standard deviations).

Bone remodelling markers in peripheral blood (osteocalcin, procollagen type 1 propeptide (P1NP), collagen type 1 cross-linked C-telopeptide (β-CTx)) were defined by electrochemiluminescence method on the analyser Elessys 2010 (Roche Diagnostics, Germany) using cobas test-systems.

The statistical analysis was performed using Statistica 10 software. Results were presented as mean values and standard deviation (M±m). The difference in parameters between the groups was defined using one-way analysis of variance ANOVA. Intergroup differences were evaluated by Scheffe's test. The difference in parameters was statistically significant at $p<0.05$.

Results

The results of ultrasound densitometry of the individuals of the main group proved low bone mineral density (Z-score≤-2.0 Sd) in all women of reproductive age (n=21) and all men younger than 50 years old (n=25). Osteoporosis (T-score≤-2.5 Sd) was evidenced in 12 postmenopausal women and 13 men older than 50 years. None of the patients with spinal cord injuries had normal parameters of bone tissue structural-functional state. In the patients of control group, osteoporosis was not diagnosed, low bone mineral density was revealed in 10 individuals (3 men and 7 women), in all postmenopausal women, and 2 men older than 50 years.

The results of ultrasound densitometry in the patients with spinal cord injuries proved significantly lower parameters of bone density compared with those of the control group (Tabl. 2).

It should be noted that the analysis of data in Tabl. 2 proved no impact of gender on the parameters of bone mineral status ($p>0.05$). there was also no significant dependence of bone parameters on age, height, injury level and duration.

Significant moderate correlation between SI and body weight was obtained in men of the main group. Relationship was described by the linear regression equation: SI (%)=8.01+0.55* weight (kg); r=0.41; p=0.017; r2=0.17. In the women no influence of anthropometric data on bone mineral status was revealed.

Bone mineral density was found to depend on the trauma age in men (r=0.41; p=0.019; r^2=0.16) and women of reproductive age (r=0.40; p=0.048; r^2=0.16).

It is established that complete spinal cord injury leads to intensive bone loss in the first years after the injury [5]. Furthermore, the rate of loss slows down and the studied bone parameters are less dependent on the duration

Table 1. Characteristic features of the examined SCI patients and persons of control group

Parameter	Main, n=73		Control, n=57		P	
Gender	Men, n=38	Women, n=35	Men, n=21	Women, n=36	Men	Women
Age, years	40.10±12.20	41.30±11.30	39.70±13.80	46.80±12.20	0.91	0.06
Height, cm	178.60±7.20	164.10±5.60	177.30±5.60	163.20±5.80	0.48	0.52
Weight, kg	72.90±9.10	59.20 ± 5.70	71.20±10.60	62.30±9.60	0.51	0.11
BMI, kg/cm^2	22.90±2.90	22.10±2.20	22.70±3.30	23.50±4.00	0.77	0.01
Trauma age, years	23.60±7.50	26.50±8.30	-	-	-	-
Trauma duration, years	16.50±11.40	14.90±6.60	-	-	-	-
Trauma level	C_{4-6}=9 Th_{2-12}=19 L_1=11	C_6=1 Th_{4-12}=22 L_1=12	-	-	-	-

Table 2. Bone mineral status in the patients with chronic spinal cord injury and persons of control group

Parameter	Men		Women	
	Main group	Control group	Main group	Control group
T-score	-3.20±0.80*	-0.40±0.80	-3.10±0.50*	-0.60±0.60
Z-index	-2.70±0.70*	0.40±0.20	-2.90±0.50*	-0.40±0.60
SI	51.40±11.80*	98.50±16.60	50.10±9.80*	92.90±11.10
extrapolated BMd	0.25±0.08*	0.55±0.10	0.24±0.06*	0.51±0.07
BUA	40.50±13.80*	78.70±15.70	37.40±12.70*	76.90±11.30
SUP	1477.00±19.00*	1554.00±26.00	1473.00±25.00*	1542.00±18.00

Note: – p<0.05, differences in parameters of the main and control groups.*

of post-traumatic period, but there is a proven relationship of osteoporosis risk and bone characteristic features at the time of the injury [6].

The results of the study performed by Professor VV Povoroznyuk in 1998 prove that in the Ukrainian population women reach peak bone mass at the age of 23-24 years old and men – at the age of 25-26 [7]. In present study, patients were divided into 2 subgroups according to their trauma age. A cutting-off point for men was 25 years and for women – 23 years of age.

In the patients with the injury that occurred before reaching peak bone mass, the bone mineral status according to ultrasound densitometry was significantly worse than that of the patients of corresponding age, anthropometric parameters and duration of the posttraumatic period with the onset of injury at the age over 23 and 25 years, respectively (Fig. 1, 2).

No difference was proved in the bone mineral status of the women depending on their age at the injury onset, but after excluding postmenopausal women from both study groups, it was found out that bone mineral status was significantly worse in the women with trauma at the age of 23 years old (Fig. 2).

A significant difference in stiffness index in the women of reproductive age and postmenopausal women was not evidenced due to small size and heterogeneity of sample. In addition, the impact of menopause should be considered in certain groups of women, depending on the age at the injury onset before or after reaching peak bone mass.

Among the examined individuals, 18 patients had fractures of hip (11 patients) or tibia fractures (7 patients) due to a low-energy trauma in the period after a spinal cord injury. 66% of them had a spinal cord injury at the age of 25 years old (p=0.001). Other differences between the group of patients with fractures and without fractures were not detected.

Bone metabolism according to remodelling markers was analysed separately for women and men, and compared with the reference data for the Ukrainian population (Table 3) [8].

According to the results of previous studies, bone metabolism is much increased after SCI, and then it gradually decreases, but remains high in the first years after the injury [4]. This paper proves that bone remodelling markers remain elevated in the later period of the injury as well (traumatic disease duration from 5 to 38 years, average duration – 14.7±1.2). In both women and men with SCI, tP1NP levels are significantly higher than the reference values. Men are also characterized by higher levels of bone resorption marker (β-CTx). the level of

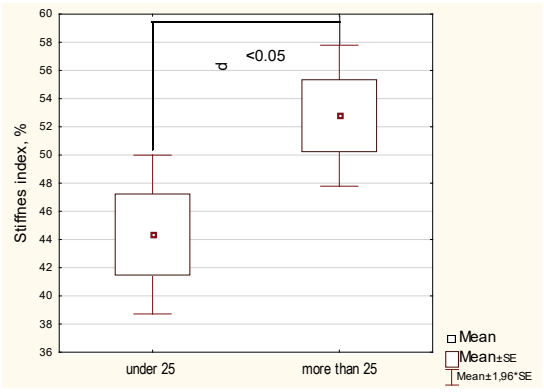

Fig. 1. Bone mineral status of men depending on the spinal cord injury age.

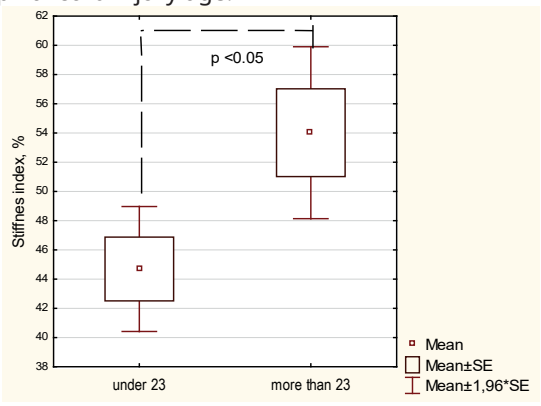

Fig. 2. Bone mineral status of reproductive age women with SCI depending on the age at trauma incidence.

vitamin D3 is significantly lower in the SCI patients than the reference values. no significant relationships of the levels of bone remodelling markers and SCI duration were evidenced, but upon division of patients into 2 groups depending on the post-traumatic period duration: subgroup A<14.7 and subgroup B>14.7 years, the β-CTx level was significantly higher in subgroup A, 0.55±0.07 pg/ml, in subgroup B – 0.39±0.04 pg/ml, t=2.4, p<0.05, proving gradual slowing of the destruction process in the late posttraumatic period.

Discussion

Increased bone resorption in the SCI patients occurs very quickly after the injury and affects bones below the spinal cord injury. According to the results of previous studies, the patients with signs of complete spinal cord injury, who had a fragility fracture, were diagnosed with a significant loss of bone mineral density in the distal femur (54%) and distal tibia bone (73%), which took place for the first 5-7 years after the injury [5]. Interestingly that bone loss is greater in the distal section. The above trend suggests that at the level of calcaneal bone, the loss will be even greater, also due to a higher content of spongy bone in the calcaneal bone. The use of ultrasound densitometry of calcaneal bone as a screening method in the diagnosis of osteoporosis is based on the close relationship of parameters of vertebral bone and calcaneal bone. In the SCI patients, this relationship is missing. After the complete spinal cord injury, bone changes differently depend on the location. In parts of the skeleton, located above the injury level, bone mineral density decreases immediately after the injury, but gradually returns to normal parameters and even increases with increased load, such as bone mineral density of upper limbs in the patients with paraplegia. In the sections below the injury, with impaired innervation and reduced static load, such as lower limbs, bone mineral density decreases progressively [4, 9]. Thus, regardless of the injury level, the lumbar vertebrae and calcaneal bone is in different conditions and changes in them are opposite. distal femur and proximal tibia are the most frequent localization of fractures in these patients and therefore an area of interest. These areas consist mainly of spongy bone and are closer in composition to calcaneal bone, therefore lose bone tissue in a similar way in the absence of axial load and adequate trophic effects.

The results of ultrasound densitometry of calcaneal bone can also diagnose osteoporosis, but according to the literature the capabilities of this method in the follow up of SCI patients are limited [8-10]. The loss of bone tissue after a severe spinal injury develops rapidly, resulting in the development of osteoporosis and significantly increased fracture risk, but after 3-5-10 years, the rate of bone resorption slows down. Further, the post-traumatic period duration has less effect on the bone mineral status [4]. Inverse correlation was proved between the post-traumatic period duration and the number of proximal tibia trabecules that confirmed the continued predominance of resorption processes in spongy bone. Simultaneously, the thinning of cortical layer of long bones of lower extremities took place, but the loss of cortical layer occurred much more slowly than that of trabecular bone. Slow cortical layer thinning may explain the fact that the average period until the first fracture in SCI patients is 9 years after the injury [3]. Thus, correction of structural-functional state of bone as fracture prevention has quite a large therapeutic window – 9 years. Among the leading risk factors for osteoporosis in the SCI patients along with severity of the injury and absence of axial load in the post-traumatic period, the low baseline values of mineral density and high levels of markers of bone remodeling are present [11]. This coincides with the results of our study. The patients, who had injury before bone mass peak, have lower parameters of structural and functional state of bone and increased risk of fractures. All patients in our study had low bone mineral density (according to Z-criterion or T-score, depending on the age). According to the 2014 National Osteoporosis Foundation guidelines, SCI is one of the diseases and conditions that contribute to the development of osteoporosis and fractures, and therefore the patients with SCI and low bone mineral

Table 3. Bone turnover markers and vitamin D level in the SCI patients

Bone metabolism parameters	Bone metabolism reference values		Bone metabolism parameters in the SCI patients		P	
	For men	For women	In men	In women	men	women
tP1NP, ng/ml	34.50±5.80	44.70±4.40	43.80±4.30	57.40±4.50	**<0.05**	**<0.05**
OC, ng/ml	24.30±1.70	23.40±1.50	24.50±1.90	19.60±1.50	>0.05	**<0.05**
β-Ctx, ng/ml	0.37±0.06	0.41 ±0.07	0.58±0.05	0.36±0.04	**<0.05**	>0.05
25(OH)D3, nmol/ml	44.60±5.20	40.40±5.60	26.80±7.80	20.30±4.50	<0.05	<0.05

density can be diagnosed with secondary osteoporosis. The prevalence of this condition among the SCI patients encourages further researches.

Conclusions

SCI leads to increased bone resorption with development of secondary osteoporosis (according to the ultrasound densitometry of calcaneal bone). In chronic SCI, bone resorption is higher than in the individuals with combined low levels of vitamin d, and the absence of axial load results in continued loss of bone mass.

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I appreciate the help of the Ukrainian Association of Osteoporosis (the President – Professor Povoroznyuk V. V.) and Association of Disabled Persons with Spinal Cord Injuries (the President – Marusevych I. M.) in conducting research.

ЗМІНИ МІНЕРАЛЬНОЇ ЩІЛЬНОСТІ ТА МЕТАБОЛІЗМУ КІСТКОВОЇ ТКАНИНИ В ПАЦІЄНТІВ ЗІ СПІНАЛЬНОЮ ТРАВМОЮ

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УКРАЇНСЬКИЙ ЦЕНТР ЛІКУВАННЯ ОСТЕОПОРОЗУ, КИЇВ, УКРАЇНА

Вступ. *Вивчення пізніх ускладнень при травмах спинного мозку, у тому числі остеопорозу, є актуальним через збільшення тривалості життя таких пацієнтів.*

Мета дослідження *визначити мінеральну щільність та особливості метаболізму кісткової тканини у хворих із травматичною хворобою спинного мозку.*

Методи. *Обстежено 73 хворих із травмами спинного мозку та 57 здорових осіб. За допомогою ультразвукової денситометрії визначили мінеральну щільність кісток; маркери ремоделювання кісткової тканини та вміст вітаміну Д стан – методом електрохімілюмінесценції.*

Результати. *У хворих із травмами спинного мозку стан мінерального компонента кісткової тканини був значно нижчим порівняно з хворими контрольної групи. Індекс міцності кісткової тканини становив 51,4±11,8% у порівнянні з 98,5±16,6 % (p<0,05) у чоловіків і 50,1±9,8 % у порівнянні з 92,9±11,1 % (p<0,05) у жінок. У хворих із травмами спинного мозку рівень маркерів ремоделювання кісткової тканини був значно вищим, ніж у контрольній групі. Стан мінерального компонента кісткової тканини у хворих із травмами спинного мозку значно гірший, порівняно з особами з вже розвинутою піковою кістковою масою, що дає змогу їх віднести до групи підвищеного ризику виникнення переломів.*

Висновки. *Травми спинного мозку зумовлюють посилення резорбції кістки з розвитком вторинного остеопорозу (за даними ультразвукової денситометрії п'яткової кістки). Процеси резорбції є значно інтенсивнішими при травмах спинного мозку у хворих із низьким рівнем вітаміну D, а також при відсутності осьового навантаження.*

КЛЮЧОВІ СЛОВА: **травма спинного мозку; остеопороз; тетраплегія; параплегія; маркери ремоделювання кісткової тканини; ультразвукова денситометрія**

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PATHOGENETIC APPROACHES AND WAYS OF PREVENTION OF THROMBOEMBOLIC COMPLICATIONS IN TRAUMA PATIENTS

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Background. *The study of thromboembolic complications prevention in trauma patients, who underwent surgery, is presented in the research***.**

Objective. *Patients were examined in the Department of Traumatology of Ternopil Municipal Hospital. The first group, 263 people, (18.6 %) consisted of patients with polytrauma and unfavorable prognosis and significant disease severity. The second group comprised patients with combined trauma, 462 people (32.8 %) – a doubtful prognosis for life. The third group, 685 people (48.6 %) involved patients with isolated trauma and positive treatment outcome.*

Methods. *All patients, besides general clinical examination, underwent evaluation of the number of platelets, clotting time, duration of bleeding and study of coagulation (prothrombin index, prothrombin activity thrombotest, total fibrinogen, fibrinogen A, activated recalcification time). The venous system of lower limbs was examined using distal ascending phlebography, color Doppler and duplex ultrasonography SIMENS ACUSSON X 300.*

Results. *A comprehensive prophylaxis of thromboembolic complications was carried out using low-molecular weight heparin as well as essential complex kinetic treatment. Bemiparin in an appropriate dose was administered once a day for 10-14 days of postoperative stay in the hospital. For the patients with moderate risk and high surgery risk (major surgery, over 40 years old in age, obesity, and serious comorbidities) Bemiparin was administered at a dose of 5000-7500 IU per day during patients' stay in the hospital. In individuals with sub-acute and chronic thrombophlebitis of subcutaneous veins the surgical prophylaxis of thromboembolic complications was performed.*

Conclusions. *The combination of physical, drug and surgical prophylaxis prevented the thromboembolic complications in trauma patients.*

KEY WORDS: **thromboembolism; trauma; polytrauma; prevention; bemiparin.**

Introduction

Thromboembolism is one of the most formidable complications after surgical interventions that cause high mortality. The frequency of thromboembolism depends on the duration of traumatic procedures and increases with the larger its dimension and expansion of indications for them, especially in the elderly patients [1]. The situation in the Departments of Traumatology and Orthopedics, where thromboembolic complications are present in more than a half of patients, is especially threatening [5].

Diagnosis, treatment and prediction of the consequences of thromboembolism in this category of patients are accompanied by considerable difficulties. It is noteworthy that the largest number of fatal cases (up to 75.4 %) was registered not at the day of injury, but in 3-5 days after it or later [2, 4]. The etiology of thromboembolism has a multifactorial nature: varicose veins of lower extremities, phlebothrombosis of small pelvis veins, venous stasis of lower extremities, postoperative violation of blood rheology are the most important.

Significant cause of thromboembolic complications is the body's response to surgical trauma, which is accompanied by spasm of peripheral vessels, hypercoagulation and vascular wall injury, especially in cases of surgeries on lower extremities, hip joint and pelvic bones [6, 7, 8].

The deep veins thrombosis of lower extremities and pelvis complicates the postoperative period by 10-30% in the elder age group [3].

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In many patients, the first clinical manifestation of deep vein thromboembolism may be thromboembolism of pulmonary artery [11]. With age in the body there are a number of hemodynamic (decreased shock, respiratory minute volume and blood circulation, increased peripheral vascular resistance) and hematologic changes (increase of aggregation and adhesion ability of platelets, activity of plasma coagulation factors, and concentration of fibrinogen), which contribute to the presence of deep vein thrombosis [9, 10].

Methods

The patients of the Department of Traumatology of the Ternopil City Communal Emergency Hospital were examined. Over the last year, 1410 patients of different age groups were hospitalized: 588 (41.7 %) men, 822 (58.3 %) women.

During the study the patients were divided into 3 groups depending on the severity of the injury. The first group, 263 men (18.6 %), consisted of patients with polytrauma and unfavorable prognosis due to the significant disease severity. The second group consisted of patients 462 people (32.8 %), with a combined injury and doubtful prognosis for life. The third group, 685 people (48,6 %), involved patients with isolated trauma and positive treatment outcome (Table 1).

The patients of the first group were in a state of severe combined shock. They were given resuscitation aimed at correction of hemodynamics and function of external respiration. The comprehensive examination of those patients included clinical, laboratory and radiological methods by means of laparocentesis, thoracentesis, sonography, computed tomography scan.

Table 1. The number of trauma patients, who were hospitalized

Group	Number of patients	% of patients
I	263	18,6
II	462	32,8
III	685	48,6

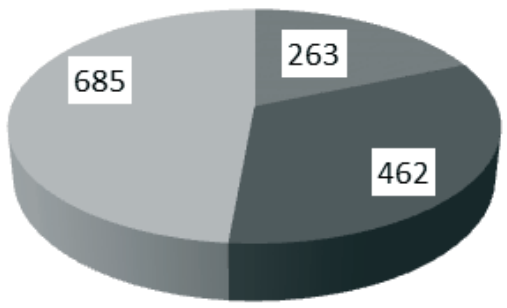

Fig. 1. The number of trauma patients, who were hospitalized.

In diagnostics of prolonged bleeding without delay, against the background of anti-shock therapy, surgical interventions aimed at stopping it were performed. On the damaged segments of musculoskeletal system, with satisfactory standing of fragments, plaster casts and skeletal extracts were used in the presence of displacements.

In the second group of patients, treatment also began with actions for vital signs support (fight against bleeding, respiratory failure). All measures were carried out against the back_ ground of intensive anti-shock therapy and elimination of dominant internal organs damage after appropriate preoperative training. Later, with satisfactory hemodynamic parameters, urgent surgeries were carried out on injuries of the musculoskeletal system.

In cases of surgical interventions in the patients of the third group with severe predominant trauma of musculoskeletal system, among which there were fractures of thigh, leg bones, shoulder, forearm, foot, brush, shoulder girdle, metal osteosynthesis was carried out. Exceptions were the cases of severe placement and tear of limbs. Such patients underwent a primary surgical treatment of the wound or limb amputation without delay.

All patients, besides general clinical examination, underwent evaluation of the number of platelets, clotting time, duration of bleeding and study of coagulation: prothrombin index, prothrombin activity thrombotest, total fibrinogen, fibrinogen, fibrinogen A, activated recalcification time.

The venous system of lower extremities was studied by means of distal ascension phlebography, color dopplerography and duplex ultrasonography SIMENS ACUSSON X 300.

Accompanying pathology of cardiovascular, respiratory and endocrine systems was found in 1167 (82.8 %) persons.

In patients with decompensation of accompanying pathology (coronary heart disease, hypertension, non-specific chronic diseases of lungs, diabetes mellitus), preoperative preparation was conducted in a hospital setting.

The number of surgical interventions during the period of research in the department is 865 surgeries, including 410 urgent. The other patients were treated conservatively (Table 2).

Table 2. Types of surgical interventions carried out in the Department of Traumatology

Type of surgery	Quantity	Number of thrombo-embolic complications
1. Surgery of the segments of musculoskeletal system:		17.2 %
1.1. Open reposition and metal osteosynthesis	376	
1.1.1. Intramedullary blocking and metal osteosynthesis	44	
1.1.2. External fixing devices	25	
1.1.3. Repositioning	12	
1.1.4. Sketch plates	295	
1.2. Primary surgical treatment and metal osteosynthesis	74	
1.3. Primary surgical treatment without metal osteosynthesis	191	10.0 %
2. Joints surgery	**93**	44.2 %
2.1 Open reposition and metal osteosynthesis	38	
2.2 Removal of metal fixators	3	
2.3 Arthroscopy	3	
2.4 Endoprothesis	49	
3. Reconstructive surgery	**31**	24.1 %
3.1. of soft tissues	27	
3.2. of bones	4	
4. Removal of metal sections	**56**	-
5. Minimally invasive intervention	**19**	-
6. Palliative treatment of metastatic bone lesion	**12**	4.5 %
7. Other	10	-

Results

In the examined patients, a number of factors of postoperative thrombotic complications were defined: thrombotic complications in history, coagulation disorders, cardiovascular and malignant diseases, overweight, traumatic complications, and traumatic nature. Malignant diseases, breast cancer, prostate and rectum cancer were most commonly associated with pathological fractures of femur and shoulder bones.

Chronic cardiac insufficiency of the I-II degree was diagnosed in 651 (46.2 %) patients, varicose enlargement under skin of lower limbs veins – in 624 (42.2 %) patients. Obesity was evidenced in 237 (16.8 %) patients. Hypercoagulation was revealed in 270 (18.2 %) patients. Changes in the coagulogram did not always correspond to the severity and features of phlebothrombosis. Only in 6 out of 33 pa_ tients fibrinogen B was present within 8 days after the surgery. A slight increase in fibrinogen A was observed in 22.3 % and significant one (up to 9.9 g/l) in 24.2 % of patients. In all other patients, its content was decreased up to 1.45 g/l. the changes in other indicators were not significant that did not cause the need for additional instrumental survey methods.

Diseases of thrombophlebitis of subcutaneous or deep veins of lower extremities were revealed in 72 (15.3 %) persons.

The choice of anesthetic method also affected the number of thrombotic complications. One of the main causes of thromboembolic complications in trauma patients in the postoperative period is spasm of peripheral vessels caused by operational stress, which leads to tissue ischemia and thrombosis. The stress response of the body in general with anxiety is the result of insufficiency of blockade of nerve impulses that are transmitted through the sympathetic nervous system.

The number of phlebothrombosis in patients who underwent surgery under general anesthesia with artificial ventilation was high – 22 cases (68.3 %). The decrease in the number of complications took place due to significant reduction of stress pulses in cases of spinal anesthesia (9 patients (27.2 %)) and conductive anesthesia in combination with intravenous anesthesia (2 patients (4.5 %)).

Discussion

Prevention of thrombotic complications was aimed at elimination of pathogenetic parts of phlebothrombosis, such as decreased blood flow, increased blood coagulation, damage of vascular wall and dependence on the risk factors presence.

The mechanism of action was physical, drug and surgical methods of prevention of thrombophlebitis of lower extremities. Over time,

these methods were applied preoperatively, sub-operatively and postoperatively.

Preventive measures were taken on the 1st–2nd day of hospital stay.

All patients fully underwent a prophylaxis of thromboembolic complications by means of low molecular weight anticoagulants and compulsory complex of kinetic therapy. Bemiparin (low molecular weight heparin (LMWH), Zibor 2500, Berlin-Chemie AG) at an appropriate therapeutical dose was prescribed from the 1st day of patients' stay in the hospital for 10-14 days of the postoperative period once a day subcutaneously in the stomach area. For the patients with moderate risk and high surgery risk (major interventions, over 40 years old in age, obesity, and serious co-morbidities) Bemiparin was prescribed at a dose of 5000-7500 IU per day while patients stay in the hospital [12, 13].

In case of changes of venous tract of legs, in the patients with a great anamnesis, elastic stockings and elastic bandings were worn.

Suboperative procedures into ankle joint, early arousal of the patient after surgery, medical physical exercises were performed for all patients.

Venotonics (Detralex, Phlebodia-600, etc.) were used in the complex treatment of the patients with chronic venous insufficiency [14].

In persons with subacute and chronic thrombophlebitis of subcutaneous veins the surgical prophylaxis of thromboembolic complications was performed. A crossectomy was carried out in 9 (1.9 %) patients, as the first stage before surgical intervention.

Not taking into account the complex of preventive measures, in 4-5 days after surgery, in 10 patients (1.1 %) thrombophlebitis of shin varicose veins, in 12 (1.3 %) – iliofemoral thrombosis developed, in 8 (0,9 %) patients deep thrombosis of lower extremities veins was diagnosed. Drug therapy was effective in 16 people. 4 patients had ligation of thighs large subcutaneous vein. In 1 case, small intestinal tract was observed. There were no cases of lethal death associated with thromboembolic complications. No hemorrhagic complications were present.

Thus, the development of pathogenetic mechanisms of thromboembolic complications in the complex measures for their prevention allowed decreasing the number of postoperative complications and mortality in trauma patients.

Conclusions

In trauma patients, who undergo surgical intervention, a high risk of thromboembolic complications is established that is a prerequisite for the preventive measures.

Prevention of thromboembolic complications should be complex and individual, taking into account the nature of the injury, the dimension of surgical intervention, and the associated risk factors.

The combination of physical, drug and surgical prophylaxis prevent thromboembolic complications in trauma patients.

ПАТОГЕНЕТИЧНІ ПІДХОДИ ТА ШЛЯХИ ПРОФІЛАКТИКИ ТРОМБОЕМБОЛІЧНИХ УСКЛАДНЕНЬ У ТРАВМАТОЛОГІЧНИХ ХВОРИХ

Л. Ю. Іващук

ТЕРНОПІЛЬСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Розлянуто питання профілактики тромбоемболічних ускладнень у травматологічних хворих.*

Мета. *Пацієнтів відділення травматології Тернопільської міської лікарні було поділено на кілька груп групи. Перша група налічувала 263 особи (18,6%) з політравмою, несприятливим прогнозом і у тяжкому стані. Другу групу (462 хворих або 32,8% вибірки) склали пацієнти з комбінованою травмою та сумнівним прогнозом для життя. Третя група налічувала 685 осіб (48,6% вибірки) - пацієнти із ізольованою травмою та сприятливим прогнозом.*

Методи. *Усім пацієнтам, окрім загального клінічного обстеження, визначали наступні показники: протромбіновий індекс, протромбінову активність, тромботест, загальний фібриноген, фібриноген В, фібриноген А, активований час рекальцифікації. Венозну систему нижніх кінцівок було досліджено за допомогою дистальної висхідної флебографії, кольорового допплерівського та дуплексного УЗД із Simens Acusson X 300.*

Результати. *Усім хворим у повному об'ємі було проведено профілактику тромбоемболічних ускладнень шляхом застосування низькомолекулярних гепаринів і обов'язковим комплексом кінетерапії. Беміпарин у відповідній дозі вводили один раз на день протягом 10-14 днів під час післяопераційного перебування в стаціонарі. Для пацієнтів з помірним і високим ризиком (обширне оперативне втручання, вік понад 40 років, ожиріння і серйозні супутні захворювання) беміпарин вводили у дозі 5000-7500 МО на добу під час перебування пацієнтів у стаціонарі. У осіб з помірно гострим і хронічним тромбофлебітом підшкірних вен проводили хірургічну профілактику тромбоемболічних ускладнень.*

Висновки. *Поєднання фізичної, фармакологічної та хірургічної профілактики запобігало тромбоемболічним ускладненням у травматологічних хворих.*

КЛЮЧОВІ СЛОВА: **тромбоемболія; травма; політравма; профілактика; беміпарин.**

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MIXED GERM CELL TERATOMATOUS TUMOUR OF TESTIS IN ADULTS: DIAGNOSTIC CHALLENGES FOR A HISTOPATHOLOGIST (case report)

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Background. *Testicular tumours account for approximately 1-2 % of the total cancer cases in the male population globally and show higher incidence in the younger male age group of up to 15 years. The majority (~98 %) of testicular tumours are observed to be of the germ-cell origin which can either be of seminomatous type or non-seminomatous type. The non-seminomatous germ cell neoplasm may be pure or of mixed subtype. Objective was to emphasize the rare case of mixed germ cell teratomatous tumour of testis in adult man.*

Methods. *A mixed germ cell teratomatous tumour of testis comprising of yolk sac tumour and embryonal carcinoma in an adult Indian male is reported in the research.*

Results. *A 45 year-old Indian male presented with enlargement of right testis which was found to be an encapsulated right testicular tumour on exploratory surgery which was followed by radical orchiectomy. Serum AFP and β-hCG levels were elevated to 380 ng/ml and 590 mg/ml respectively. Histopathology revealed a mixed germ cell teratomatous tumour of testis comprising of yolk sac tumour and embryonal carcinoma.*

Conclusions. *In adults teratomas occur usually as a component of mixed germ cell tumours. However in the present case teratomatous embryoid yolk sac germ cell tumour of testis was observed in an Indian adult male. The prognosis of embryoid germ cell tumours of testis is generally poor. The possibility of this condition should always be considered in all cases that present with a testicular lump.*

KEY WORDS: **testicular tumours; mixed germ cell tumor; embryoid bodies; adult male**.

Introduction

Testicular tumours account for approximately 1–2 % of the total cancer cases in the male population globally [1]. Testicular neoplasia present in a very distinctive age group of distribution and prove higher incidence in the younger male age group of up to15 years old [2]. The majority (~98 %) of testicular tumours are observed to be of the germ-cell origin [3]. Germ cell neoplasia includes the seminomatous (SGCT) and non-seminomatous germ-cell tumour (NSGCT) types. NSGCT may present as pure (one cell type), or mixed cell (multiple cell type) tumours, depending upon the origin from the adult germ cells or dedifferentiated embryonal cells or from both [2, 4]. The adult germ cell tumours include seminoma, spermatocytic seminoma, while the dedifferentiated NSGCT present a wide spectrum including embryonal carcinoma, embryonal-like somatically differentiated teratoma, extra-embryonally differentiated choriocarcinoma, and yolk sac tumour phenotypes [2]. The embryonal tumours usually occur in the infant and child groups [5].

A mixed germ cell teratomatous tumour of testis comprising of yolk sac tumour and embryonal carcinoma in an adult Indian male is reported in the research.

Case History

A 45 year-old Indian male was admitted in Jawaharlal Nehru Medical College, Aligarh Muslim University, India with the complaints of enlargement of right testis in the scrotum for six months. Patient was conscious about testicular mass and had no other constitutional symptoms. On clinical examination, a non-tender, firm, freely mobile, and oval lump measuring 6×4×3 cm was palpable, diffusely involving the right testis. The left side showed normal descended testis of normal size, shape, and consistency. Other external genitalia were normal.

Ultrasonographic examination of testis revealed a well-defined encapsulated mass of a size of 54×44 mm with solid and cystic components. No inguinal lymph node enlargement was evidenced. Chest radiography results

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were normal. Serum AFP and β-hCG levels were elevated to 380 ng/ml and 590 mg/ml respectively. Exploratory surgery revealed an encapsulated right testicular tumour. Radical orchiectomy was performed and excised mass was submitted for final diagnosis.

Gross examination proved a firm, encapsulated globular swelling measuring 4.5x4x3 cm. The cut surface presented greyish brown appearance with cystic and solid areas (Fig. 1).

Haematoxylin and Eosin (H&E) stained paraffin sections revealed mixed cell tumour comprising of large pleomorphic atypical cells with vesicular nucleus, prominent nucleoli, abundant cytoplasm, and poorly defined cell borders forming embryoid bodies pathognomonic of embryonal carcinoma of testis (Fig. 2a and 2b). The interspersed areas were of epithelial tubulo-papillary structures with Schiller-uval bodies and presence of hyaline globules characteristic of yolk sac tumour (Fig. 3).The findings were diagnostic of teratomatous mixed germ cell tumour of testis comprising of yolk sac tumour with embryoid carcinoma of testis.

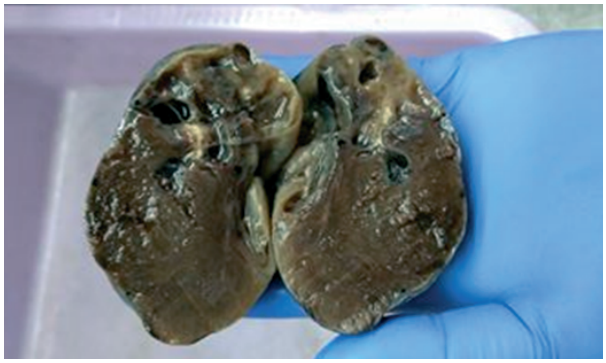
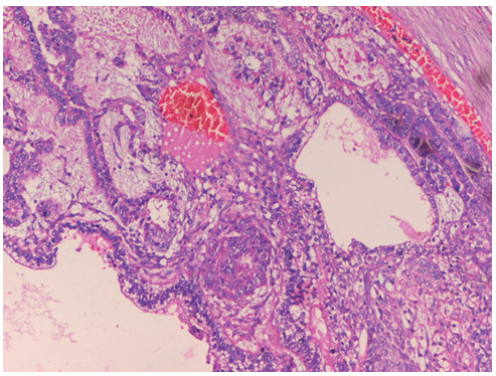
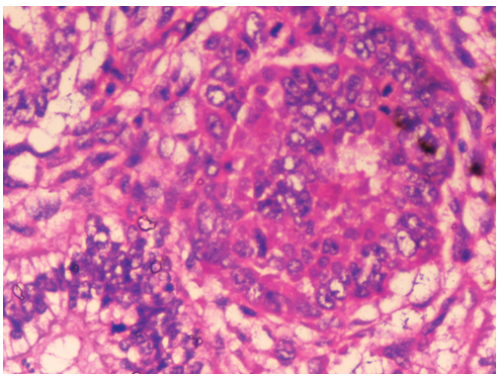


Fig. 1. Gross examination showed a firm, encapsulated globular swelling measuring 4.5x4x3 cms. The cut surface presented greyish brown appearance with solid-cystic areas

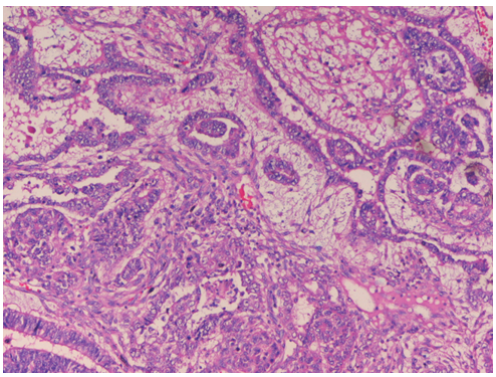


2a

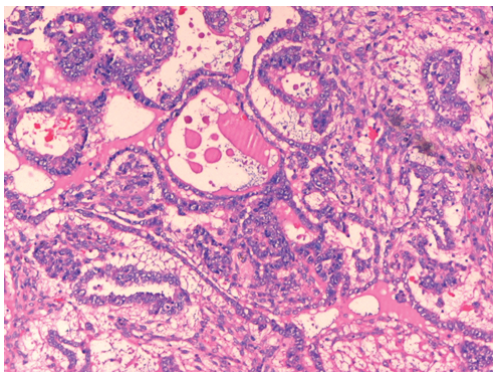


2b

Fig. 2a and 2b. H&E stained sections revealed mixed cell tumour comprising of large pleomorphic atypical cells with vesicular nucleus, prominent nucleoli, abundant cytoplasm, and poorly defined cell borders forming embryoid bodies pathognomonic of the embryonal carcinoma of the testis (100x and 400x).



3a



3b

Fig. 3a and 3b. H&E stained sections showing epithelial tubulo-papillary structures with Schiller-Duval bodies and presence of hyaline globules characteristic of Yolk sac tumour (100x).

Follow up. Post-surgical recovery of the patient was uneventful. He was referred to Medical Oncology Department for platinum chemotherapy and reporting well under follow up for the last 4 months.

Discussion

Histopathological findings in the present case were diagnostic of teratomatous mixed germ cell tumour of testis comprising of yolk sac tumour and embryoid carcinoma of testis. Diagnosis of gonadal germ cell tumours presents diagnostic challenges for the histopathologist. Microscopically, more than a half of germ cell tumours consist of more than one cell type, requiring appropriate sampling for the correct diagnosis [6]. Usually these tumours present one or more of the 4 basic histological patterns namely, seminoma, embryonal carcinoma, choriocarcinoma, and teratoma [7]. The correct diagnosis often has major important therapeutic and prognostic implications.

About one-third of all the testicular tumours are mixed germ cell tumours and 50 % of the mixed cell testicular tumours contain teratomatous components [8]. Very often a random admixture of elements is noted. Yolk sac tumour is a common component of mixed germ cell tumours of testis, accounting for about 1 % of the testicular germ cell tumours [7]. In one series the most frequent combination of mixed germ cells in the tumours was of teratoma and embryonal carcinoma, the major component being teratomatous embryoid elements with minor foci of yolk sac tumour [8]. Another study reported histologically that nearly 59 % of the mixed germ cell tumours contained seminoma, 41 % yolk sac tumour, and 47 % embryonal carcinoma and teratoma components [3].

Although mixed germ cell tumours are common in testis; the presence of embryonal carcinoma and yolk sac elements in the orchidectomy specimen have been a histopathological curiosity, emphasizing the pluripotentiality of the testicular germ cells. Some workers related this pluripotency of testicular germ cells either to testicular embryonal cell-rests, or to mutations of gonocytes acquired during the fetal development, or to acquired germ cell chromosomal abnormalities during the adulthood [9, 10]. Irrespective of the developmental pathway the tumour cells can histologically differentiate into spermatic germ cell like differentiated (seminoma), primitive zygotic (embryonal carcinoma), embryonal-like somatically differentiated (teratoma), and extra-embryonally differentiated (choriocarcinoma and yolk sac tumour) phenotypes [7].

Human chorionic gonadotropin (HCG) and α-Fetoprotein (AFP) serve as helpful tumour marker for testicular germ celltumours in the adult population [11].

In adults teratomas occur usually as a component of mixed germ cell tumours. The age range in adults is same as of other malignant germ cell tumours. Pure teratomas are uncommon in adults but when present behave in a malignant fashion [12]. Histologically, teratomas consist of tissues representing the 3 germinal layers: endoderm, mesoderm, and ectoderm. The embryonal carcinoma with teratoidembryoid bodies and yolk sac tumour elements are characteristic histopathological findings which distinguish the teratomatous germ cell tumours of testis from the other germ cell tumours. Usually teratomatous germ cell tumours occur in the infant and early childhood [5]. However in the present case teratomatous embryoid yolk sac germ cell tumour of testis was observed in an Indian adult male. The prognosis of embryoid germ cell tumours of testis is generally poor [13, 14].

Conclusions

We wish to emphasize through this case report that even though the occurrence of mixed germ cell tumour in adults is a rare phenomenon, the possibility of this condition should always be considered in all cases that are present with a testicular lump.

ЗМІШАНІ ГЕРМІНОГЕННІ ПУХЛИНИ ЯЄЧКА У ДОРОСЛИХ: ДІАГНОСТИЧНИЙ ВИКЛИК ДЛЯ ГІСТОЛОГА (КЛІНІЧНИЙ ВИПАДОК)

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Вступ. *Питома вага гермiногенних пухлин яєчка становить приблизно 1-2% від загальної кількості злоякісних пухлин у чоловіків, і частіше зустрічаються у віці до 15 років. Більшість (~ 98%) пухлин яєчок, які мають походження із зародкових клітин, можуть бути семіномними і несеміномними пухлинами. Останні можуть бути представлені одним компонентом (чисті) або кількома – змішані пухлини.*

Мета дослідження *ознайомити широкий загал лікарів з рідкісним випадком змішаної гермiногенної пухлини яєчка у дорослого чоловіка.*

Методи дослідження. *Описано клінічний випадок змішаної тератоматозної гермiногенної пухлини, що складалася з пухлини жовткового мішка і ембріональної карциноми у дорослого індійського чоловіка.*

Результати дослідження. *У 45-річного чоловіка із збільшенням правого яєчка діагностовано інкапсульовану пухлину правого яєчка, після якої проведено радикальну орхіектомію. Рівні сироваткового альфа-фетопротеїну та хоріонічного гонадотропіну були підвищені до 380 нг/мл і 590 мг/мл відповідно. Гістологічний аналіз виявив змішані зародкові клітини тератоматозної пухлини яєчка, що містили клітини жовткового мішка і ембріональної карциноми.*

Висновки. *Зазвичай у дорослих тератоми виникають як компонент змішаних пухлин ембріональних клітин. Проте в даному клінічному випадку тератоматозна гермiногенна пухлина спостерігалася у дорослого індійського чоловіка. Прогноз при таких пухлинах, як правило, несприятливий. Незважаючи на те, що подібні пухлини зустрічаються вкрай рідко, це необхідно враховувати при диференціальній діагностиці.*

КЛЮЧОВІ СЛОВА: **пухлини яєчок; змішана герміномна пухлина; ембріональні тільця; дорослий чоловік.**

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IS METABOLOMICS THE DIAGNOSTIC TOOL FOR MEDICAL DIAGNOSTICS OF CANCER? AN EXAMPLE BASED ON LUNG AND BREAST CANCER

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Background. *Metabolomics is a relatively new diagnostic tool that allows a deep insight into the body metabolism at a cellular level.*

Objective. *This paper provides a comprehensive view into the metabolomics methodology and shows usefulness of this approach in diagnosing and stratifying lung and breast cancers.*

Methods. *Literature review of metabolomics studies and its clinical application in the diagnosis of cancer-selected studies.*

Results. *In general, the metabolomic approach comprises three steps: 1) sampling and preparing biofluids or tissue homogenates, 2) identification of low-molecular weight compounds up to 1.0 kDa using nuclear magnetic resonance, mostly ^{1}H-NMR and/or mass spectrometry, and finally 3) data processing and analysing. It is possible to identify a set of metabolites, which is specific for a certain metabolic status (the metabolic fingerprint). Furthermore, this set of metabolites provides information of possible pathomechanisms involved in the disease process i.e. information about the disease etiology. It has been proven that the change in metabolome precedes; not only clinical symptoms but other laboratory findings as well. Consequently, this approach, if sufficiently validated, seems to be very promising especially in screening and early diagnosing.*

Conclusions. *It was demonstrated that metabolomic approach allows to discriminate patients with cancer from healthy persons, as well as to differentiate between clinical stages of the cancer.*

KEY WORDS: **metabolomics; metabolome; breast cancer; lung cancer**.

Introduction

To understand the complex processes occurring in the living systems the holistic approach should be used within employing all established multilevel approaches, currently is called 'omics science'. In general, there are more than 100 types of omics subjects, including sciences such as: genomics, transcriptomic, proteomics, and metabolomics. This approach includes all-important live processes from information storage transcription processes, protein production, up to enzymes actions transforming certain substrates to products maintaining the cell living processes. While genomics and proteomics have been successfully introduced to routine diagnostics, metabolomics is still on the runway despite its assessed potential [1].

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Metabolomics refers to the analysis and interpretation of ongoing processes in living organisms at the end of life chain consisting of genomics-transcriptomics-proteomics-metabolomics science. Metabolomics might be strongly influenced by external factors such as environment, life style, diet, medications, as well as by additional metabolite input from the body's microbiomes. Metabolites are delineated as low-molecular weight compounds (LMWC) up to 1.0 kDa. Those compounds are carbohydrates, fatty acids, lipids, amino acids, nucleosides, or other organic molecules which are involved in biochemical reactions as substrates, intermediates, and/or final products. The set of body's metabolites ultimately form its characteristic metabolome. Metabolome is a dynamic system, which is largely dependent on internal biochemical reaction and external factors mentioned above [1, 2]. Metabolomics is a comparative science, e.g. the obtained information should be compared to the referenced one or vice versa. With this approach metabolomics can be

regarded as a diagnostic tool, which in principle enables to describe current metabolic status of cells, tissues, and organs. Simply put, it can define the general health of a living organism. This is done by analysing body fluids, either secreted like urine, saliva, mother milk, stool, or obtained during standard medical sampling procedures such as serum, plasma, cerebrospinal fluid, tissue samples etc.. The samples collection for metabolomics studies is relatively simple, however certain procedures must be followed [3]. The patients must follow standard requirements: they should be fasting and reports all designated therapy, addictions, and medical history. The samples taken should be stored at -80 °C or preserved in liquid nitrogen [2, 4]. This low storage temperature ensures the maintaining of metabolic composition, i.e. minimalizing possible changes in the metabolic profile after sampling, and protects against potential influence of the donor's microbiome [5, 6].

Comprehensive or even partial determination of the metabolome is not possible with a single analytical method due to different levels of detection limits and different group determination. There are many analytical methods employed for metabolomics, each of them having both advantages and disadvantages. However, there are two main methods, which are routinely used in metabolomics studies: NMR (Nuclear Magnetic Resonance, mostly ^{1}H-NMR) and MS (Mass Spectrometry) hyphenated with separations techniques [2]. Studies based on both NMR and MS have a common pathway:

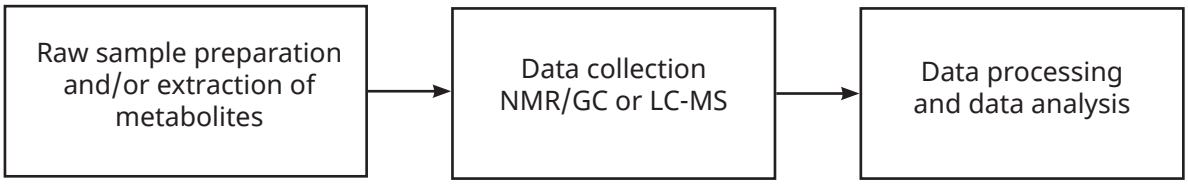

Fig. 1. Typical workflow for metabolomics studies [2].

One of the most important advantages of the MS method is the sensitivity to femtomole concentration, which allows analysing up to several thousand molecules in a certain sample, resulting in precise molecular description in the tested specimen. On the other hand, reproducibility with MS is usually worse as compared to NMR spectroscopy. Unlike the MS, the NMR method provides high reproducibility with the ability to combine structure analysis and detection of the range of 30-90 molecules without sample destruction (biofluids). However, the detection limit of individual compounds is shifted several rows and strongly depend on the time of measurements (acquisition). The advantage of this method is the ability to measure different compounds such as amino acids, nucleosides, amines, acids, etc. all at the same time. The data obtained with both methods are both quantitative and qualitative among the set of detected metabolites [7].

The results obtained are further elaborated using chemometric multidimensional discriminatory methods. For this type of studies, the methods most commonly used are: unsupervised PCA, supervised PLS (Partial Least Squares Discriminant Analysis), and OPLS-DA (orthogonal version of PLS-DA). These approaches allow describing each subject not with 2 or 3 variables (chemical compounds or characteristic parameters) as in routine statistical analysis, but even hundreds of variables (chemical compounds - metabolites) can be analysed with selection of the most specific ones. Those compounds or more often sets of compounds can be employed as biomarkers for discriminating patients from healthy individuals or for staging of the disease [8].

Application of metabolomics in the diagnosis of cancer – selected studies

Lung cancer

In Europe, lung cancer accounts for approximately 20 % of all cancer deaths, with 376 000 deaths in 2008. Unfortunately, the early stages of this cancer are usually low-symptomatic and final diagnosis is made late in the natural history of the disease. Consequently, effective treatment is not possible up to 90 % of cases, and the overall 5-year survival rate is 11.2 % for men and 13.9 % for women [9]. The metabolomic studies of lung cancer are based on various body fluids, predominantly blood serum, urine, and saliva. The most

common type of lung cancer is non-small cell lung cancer (NSCLC). The main recognized risk factor for NSCLC is cigarette smoking. Additionally, COPD is considered an independent risk factor of lung cancer [10]. Our team has demonstrated a possibility to differentiate between patients with COPD and with two stages of NSCLC: an early NSCLC (E-NSCLC) and an advanced NSCLC (A-NSCLC). Using ^{1}H NMR method we were able to identify 45 metabolites in patients' serum. Concentrations of acetate, citrate, and methanol were significantly reduced in lung cancer subjects. In contrast, concentrations of N-acetylated glycoproteins, leucine, lysine, mannose, choline, lipids (L3 + L4), and two other unknown compounds were increased in for patients suffering cancer in all three performed comparisons: NSCLC vs. COPD, E NSCLC vs. COPD and ANSCLC vs. COPD [10]. Similar results were obtained by Musharraf et al [11]. Using GC-MS method for tracing metabolites in plasma, they were able to discriminate lung cancer patients from COPD patients, as well as healthy non-smokers and healthy smokers with high sensitivity (96.2 %) and specificity (92.05 %).

Another research group focused on differentiating early lung cancer patients from healthy controls. They used ^{1}HMR and rapid resolution liquid chromatography (RRLC) methods to investigate metabolites in serum and identified 25 metabolites, which were up or down regulated. Those findings proved disorders in glycolysis, lipid metabolism, choline phospholipid metabolism, one-carbon metabolism, and amino acid metabolism. The use of both methods enabled diagnosing early stage of lung cancer with a very high accuracy [12]. Mass spectrometry hyphenated with gas chromatography (GC-MS) was used by Horia and co-workers [13]. They demonstrated that the levels of 23 of 58 serum metabolites and 48 of 71 sampled from tissue were significantly changed in patients with lung cancer with I-IV stage as compared, with healthy volunteers. An early cancer stage was also investigated by other group using LC-MS method. In this study, the achieved AUC value based on 12 metabolites was 0.836 [14].

Urine also has a strong discriminative potential in lung cancer diagnosing. This was proven in a study performed using ^{1}H NMR method by Carrola and colleagues [15]. The main metabolites differentiating between healthy controls and lung cancer patients were: hippurate, trigonelline, β-hydroxyisovalerate, α-hydroxyisobutyrate, *N*-acetylglutamine, and creatinine. They were able to develop a classification model, which confirmed 93 % sensitivity and 94 % specificity with overall classification rate of 93.5 %. [15]. The studies performed by LC-MS method on urine samples from cohort of 469 patients with lung cancer and 536 controls revealed two biomarkers: creatine riboside and *N*-acetylneuraminic acid, which were significantly increased in non-small cell lung cancer and were associated with worse prognosis [16].

Exhaled breath condensate (EBC) seems to be naturally associated with lung cancer. Indeed, studies on EBC performed with GC-MS [17] and LC-MS [18] proved diagnostic usefulness of EBC differentiating lung cancer patients, COPD patients, smokers, ex-smokers, and healthy controls. In addition to the above mentioned biofluids, also sweat should be included, which was successfully used to differentiate the lung cancer patients from control individuals with risk factors and without them by its analysis by LC-MS system [19].

Recently, a very promising study has been published by Shen et al.. Using two-stage study design and advanced metabolon platform they were able to identify four metabolites, which may be useful biomarker candidates for identifying patients, who may benefit from platinum-based chemotherapy in advanced NSCLC [20]. The comprehensive review on lung cancer biomarkers and metabolomics methods has been published recently [21].

Breast cancer

Breast cancer (BC) is the most common cancer in women worldwide and the morbidity keeps rising. Incidence vary from 19.3 per 100,000 women in Eastern Africa to 89.7 per 100,000 in Western Europe [22, 23]. Some decrease in the number of death caused by BC is observed mainly due to the improvement in early diagnostics [24]. Nevertheless, routine methods currently used to detect BC usually are not effective, especially in the very early stages [25].

A new approach in cancer diagnostics involves the use of metabolomics tools for blood serum and urine analysis [25, 26, 27]. Metabolomic-based diagnosing not only seems to allow discriminating healthy controls from BC subjects, but also differentiating metastatic BC from early stages of BC as well [28, 29].

Previous studies proved that BC can be diagnosed by NMR spectroscopy and by MS, both by use of serum and urine [27, 30, 31].

However, those metabolomic studies involved relatively small groups and, utilized various protocols and methods [32]. Therefore, a more integrated and coherent methodology should be used [33].

The GC-MS method can be used for metabolic profiling of serum. Using this method, it was possible to differentiate patients with BC from patients with non-malignant tumours, and from healthy controls. Sets of amino acids, fatty acids, and lysolipids allowed differentiating these three groups [31]. There is also evidence that polar compounds analysis has been successfully applied for metabolomic-based BC diagnosing in tissue samples. Chae and co-workers used HR-MAS ^{1}H NMR method for retrospective analysis in the patients with ductal carcinoma in situ (DCIS) diagnosed on preoperative biopsy. The univariate analysis proved that choline-containing compounds did not differ between the groups, while GPC/PC ratio, myo-inositol, and succinate were higher in the 'pure' DCIS group as compared to the invasive carcinoma subjects. Multivariate analysis OPLS-DA could discriminate to some degree between these two groups [34]. Comparable results were obtained in other groups. In a study based on add space ^{1}H NMR method the authors were able to distinguish between the non-invasive intraductal carcinoma and invasive ductal carcinoma patients. Histidine concentration was significantly lower in the patients with invasive ductal carcinoma. In contrast, those patients presented higher concentrations of glucose, lactate, tyrosine, and lipids in plasma samples, as compared to the non-invasive carcinoma group [35].

It is also possible to differentiate metastatic BC patients from the ones with a localized, early-stage disease. Serum analysis with NMR spectroscopy identified 9 metabolites: histidine, acetoacetate, glycerol, pyruvate, glycoproteins (N-acetyl), mannose, glutamate, and phenylalanine concentrations, all of which were significantly different, than those in the other studied groups [26].

Volatile organic compounds (VOC) from urine samples can also be used for BC diagnosis. In the study of Silva et al., gas chromatography-mass spectrometry was used to obtain metabolomic patterns of 26 BC patients and 21 healthy individuals. Of the 79 volatile identified metabolites six compounds were of diagnostic power: (–)-4-carene, 3-heptanone, 1,2,4-trimethylbenzene, 2-methoxythiophene, phenol, and dimethylsufide. All of them were able to successfully discriminate between the groups [30].

More holistic approach was suggested by Bro et al. In a retrospective study they combined ^{1}H NMR data with other relevant biological and phenotypic information to construct a patient's biocountour. With this approach the authors could predict an increased risk of BC, a few years before its occurrence with sensitivity and specificity well above 80 % [36]. A very interesting paper aiming on possible associations of diet-related metabolites with BC risk was published recently by Pleydon et al.. Using CE-MS method they identified a bunch of metabolites, which were moderately correlated with increased risk of estrogen receptor related to BC development [37]. The annually increasing literature data proves the usefulness of metabolomics in BC [33, 38].

Conclusions

Data from the literature clearly demonstrate the usefulness of metabolomic approach in diagnostics of lung cancer, breast cancer, and many other diseases. Despite this, the metabolomic approach is still not routinely implemented into medical protocols. This results from many factors. Analytical methods used for metabolomic research are neither standardized nor validated. Consequently, it is difficult to: compare results obtained by various research groups, and determine the definitive clinical recommendations. Threfore we need more and more research to overcome these difficulties. However, in the future the metabolomic approach with fingerprinting and profiling-based methods, combined together with predictive-discrimination statistical models should be the method of choice for preventive, screening, and treatment research.

МЕТАБОЛОМІКА – НОВИЙ ПІДХІД ДО ДІАГНОСТУВАННЯ РАКУ? НА ПРИКЛАДІ РАКУ ЛЕГЕНЬ ТА МОЛОЧНИХ ЗАЛОЗ

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Вступ. *Метаболоміка це відносно новий діагностичний підхід, який дозволяє аналізувати метаболічні процеси на рівні клітини.*

Мета дослідження та методи – *огляд технології аналізу метаболоміки та її використання у клінічній практиці для діагностики певних злоякісних захворювань.*

Результати. *Загалом, метаболомічний підхід включає три етапи: 1) відбір проб біоологічних матеріалів, 2) ідентифікація низькомолекулярних сполук масою до 1,0 кДа з використанням ядерного магнітного резонансу та/або мас-спектрометрії, 3) обробка та аналіз даних.*

Сьогодні ми можемо визначити набір метаболітів, що є специфічним для певного метаболічного статусу (так званий «метаболічний відбиток»). Цей набір метаболітів дасть можливість оцінити певні патогенетичні ланки та визначитися з етіологією захворювання. Доведено, що зміни в метаболомі передують, не лише появі перших клінічних ознак та симптомів, але й будь-яким змінам у клініко-лабораторних аналізах. Отже, метаболомічний підхід може бути перспективним для скринінгу та ранньої діагностики.

Висновки. *Доведено, що метаболоміка дозволяє первинно діагностувати та провести диференціальний діагноз стадії злоякісного процесу.*

КЛЮЧОВІ СЛОВА: **метаболоміка; метаболіти; рак молочної залози; рак легень.**

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HEMOPHAGOCYTOSIS SECONDARY TO PHARYNGEAL ABSCESS IN AN IMMUNOCOMPETENT PATIENT (case report)

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Background. *Hemophagocytosis is a rare, potentially fatal disorder, comprising pancytopenia, liver dysfunction, hepatosplenomegaly, hypertriglyceridemia, and hyperferritinemia presenting as fever, lymphadenopathy and skin rashes.*

Objective. *To attract the clinicians' attention to a problem of hemophagocytosis in Critical Care management*.

Methods. *Hemophagocytosis secondary to pharyngeal abscess in a 58 year old male is being reported.*

Results. *A 58-year-old immunocompetent patient presenting with hemophagocytosis secondary to pharyngeal abscess, was managed on ventilator and inotropic support, when he developed heathcare-associated urinary tract infection by Escherichia coli and ventilator-associated pneumonia by Acinetobacter baumanii. He developed neutropenic septic shock and multi-organ dysfunction and went through a downhill course leading to demise.*

Conclusions. *Hemophagocytosis remains a sinister entity in modern intensive care despite astute clinical management. Secondary superinfections with opportunistic multidrug resistant pathogens are difficult to treat. A high index of clinical suspicion, aggressive diagnosis and prompt treatment for hemophagocytosis and polymicrobial opportunistic superinfections with multidrug-resistant healthcare-associated pathogens needs to be addressed upfront.*

KEY WORDS: **hemophagocytosis; pharyngeal abscess; *Acinetobacter baumanii*; pancytopenia; ventilator-associated pneumonia; sepsis.**

Introduction

Hemophagocytosis is a rare, potentially fatal disorder, comprising pancytopenia, liver dysfunction, hepatosplenomegaly, hypertriglyceridemia, and hyperferritinemia presenting as fever, lymphadenopathy and skin rashes. Primary hemophagocytosis may be genetic (X-linked lymphoproliferative syndrome and Chediak-Higashi syndrome), while secondary hemophagocytosis may occur due to infections, malignancies (lymphomas) and autoimmune diseases (sarcoidosis). Bacterial infections causing hemophagocytosis are tuberculosis, typhoid, brucellosis and ehrlichiosis. Many viruses such as swine influenza H1N1, avian influenza, measles, Epstein-Barr virus, human immunodeficiency virus, parvovirus, hepatitis viruses, herpes viruses and Varicella zoster virus, parasitic diseases such as leishmaniasis, systemic mycosis and various emerging pathogens can also cause hemophagocytosis [1, 2, 3, 4, 5]. Antigenic stimuli cause a progressive immune reaction with a cytokine storm, activate macrophages phagocytose erythrocytes and leucocytes in bone marrow and lymphoid tissues. Hemophagocytosis secondary to pharyngeal abscess in a 58 year old male is being reported.

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Case Report

A 58 year old male with mild odynophagia and nasal congestion of three year duration along with high grade continuous fever of one month duration, was referred to a tertiary-care facility. On examination at arrival, the patient had an intoxicated appearance with tachycardia, tachypnea, fever 105.3 °F, pallor and weight 66 kg. He was nursed in intensive care after tracheostomy with ventilator support in synchronized intermittent mandatory ventilation (SIMV) mode along with inotropic support for worsening hypotension. Ventilator was readjusted to continuous positive airway pressure (CPAP) mode. Central venous pressure was 8 mm Hg. Hot potato voice and nasal twang were evidenced along with oral mucositis and coated tongue. Oedematous and erythematous soft palate with superficial slough was present.

Ulcer on posterior pharyngeal wall extending from soft palate to floor of vallecula and multiple aphthous ulcers along the lateral border of tongue were evidenced. Bilateral congested palpebral conjunctiva and arcus senilis were also present. Ultrasonography revealed subcutaneous emphysema chest, hepatomegaly, distended gall bladder and grade II prostatomegaly. CECT chest revealed bilateral ground glass opacities in lung bases and early interstitial lung disease in posterobasal segment of right lung. Repeat NCCT neck revealed mass in right pyriform fossa which was confirmed by spiral CT neck, obliteration of right pyriform sinus, thickening of right aryepiglottic folds with restriction of right true vocal cord movement suggestive of mass lesion. MRI showed diffuse oedema and thickening of posterior naso-oro-hypopharyngeal walls with effacement of right pyriform sinus and significant compromise of lumen. Acute pansinusitis, bilateral mastoiditis, bilateral pleural effusion and fibrotic opacity right lung were evidenced. Subcutaneous emphysema of right subclavian region was present. History of low-grade continuous fever two months prior to high-grade fever was elicited for which he was treated with injectable antimicrobials with little improvement. There was history of loose stools 10–12 times and weight loss 2.5 kg over past three months. He underwent septoplasty three years earlier.

During the course of his illness, haemoglobin dropped from 10 gm/dl to 7.5 gm/dl with leukopenia reducing from 3600/cu mm to 900/cu mm with neutrophil count 600/cu mm. Erythrocyte sedimentation rate increased from 45 to 96 in one hour. Anaemia workup revealed mean corpuscular volume 69 fl, serum iron 23 µg/dl, total iron binding capacity 936 µg/dl, ferritin 280 µg/dl. Liver function tests included total bilirubin 3.4 mg/dl, direct bilirubin 0.6 mg/dl, alanine and aspartate aminotransferases 125 and 55 mg/dl, alkaline phosphatase 78 mg/dl and gamma glutamyl transferase 180 mg/dl. Coagulation profile was deranged with INR 2.6, d-dimers 3.17 µg/l and features of early disseminated intravascular coagulation. Dyselectrolytemia and hypocalcaemia persisted. Serum IgA and IgM were raised. Serum protein electrophoresis revealed polyclonal bands in gamma regions. Microbiological work up including Mantoux test, tuberculosis polymerase chain reaction, widal test, malarial serology, tests for HIV, syphilis, Hepatitis B and *Clostridium difficile* toxin were negative. Blood cultures were negative, urine cultures revealed *Escherichia coli.* Throat swab revealed *Streptococcus viridans* and budding yeast cells. Tracheal aspirates revealed *Acinetobacter baumanii* susceptible to colistin and *Cryptococcus laurentii* which was susceptible to all antifungals. Histopathological work up involving punch biopsy from pharyngeal wall and nasopharynx revealed chronic inflammatory posterior pharyngeal wall abscess. Repeat biopsy revealed acute ulceration with granulomatous tissue without malignant cells. Bone marrow biopsy revealed reactive marrow with brisk haemophagocytosis. Vasculitis work up was negative. No blasts or atypical cells were seen. Rheumatologic workup for both anti-neutrophil cytoplasmic antibodies and myeloperoxidase were not contributory, however C-reactive proteins and gamma glutamyl transferase were raised. Whole body dual-time Positron Emission Tomography (PET)-fludeoxyglucose F 18 (FDG) scan revealed avid inflammatory lesions in oropharynx and non-FDG avid mass in left pharyngeal spaces along with bony erosions in cervical vertebral bodies with adjacent soft tissue attenuation. A host of other investigations such as thyroid profile, vitamin B-12 assay, upper gastrointestinal endoscopy were not contributory. Neutropenic septic shock and multi-organ dysfunction developed and the patient went through a downhill course leading to demise.

Discussion

Deep seated abscesses in pharyngeal spaces can be present insidiously with prolonged fever which may be difficult to localize and treat. Apart from features of infection such as fever, they can cause pressure effects leading to variable degrees of dysphagia, odynophagia and respiratory obstruction and/or compromise. The abscess can spread to contiguous areas or the inflammation can affect surrounding tissues. Pre-existing comorbidities can lead to rapid worsening of general condition despite the infection being localized, which may also preclude surgical intervention [6, 7]. Conditions leading to hemophagocytosis can also cause granulomatous hepatitis. Hemophagocytosis in this patient appears to be due to multiple etiology causing immunological stimulation in parallel. Hemophagocytosis is known to occur in patients under intensive-care leading to sepsis and multiple organ failure as evidenced in this patient [8]. Unexplained hypotension requiring inotropic support in this patient is likely to be due to pre-existing disease conditions

such as pulmonary emphysema and liver disorder. Pancytopenia in hemophagocytosis can lead to immunocompromised state facilitating opportunistic infections such as healthcare-associated urinary tract infection and ventilator-associated pneumonia. *Acinetobacter baumanii* was multidrug resistant and was susceptible only to colistin. Secondary superinfections with opportunistic multidrug resistant pathogens were difficult to treat [9, 10, 11, 12]. CT, MRI and histopathology localized the same lesion without detection of any other foci [13]. Whole body dual-time PET-FDG scan revealed avid inflammatory lesions in oropharynx which could suggest active disease and non-FDG avid mass in left pharyngeal spaces hints towards a latent tuberculosis infection. Also the bony erosions in cervical vertebral bodies with adjacent soft tissue attenuation point towards involvement of cervical spine.

Retropharyngeal space abscesses are a disease of infancy, usually resulting from abscess of lymph nodes draining infection of ear, nose or throat [14,15]. Chronic inflammatory posterior pharyngeal wall abscess is usually evidenced in adults or slightly elder children. Retropharyngeal abscess in adults is often pyogenic and usually secondary to pharyngeal or oesophageal perforation, sepsis in the throat or sinuses, penetrating injury, oral endotracheal intubation or trauma to pharynx. Retropharyngeal tuberculous abscess can rarely be present with odynophagia, neck pain, stridor, mediastinitis and life-threatening respiratory obstruction. Diagnostic limitations preclude diagnostic outcome despite clinical intuition, especially in emerging and rare pathogens [16, 17, 18, 19, 20, 21].

Conclusions

Hemophagocytosis remains a sinister entity in contemporary intensive care despite astute clinical management. A high index of clinical suspicion, aggressive diagnosis and prompt treatment for hemophagocytosis and polymicrobial opportunistic superinfections with multidrug-resistant healthcare-associated pathogens needs to be addressed upfront.

ГЕМОФАГОЦИТОЗ ВНАСЛІДОК ГЛОТКОВОГО АБСЦЕСУ В ІМУНОКОМПЕТЕНТНОГО ПАЦІЄНТА (КЛІНІЧНИЙ ВИПАДОК)

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Вступ. *Гемофагоцитоз – це рідкісний, потенційно небезпечний для життя розлад, який включає панцитопенію, порушення функції печінки, гепатоспленомегалію, гіпертригліцеридемію і гіперферритинемію, та проявляється у вигляді лихоманки, лімфадентопатії та шкірних висипань.*

Мета дослідження *привернути увагу лікарів до проблеми гемофагоцитозу у відділеннях невідкладної допомоги.*

Методи. *Описано клінічний випадок гемофагоцитозу, який розвинувся на фоні глоткового абсцесу в чоловіка 58 років.*

Результати. *58-річний імунокомпетентний пацієнт з гемофагоцитозом на фоні глоткового абсцесу перебував на штучній вентиляції легень та отримував інотропну фармакотерапію (кардіотоніки), коли у нього розвинулась інфекція сечовивідних шляхів, спричинена Escherichia coli та вентиляційна пневмонія, спричинену Acinetobacter baumanii. У пацієнта розвинувся нейтропенічний септичний шок і мультиорганна недостатність, які призвели до його смерті.*

Висновки. *Незважаючи на інтенсивну терапію та сучасні засоби і методи лікування, гемофагоцитоз залишається небезпечним для життя станом. Вторинні суперінфекції, спричинені опортуністичними мультирезистентними патогенами, важко піддаються лікуванню. Клінічна настороженість, агресивна діагностика, раннє лікування гемофагоцитозу та мультирезистентних опортуністичних суперінфекцій є необхідністю у відділеннях інтенсивної терапії.*

КЛЮЧОВІ СЛОВА: **гемофагоцитоз; глотковий абсцес; Acinetobacter baumanii; панцитопенія; вентиляційна пневмонія; сепсис.**

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REACTIVE OXYGEN AND NITROGEN SPECIES ROLE IN EXPERIMENTAL PERIODONTITIS DEVELOPMENT

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Introduction. *Activation of lipid peroxidation is one of the trigger mechanisms of periodontium injury, which is primary caused by cellular damage. Reactive oxygen and nitrogen species (RONS) are able to cause damage to a cell as well as final products of lipid peroxidation, including unsaturated aldehydes and other metabolites.*

Objective. *The aim of the research was to determine the role of RONS and accumulation of lipid peroxidation derivatives in initial development and formation of chronical inflammatory process in periodontium.*

Methods. *Experimental periodontitis was modeled in animals by injection of complex mixtures of microorganisms diluted in egg protein into periodontal tissues. The results of biochemical studies of free radical processes activity in blood serum were evaluated by content of diene, triene conjugates, TBA-active products and total quantity of metabolites of nitric oxide (NO_2^-+NO_3^-), which were determined on the 7th, 14th and 30th days of the experiment.*

Results. *Generation of active forms of oxygen is more influential, providing longevity of inflammatory process. This pays attention to typical dynamics of changes in active processes of lipid peroxidation in the development and course of experimental periodontitis. The study of inflammatory process with a bacterial-immune component in the rats' periodontal complex proved accumulation of lipid peroxidation and nitric oxide metabolites in blood serum.*

Conclusions. *The preservation of increased lipid peroxidation and nitric oxide metabolites in blood serum of the experimental animals with acute periodontitis conduce enhance of alteration and delayed healing that result in its sequel into chronical periodontitis.*

KeY Wo Rd S: **periodontitis; nitric oxide metabolites; TBA-active products; diene conjugates; triene conjugates.**

Introduction

Improvement of the established and creation of new methods of generalized periodontitis treatment is one of the urgent matters of contemporary dentistry [1, 2]. inflammatory processes in the complex of periodontal tissues are the most common among inflammatory complications of maxillofacial area [3, 4]. it is indisputable that pathogenic infection is crucial in this case, as well as their combinations, in particular Staphylococcus and Streptococcus, which develop in cases of reduced resistance of oral tissues to infectious agents. [5, 6]. The etiology and pathogenesis of periodontal diseases are complicated and studied insufficiently however, the infectious factor and ability of immune protective mechanisms (local cellular unspecifi and general adaptive) are essential; the features of pathological process development, its subsequent treatment and prophylaxis affects depend on them [7]. It is unclear as well as the mechanisms, due to which different by nature and character of action local and general factors lead to inflammatory and destructive lesions of periodontal tissues [8]. The investigation of inflammatory process mechanisms in the tissues of periodontal complex is one of the current issues of contemporary dentistry due to a relatively wide spread and unfavorable prognosis, because the frequency of periodontal disease in world is within 5–20 % and increases with age up to 75 % [9, 10].

t he development of inflammatory-destru - tive changes in periodontal tissues is caused by disturbances of microcirculation and transcapillary exchange with underlying severe hypoxia. activation of reactive oxygen and nitrogen species (Ron S) and exhaustion of antioxidant defense in biological tissues is the most serious of all effects and outcomes of

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hypoxia. activation of lipid peroxidation (l Po) is a trigger mechanism for oxidative stress with cellular metabolism disorders, which are primary caused by damage of cellular and subcellular membranes [11].

activation of l Po and decrease of antioxidant protection contribute to accumulation of deleterious free cholesterol, lysophosphatides, phosphatidylcholine, that changes the dynamic stability of cellular membranes due to pathological process development in periodontal complex [13].

all these facts about the influence of oxidative stress on the pathogenesis of periodontitis are present in the activity of lipid peroxidation as potential predictors of escalation of inflammatory lesions in periodontal disease. t he disturbance of antioxidant protection in the patients with hypertension, which was proved by changes in the activity of catalase, ceruloplasmin and saturation of transferrin by iron, and the increase in the level of diene conjugates and TBA-active products in serum, which leads to the development of endogenous intoxication syndrome in the patients with general periodontitis. One of the parameters that allow estimating the state of free radical processes is the content of lipids hydroperoxides and t Ba-active products formed by oxidation of unsaturated fatty acids, and aldehyde and ketone derivatives, which are developed by the action of active radicals on the amino acid residues in protein molecules [14].

t he components of bacterial toxins (especially lipopolysaccharide) and proinfla matory cytokines (mainly tnf -α, il -1 and interferon gamma-ifn -γ) produced by the affected tissues stimulate the production of nitric oxide (no) by the inducible nitric oxide synthase (ino S) in different cell types [15]. It is proved that parodonotopathogenic bacteria are capable of inducing NO formation by inducible NO synthase. excessive n o formation, which occurs when ino S is stimulated by proinflammatory cytokines and endotoxins of pathogenic microfl ra of oral cavity, leads to nitrooxidative stress which, together with the activation of lipoperoxidation and oxidative modification of proteins, can cause increased disintegration of connective tissue components and progressing of periodontitis [16].

The aim of this investigation was to determinate the pathogenic influence of Ron S and accumulation of lipid peroxidation derivatives in regard to initial development and formation of chronical inflammatory process in periodontal complex.

Methods

t he experiments were carried out using white clinical healthy male rats, 150–200 g in weight, in environments of vivarium, on a standard diet balanced for the basic elements. The research related to animals' use has been complied with all the relevant national regulations and institutional policies for the care and use of animals. The investigations was conducted following the general rules and regulations of the European Convention for the Protection of vertebrate Animals Used for experimental and o ther Scientific Purposes (Strasbourg, 1986), the General Ethics on animal experimentation (Kyiv, 2001).

t he experimental animals were randomly selected and divided into 4 groups: the 1st – intact animal, controls (n=10); the 2nd – animals with experimental periodontitis on the 7th day of study (n=8); the 3rd – animals with experimental periodontitis on the 14th day of study (n=8); the 4th – animals with experimental periodontitis on the 30th day of study (n=8). experimental periodontitis (EP) was caused by introduction of complex mixtures of microorganisms diluted in egg protein into periodontal tissues [17]. Simultaneously with the injections of the pathogen a complete Freund's adjuvant was injected in the rat paw to enhance the immune response. When conducting studies with animals of group 4, on the 14th day, repeated administration of the pathogen and injection of adjuvant was carried out. At the 7th and 14th days the experimental animals were euthanized by total heart bloodletting and previous thiopental anesthesia. Serum samples were taken for further research.

In blood serum the level of diene (DC) and triene conjugates (TC), TBA-active products and total quantity of metabolites of nitrogen (II) oxide were determined. t he concentration of diene conjugates (DC) and triene conjugates (TC) was evaluated by the method based on the fact that the extracted heptane-isopropyl hydroperoxide mixture had an appropriate absorption maximum: dC at a wavelength of 232 nm; TK at a wavelength of 275 nm [18]. The total nitric oxide metabolites in blood plasma: nitrite anion (NO_2-) and nitrate anion (NO_3-), were determined by photometry using a Gray reagent (sulfanilamide solution and N-naphthyl ethylenediamine dihydrochloride in 30 % glacial acetic acid), which was used as a color reagent

giving raspberry coloring in the presence of nitrogen oxide metabolites in a liquid [19]. t he method of determining the concentration of TBA-active products consisted in the ability of malonic dialdehyde to interact with tiobarbituric acid in an acidic medium to form a colored complex which intensity is adequate to the content of TBA-active products [20]. The results were statistically analyzed by means of nonparametric indexes [21]. t he data were presented in the arithmetic mean (M) ± standard deviation of the mean value (m) for a specific number of the animals (n). Changes were considered statistically significan at p<0.05. excel 2010 (Microsoft Corporation) and Statistica 10.0 (StatSoft, USA) software were used.

Results

These studies were performed in accordance with the suggested and patented patterns for experimental periodontitis [22], which presented the influence of bacterial and immune disorders on the mechanisms of inflammation development in periodontal complex. t he study of experimental periodontitis is associated with the fact that this type and values of bacterial-immune infl mmation has not investigated before.

The results of the research proved that in the early period of inflammation development in periodontal complex, which included the period from the 1st to the 7th day of the experiment, there was an excessive accumulation of lipid peroxidation products in serum, as evidenced by increased concentration of DC (in 2.20 times, p<0.01) and TC (in 1.93 times, p<0.01) respectively, compared with the control group of experimental animals (t able 1, f ig. 1). o n the 14th day of experimental periodontitis model, there was a significant decrease of d C (in 1.53 times, p<0.01) and TC (in 1.52 times; p<0.01) in serum compared to the group of animals studied on the 7th day of the experiment, but these indices were higher than those of the intact animal group (in 1.44 times, p<0.01 and by 1.26 times, p<0.01, respectively).

In the further observation, on the 30th day of inflammatory process development in the tissues of periodontal complex, the content of DC in blood serum slightly increased in comparison with the indices on the 7th day, but the data were statistically insignificant (p>0.05), but on the 14th day this index increased in 1.53 times (p<0.01). When comparing it with the indices of the control group, it was found out that the content of this metabolite in serum was significantly higher (in 2.21 times, p<0.01).

The content of triene conjugates changed the same during the period of monitoring, however, the increase in their concentration in blood serum was less significa t – in 1.53 times (p<0.01), compared with the indices on the 14th day, and in 1.94 times (p<0.01), compared with the control group. When comparing them with the results of the group of animals with experimental periodontitis on the 7th day of the experiment, the changes were found to be statistically insignificant (p>0.05)

When determining the ratio of DK/TC content (Table 2) in blood serum, it was proved that that index significantly increased on the 7th day of the study (in 1.15 times; p<0.01)

Table 1. Concentration of diene and triene conjugates in serum of the rats in different periods of experimental periodontitis (M±m)

f orm of experiment	d uration of experiment (days)	Number of animals	DC, conditioned, units/ml	TC, conditioned, units/ml
Control, intact animals	-	10	2.383±0.071	2.756±0.022
Animals with periodontitis	7	8	5.250±0.242 p1<0,01	5.310±0.187 p1<0,01
	14	8	3.431±0.089 p1<0.01, p2<0.01	3.485±0.107 p1<0.01, p2<0.01
	30	8	5.266±0.141 p1<0.01, p2>0.05, p3<0.01	5.338±0.140 p1<0.01, p2>0.05, p3<0.01

Notes: p1 – statistical significance of differences relative to the intact animals;
p2 – statistical significance of differences relative to the animals with experimental periodontitis on the 7th day of the research;
p3 – statistical significance of differences relative to the animals with experimental periodontitis on the 14th day of the research.

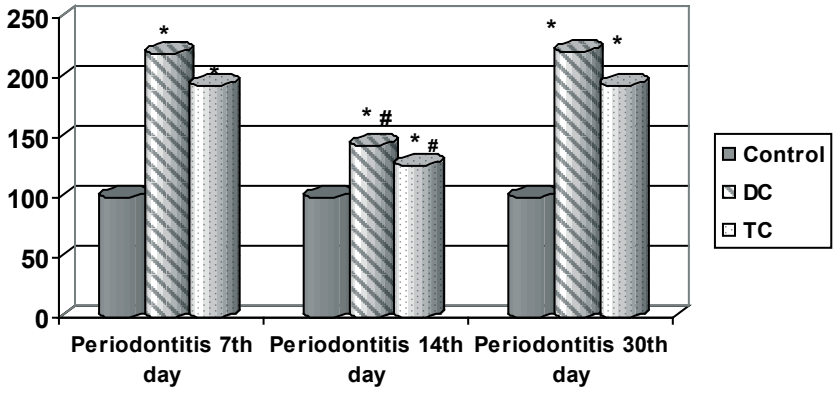

fig. 1. Changes in the indices of lipid peroxidation in rats' serum in the experimental periodontitis follow-up (% of the control).

*Notes: * – statistically significant differences relative to the intact animals (p<0.01);*
 # – statistically significant differences relative to the animals with periodontitis on the 7th day of the experiment (p<0.01);
● – statistically significant differences relative to the animals with periodontitis on the 7th day of the experiment (p>0.05);
º – statistically significant differences relative to the animals with periodontitis on the 14th day of the experiment (p<0.01).

compared to the control group and remained on the same level throughout the duration of the experiment: it was higher on the 14th (in 1.16 times, p<0.01) and on the 30th day (in 1.15 times, p<0.01) of the indices of the intact animals. When comparing the same ratios in the rats at different periods of the experiment, in particular on the 7th, 14th, 30th days, the differences were statistically insignificant (p<0.05).

As a result of the study of the main indices of lipid peroxidation – the content of t Ba-active products, significant changes were also evidenced (Table 3). In particular, it was found out that on the 7th day of experimental periodontitis development in the rats, this serum level was higher in 4.22 times (p<0.01) compared to the control group.

On the 14th day of the experimental periodontitis model, a gradual decrease in the level of TBA-active products (in 1.34 times, p<0.01) was evidenced in blood serum in comparison with the group of animals with inammatory process in periodontal tissues on the 7th day of the experiment, but these indices were still increased compared to the intact group of animals (in 3.16 times, p<0.01), that proved a signicant activation of free radical lipid oxidation processes during the entire period of inflammation development. The studies on the 30th day of the experiment proved that the content of TBA-active products in serum gradually decreased (in 1.49 times, p<0.01 and in 1.11 times, p<0.01) respectively, compared to the groups of animals with experimental periodontitis on the 7th and 14th days of the experiment. at the same time, it was higher (in 2.84 times, p<0.01) than in the intact group of white rats (Fig. 2).

at the early stage of experimental periodontitis development, that is on the 7th day, there was a signicant increase in the content of nitric oxide metabolites (no_2^-+no_3^-), which were classied as unstable products of free radical oxidation in serum (in 6.86 times, p<0.01), but on the 14th day this index changed

Table 2. Correlation of diene and triene conjugates in serum of the rats in different periods of experimental periodontitis development (M±m)

form of experiment	Control, intact animals	Animals with periodontitis		
duration of experiment (days)	-	7	14	30
Number of animals	10	8	8	8
DC / TC	0.86±0.03	0.99±0.02 p1<0.01	1.00±0.04 p1<0.01, p2>0.05	0.99±0.01 p1<0.01, p2>0.05, p3>0.05

Notes: p1 – statistically significant differences relative to the intact animals;
p2 – statistically significant differences relative to the animals with experimental periodontitis on the 7th day of the research;
p3 – statistically significant differences relative to the animals with experimental periodontitis on the 14th day of the research.

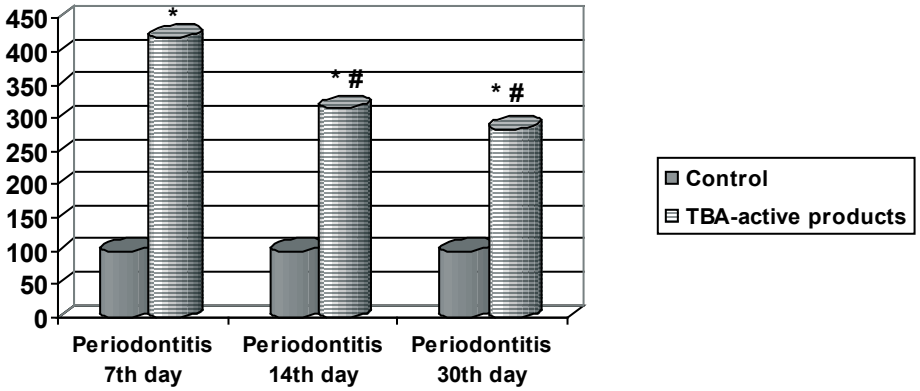

fig. 2. Changes in the indices of t Ba-active products in rats' serum in the experimental periodontitis follow-up (% of the control).

*Notes: * – statistically significant differences relative to the intact animals (p<0.01);*
 # – statistically significant differences relative to the animals with periodontitis on the 7th day of the experiment (p<0.01);
º – statistically significant differences relative to the animals with periodontitis on the 14th day of the experiment (p<0.01).

reversely, that is it decreased (in 1.31 times, p<0.01) compared with the animals on the 7th day of the experiment, but was increased compared to the intact group of animals (in 5.25 times, p<0.01) (Table 3, Fig. 3).

Characterizing the changes in the content of products of metabolism of nitric oxide in blood serum of the experimental animals with periodontitis, it should be noted that this active form of oxygen on the 30th day of the experiment along with the lipoperoxidation indices of previous studies also signicantly increased (in 3.64 times, p<0.01) compared to the results of the animals of control group. However, the data were lower than those in rats on the 7th (in 1.88 times, p<0.01) and 14th days (in 1.44 times, p<0.01) respectively.

Discussion

introduction of complex mixtures of micro-organisms diluted in egg protein into periodontal tissues caused hyperergic inam - matory process with signicant changes in soft tissue of lower jaw accompanied by edema and hyperemia of mucous membrane and the manifestations were the same as the changes in humans [23]. Inflammatory process in periodontal tissues was accompanied by cellular inltr ation of surrounding tissues and destructive changes in periodontal complex [24, 25].

The obtained data proved that generation of active forms of oxygen at a suciently high level, activation of free radical lipid oxidation were present during the entire period of

Table 3. The content of TBA-active products and metabolites of nitrogen (II) oxide (NO2–+NO3–) in serum of the rats in different periods of experimental periodontitis development (M±m)

form of experiment	duration of experiment (days)	Number of animal	TBA-active products, mcmol/l	no 2–+no 3–, mcmol/l
Control, intact animals	-	10	2.555±0.092	0.028±0.001
Animals with periodontitis	7	8	10.774±0.122 p1<0.01	0.192±0.006 p1<0.01
	14	8	8.066±0.143 p1<0.01, p2<0.01	0.147±0.003 p1<0.01, p2<0.01
	30	8	7.255±0.103 p1<0.01, p2<0.01, p3<0.01	0.102±0.002 p1<0.01, p2<0.01, p3<0.01

Notes: p1 – statistically significant differences relative to the intact animals;
p2 – statistically significant differences relative to the animals with experimental periodontitis on the 7th day of the research;
p3 – statistically significant differences relative to the animals with experimental periodontitis on the 14th day of the research.

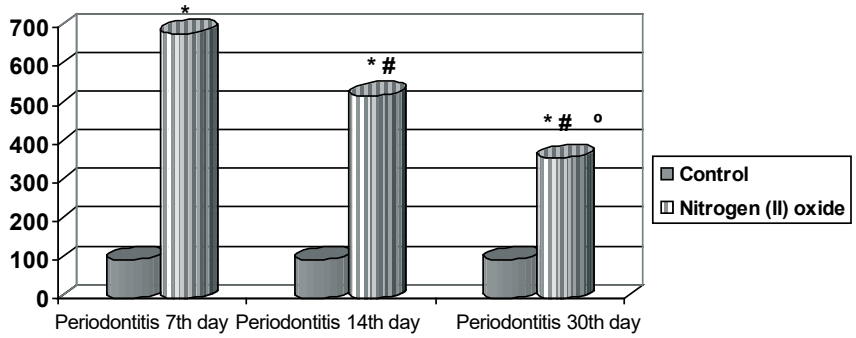

fig. 3. Changes in the indices of metabolites of nitrogen (II) oxide in rats' serum in the experimental periodontitis follow-up (% of the control).

*Notes: * – statistically significant differences relative to the intact animals (p<0.01);*
 # – statistically significant differences relative to the animals with periodontitis on the 7th day of the experiment (p<0.01);
º – statistically significant differences relative to the animals with periodontitis on the 14th day of the experiment (p<0.01).

inammatory reaction development, but the highest degree was during the peak of the inammatory process that corresponded to a more severe clinical picture in this group of animals. In a later period of periodontitis, despite a slight decrease in the intensity of l PO, a complete reduction of the inamed process in periodontal tissues did not take place, which may point to its chronicity.

t he indices of lipid peroxidation activity: the content of TBA-active products in serum proved that irrespective of the period of their study, during the development of bacterial-immune experimental periodontitis, the formation and accumulation of intermediate toxic products of lipid peroxidation in serum took place at different stages of its chain branching. also, the inam - matory reaction in periodontal complex in the acute period of development became a source of formation of reactive oxygen species, which were capable of triggering a cascade of free radical processes involving NO-radical metabolites. active form of oxygen on the 30th day of the experiment proved the continuation of no generation, the enhancement of free radical processes activity and the disturbance of dynamic equilibrium with the antioxidant defense system.

Conclusions

t he inammatory process with bacterial-immune component in periodontal complex is accompanied by increase of lipid peroxidation and nitric oxide metabolites in the blood serum that affects the course and completion of the inammatory pr ocess.

a signicant increase of diene and triene conjugates level and TBA-active products in blood serum in the acute period (on the 7th day of the experiment) and a temporary decrease on the 14th day, as well as further increase on the 30th day of the experiment evidence the increased generation of reactive oxygen species and their derivates for the entire period of inammation de velopment.

t he preservation of increased lipid peroxidation and nitric oxide metabolites in blood serum of the experimental animals with acute periodontitis conduce to enhance of alteration and delayed healing that result in its sequel into chronical periodontitis.

РОЛЬ АКТИВНИХ ФОРМ КИСНЮ ТА НІТРОГЕНУ У РОЗВИТКУ ЕКСПЕРИМЕНТАЛЬНОГО ПАРОДОНТИТУ

А. Є. Демкович

ТЕРНОПІЛЬСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Розвито оксидативного стресу є одним з пускових механізмів патогенезу ушкодженння пародонту. Активні форми кисню та нітрогену здатні викликати пошкодження клітини, так само як і кінцеві продукти перекисного окислення ліпідів, включаючи ненасичені альдегіди та інші метаболіти.*

Мета *дослідження полягала у визначенні ролі активних форм кисню та нітрогену та накопичення продуктів перекисного окислення ліпідів у формуванні хронічного запального процесу в пародонті.*

Методи. *Експериментальний пародонтит моделювали у тварин шляхом введення складних сумішей мікроорганізмів, розведених в яєчному білку. Активність вільнорадикальних процесів у сироватці крові оцінювали за вмістом дієнових та трієнових кон'югатів, ТБК-активних продуктів та метаболітів оксиду азоту (NO^{2-} та NO^{3-}) на 7-у, 14-у та 30-у доби експерименту.*

Результати. *Генерація активних форм кисню забезпечує значну тривалість запального процесу. Тому типова динаміка процесів перекисного окислення ліпідів у розвитку та перебізі експериментального періодонтиту викликає значний інтерес. Результтпти нашого дослідженння запального процесу з бактеріально-імунною складовою в періодонтальному комплексі щурів довело важливу роль накопичення продуктів перекисного окислення ліпідів і метаболітів оксиду азоту в сироватці крові.*

Висновки. *Підвищений рівень продуктів перекисного окислення ліпідів та метаболітів оксиду азоту в сироватці крові експериментальних тварин з гострим парродонтитом сприяє поглибленню патологічного процесу та уповільнює загоєння, що призводить до розвитку хронічного перебігу захворювання.*

КЛЮЧОВІ СЛОВА: **пародонтит; метаболіти оксиду азоту; ТБК-активні продукти; дієнові кон'югати; трієнові кон'югати.**

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EPIDEMIOLOGIC EVALUATION OF THYROID DISEASES MORBIDITY OF UKRAINIAN ADULT POPULATION FROM 2000 TO 2013

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Background. *The diseases of thyroid gland have been attracting considerable attention in recent decades. This is partly due to the fact that thyroid gland reacts actively to geochemical state and pollution of the environment with industrial and agricultural waste products with the subsequent incidence of certain pathological processes.*

The objective *of the research was to analyze the morbidity of adult population of Ukraine for thyroid gland diseases in the period from 2000 to 2013.*

Methods. *The methods of empirical and theoretical research of scientific information: analysis, synthesis, induction, deduction and systematization, as well as epidemiological and statistical methods were used. Using the Microsoft Office Excel (2007) and IBM SPSS StatisticsBase v.22 program the correlation and regression analyzes were conducted.*

Results. *From 2000 to 2013, high levels of adult population endocrinopathies, thyroid in general and diffuse goiter of varying degrees, general and primary morbidity were registered in the western and northern regions of Ukraine, low – in the central, eastern and southern regions. Statistically significant ($p<0.001$) positive correlation between the level of prevalence and the level of incidence of endocrine pathology, diseases of thyroid gland as a whole, as well as individual nosology was detected.*

Conclusions. *Regional peculiarities of the levels and dynamics of changes in the incidence rates of thyroid morbidity among adult population of Ukraine can be related to the urgent environmental factors for each region. This factor requires further study to develop effective methods of prophylaxis and defense.*

KEY WORDS: **morbidity; thyroid gland; adult population.**

Introduction

The diseases of thyroid gland have been attracting considerable attention in recent decades. In the structure of endocrine pathology prevalence in Ukraine they are the first: on average 44 %, and in the endemic with iodine deficiency western regions – up to 70 % [1]. This is partly due to the fact that thyroid gland reacts actively to geochemical state and pollution of the environment with industrial and agricultural waste products with the subsequent occurrence of certain pathological processes [2]. In the structure of endocrinological pathology, different types of goiter are the largest share [3, 4]. Minimizing thyroid dysfunction rates is an important task for most countries of the world, since, according to the WHO publications, nearly 2 billion planet's inhabitants are at risk for thyroid diseases, including iodine-dependent diseases [4, 5].

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The objective of the research was to analyze the morbidity of adult population of Ukraine for thyroid gland diseases in the period from 2000 to 2013.

Methods

The object of our research was the level and the dynamics of endocrine system (Class IV, E00-E90 according to the International Diseases Classification of the Tenth Revision) and thyroid gland (E00-E07) general morbidity (prevalence[1]) and primary morbidity (incidence[2]) of adult population in 24 regions of Ukraine, the Crimea as well as Kyiv and Sevastopil cities, in the period from 2000 to 2013.

The sources of information were the reports of the Endocrinology Service of Ukraine 'The main indices of the activity of the endocrinology service of Ukraine...' for the period of 8 years: 2000, 2004, 2005, 2006, 2009, 2010, 2011 and 2013 [7–14], according to which the evaluation of general endocrinology morbidity, incidence of diffuse nontoxic goiter degree I, diffuse non-

1 The term recommended by WHO.
2 The term recommended by WHO.

toxic goiter degree II–III, nodule goiter, hypothyroidism, thyrotoxicosis, thyroiditis and thyroid cancer (II class of neoplasm, C73) was carried out. The spreadsheet was developed in Microsoft Office Excel (2007).

The methods of empirical and theoretical research of scientific information: analysis, synthesis, induction, deduction and systematization, as well as epidemiological and statistical methods were used.

For the analysis of changes in the level of general and primary thyroid disease, the average absolute increase (AI), which characterizes the average annual increase rate, increase rate (AIR,%) and increase rate (IR,%), were evaluated. The estimation of the basic (relative to the level of the initial year) and the chain (relative to the level of the previous year) indices were carried out.

Using the Microsoft Office Excel (2007) and IBM SPSS StatisticsBase v.22 program correlation (with Pearson correlation coefficient – r) and regression analyzes to estimate the dynamics of population morbidity over a fourteen-year period, the ranking of investigated administrative territories (AT) of Ukraine according to the levels of prevalence and incidence for 2000–2013 years and the rate of their increase, the identification of the relationship between the incidence rate of individual thyroid diseases and the relationship between the levels and the rate of morbidity index increase according to the calculation of the Spearman rank correlation coefficient (r_s), were conducted

Results

The current WHO statistics prove that the pathology of endocrine system takes the third place after cardiovascular and cancerous diseases in the structure of the overall morbidity and causes of mortality in most countries of the world and it continues to increase [1]. A similar situation is evidenced in Ukraine also, where the increase in the number of patients with various endocrinopathies in the period from 2005 to 2010 amounted to 9.85 % [14].

It has been established that in the period from 2000 to 2013, the overall endocrinology incidence (prevalence of endocrinopathies) of adult population in 16 regions of Ukraine, the Crimea, Kyiv and Sevastopil increased, as evidenced by the statistically significant coefficients of the Pearson correlation pair ($r>r_{tabl}$; r_{tabl}=0.707 at n=8, p=0.05). Regarding the primary incidence of endocrine system diseases, in the period of monitoring, a positive correlation was established only in 6 regions, another one (Cherkasy) proved a tendency to increase ($r>r_{tabl}$; r_{tabl}=0.629 at n=8, p=0.1). At the same time, the largest AIR and IR of both general and primary morbidity were observed in Zaporizhia region (IR 137.1 and 118.6 % respectively), Kyiv (113.3 and 33.8 %), Mykolaiv (102.7 and 55.8 %), Kharkiv (100.7 and 64.8 %), and Poltava (100.1 and 57.4 %) regions (respectively, 1, 2, 3, 4 and 5 rank positions for IR of general morbidity and 1, 5 , 4, 2 and 3 – IR of primary morbidity).

Significant ($p<0.05$) decrease of the total endocrinological morbidity that correlated with the year was observed only in three regions: Volyn (administrative center is Lutsk), Sumy and Chernihiv. In the other (five) regions the statistically significant correlation between the prevalence of endocrine pathology and the year was not proved. Regarding the primary morbidity, a statistically significant inverse correlation relationship was established in 8 regions (including Volyn, Sumy and Chernihiv); in another one (Kirovohrad, administrative center Kropyvnytskyi) and Crimea regions there was a tendency to index decrease ($0.05<p<0.1$). In the other (10) investigated AT, the level of primary morbidity did not significantly change during 14 study years ($p>0.1$).

In general, between the IR of the general and IR of the primary incidence of endocrine system diseases in the period from 2000 to 2013 there was a significant positive correlation (r_s=0.916; $r_{s\ tabl}$=0.597 at n=27, p=0.001). The same correlation was found between the levels of prevalence and incidence of endocrine pathology (r_s=0.841).

In general, in the period from 2000 to 2013, the highest levels of both indices of endocrine disease were reported in Vinnytsia region and the regions of Western Ukraine: Zakarpattia (administrative center is Uzhgorod), Rivne, Volyn (Table. 1). Primary morbidity was high also in Ivano-Frankivsk, Ternopil and Khmelnitsky regions, and prevalence – in the northern regions (Kyiv, Chernihiv and Sumy regions). It should be noted that these areas have the highest incidence of diffuse goiter of varying degrees; in the northern regions of nodular goiter and thyroid cancer as well.

The seven last ranked places according to both indices of endocrine system diseases incidence were occupied by the eastern (Luhansk), southern (Zaporizhia, Kherson), central (Poltava) regions and Sevastopil. Primary morbidity was also low in Donetsk and Odesa regions, and prevalence – in Kirovohrad and Mykolaiv. The

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Table 1. Ranking of administrative territories according to the levels of thyroid general (GM) and primary (PM) morbidity of adult population during the period of 2000–2013

Regions	Administrative territory	Ranks															
		Endocrine system diseases (ESD)		Diffuse goiter of I degree (DG-I)		Diffuse goiter of II-III degrees (DG-II+III)		Nodular goiter (NG)		Thyroid cancer (TC)		Hypothyroidism (HT)		Thyrotoxicosis (TT)		Thyroiditis (TD)	
		ЗЗ	ПЗ	ЗЗ	ПЗ	ЗЗ	ПЗ	ЗЗ	ПЗ	ЗЗ	ПЗ	ЗЗ	ПЗ	ЗЗ	ПЗ	ЗЗ	ПЗ
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Western	Volyn	2**	5**	1**	2**	4**	4**	12	16	27''	25''	10	24''	17	18	25''	26''
	Zakarpattia	3**	1**	2**	3**	1**	1**	20	27''	25''	27''	14	13	2**	3**	24''	24''
	Ivano-Frankivsk	8	4**	7**	4**	17	17	18	23''	26''	26''	1**	8	4**	4**	22''	20
	Lviv	11	12	6**	6**	14	16	24''	22''	17	15	16	18	8	21''	14	14
	Rivne	5**	2**	4**	1**	2**	2**	14	14	23''	21''	22''	19	16	10	23''	22''
	Ternopil	12	6**	13	5**	10	13	19	25''	22''	22''	19	21''	15	19	27''	27''
	Khmelnitsky	9	7**	14	10	11	8	8	12	18	20	6**	4*	1**	1**	17	12
	Chernivtsi	10	8	12	12	5**	6**	22''	19	11	24''	25''	23''	10	26''	12	18
Northern	Zhytomyr	15	16	11	13	9	9	7**	13	15	12	24''	26''	13	16	19	23''
	Kyiv	4**	15	3**	11	3**	3**	2**	3**	2**	2**	8	10	21''	17	6**	4**
	Sumy	7**	9	10	9	7**	5**	4**	5**	8	4**	17	15	6**	12	7**	9
	Chernihiv	6**	19	5**	14	6**	15	3**	4**	6**	6**	21''	20	7**	13	9	10
	The city of Kyiv	16	17	21''	20	15	12	1**	1**	1**	1**	2**	1**	24''	7**	1**	1**
Central	Vinnytsia	1**	3**	8	7**	8	11	5**	2**	4**	7**	5**	5**	5**	15	8	7,5**
	Dnipropetrovsk	17	18	16	16	12	10	13	8	7**	9	4**	2**	9	6**	5**	6**
	Kirovohrad	21''	13	15	15	24''	19	21''	20	19	16	26''	27''	20	27''	13	15
	Poltava	22''	24''	18	21''	19	24''	9	6**	13	11	18	14	14	8	15	13
	Cherkasy	13	10	20	18	13	7**	10	10	12	10	11	12	12	2**	21''	19
Eastern	Donetsk	19	21''	22''	23''	21''	22''	11	9	9	13	3**	3**	3**	5**	2**	3**
	Luhansk	27''	26''	23''	22''	20	18	27''	21''	24''	23''	27''	25''	25''	14	26''	25''
	Kharkiv	18	14	17	17	16	14	25''	15	16	19	13	6**	27''	24''	3**	2**
Southern	Zaporizhia	26''	26''	24''	24''	26''	25''	16	11	10	88	23''	17	19	11	11	5**
	Mykolaiv	24''	20	19	19	22''	21''	26''	18	21''		7**	9	26''	23''	10	11

Continuation of table 1

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Southern	Odesa	20	23''	26''	27''	25''	26''	23''	26''	14	14	15	16	11	25''	20	17
	Kherson	23''	22''	25''	26''	18	23''	6**	7**	3**	3**	9	11	22''	20	4**	7,5**
	Crimea	14	11	9	8	23''	20	15	17	20	17	12	7**	18	9	18	16
	Sevastopil	25''	27''	27''	25''	27''	27''	14	24''	5**	5**	20	22''	23''	21''	16	21''

*Note.** – the highest levels of morbidity, '' – the lowest levels of morbidity.*

Table 2. Correlation between the levels of thyroid general (GM) and primary (PM) morbidity of adult population of Ukraine during the period of 2000–2013.

	Spearman's rank correlation coefficients														
	ESD-GM	ESD- PM	DG-I-GM	DG-I-PM	DG-II+III-GM	DG-II+III-PM	NG-GM	NG-PM	TC- GM	TC-PM	HT-GM	HT- PM	TT-GM	TT-PM	TD-GM
ESD-GM	1														
ESD-PM	0.841*	1													
DG-I-GM	0.882*	0.764*	1												
DG-I-PM	0.844*	0.906*	0.916*	1											
DG-II+III-GM	0.858*	0.692*	0.783*	0.714*	1										
DG-II+III-PM	0.797*	0.750*	0.717*	0.716*	0.920*	1									
NG-GM	0.360^	0.037	0.160	0.023	0.350^	0.265	1								
NG- PM	0.160	-0.124	0.023	-0.151	0.235	0.176	0.822*	1							
TC- GM	-0.114	-0.422''	-0.335^	-0.499*	-0.056	-0.144	0.640*	0.702*	1						
TC- PM	-0.184	-0.488*	-0.328^	-0.493*	-0.159	-0.219	0.691*	0.717*	0.911*	1					
HT- GM	0.234	0.184	0.041	0.063	0.034	0.073	0.323^	0.332^	0.194	0.156	1				
HT- PM	0.079	0.063	-0.104	-0.083	-0.060	-0.001	0.291	0.442''	0.325^	0.253	0.868*	1			
TT- GM	0.559*	0.469''	0.396''	0.409''	0.392''	0.308	0.211	0.021	-0.053	-0.169	0.223	0.183	1		
TT- PM	0.245	0.217	0.132	0.181	0.19	0.259	0.418''	0.313	-0.033	0.009	0.430''	0.507*	0.494*	1	
TD- GM	-0.163	-0.338^	-0.246	-0.408''	-0.122	-0.158	0.388''	0.672*	0.772*	0.673*	0.376^	0.545*	-0.118	-0.092	1
TD- PM	-0.153	-0.322	-0.248	-0.397''	-0.175	-0.184	0.379^	0.698*	0.701*	0.642*	0.456''	0.672*	-0.083	0.065	0.940*

*Note. Spearman's rank correlation coefficients (n =27): * – $r_{s\ tabl}$=0.487 at p=0.01; '' – $r_{s\ tabl\ n}$=0.381 at p=0.05; ^ – $r_{s\ tabl}$ =0.323 at p=0.1.*

lowest rates of diffuse goiter of different degrees are presented in Table 1.

However, it should be noted that in the regions that occupy the last ranked positions according to the levels of both morbidity indices, AIR and IR were high. On the contrary, in regions with a high morbidity, AIR and IR were low or even negative. This was confirmed by the Spearman's rank correlation coefficients, which proved a statistically significant backward correlation between the level and the IR of the total (r_s=-0.576; $r_{s\,tab}$l=0.478 at n=27, p=0.01) and the primary (r_s=-0.509, p<0.01) incidence of endocrinopathies among the population of 27 investigated AT of Ukraine.

In general, the incidence rates of adult population for endocrine diseases correlate with the prevalence and morbidity rates of diffuse goiter of various degrees (Table 2), which is explained by a high specific gravity of the latter in the structure of endocrine diseases. Thus, in Ukraine in 2010, in the structure of endocrinopathy, diffuse goiter of degrees I-III was the second (29.99 %) after diabetes mellitus (31.88 %), and the share of all thyroid gland diseases was 46.67 % [14].

In the analysis of both indices of thyroid morbidity, it was found out that during the period of investigation, the highest levels were registered in the western and northern regions of Ukraine, and the lowest ones were in the central, eastern and southern (except in the Autonomous Republic of Crimea) regions (Fig. 1). At the same time, in most of the western and northern regions during 14 years there was a decrease in both (Volyn, Lviv, Zakarpattia, Ivano-Frankivsk, Sumy oblasts), or one (Ternopil, Zhytomyr, Kyiv, Chernihiv) of the morbidity indices, while the changes in the other were insignificant (Fig. 2).

Kyiv was the exception: both indices, especially the level of general morbidity, increased; in Rivne and Chernivtsi regions the general morbidity significantly increased without significant changes in the primary one, and in Khmelnitsky region significant changes in both indices did not take place. The situation in the central, eastern and southern regions was the opposite, where in the majority of regions there was a significant increase in both (Cherkasy, Poltava, Kharkiv, Donetsk, Zaporizhia, Mykolaiv, Odesa) or one (Vinnytsia, Dnipropetrovsk, Kherson, Luhansk regions and Sevastopil) of indices with minor changes of the other. Kirovograd region and Crimea were the exceptions: the general morbidity did not

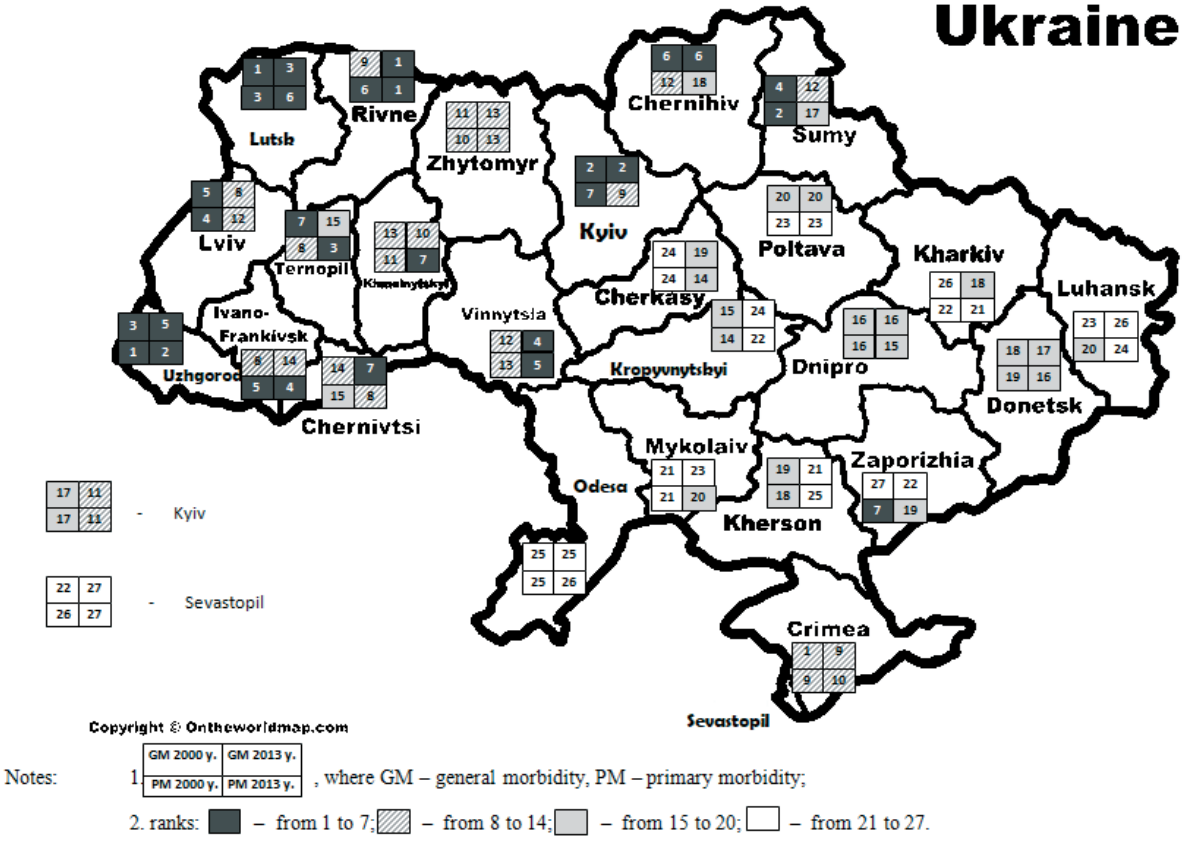


Fig. 1. Thyroid morbidity.

change significantly, and the primary one – decreased (Fig. 2).

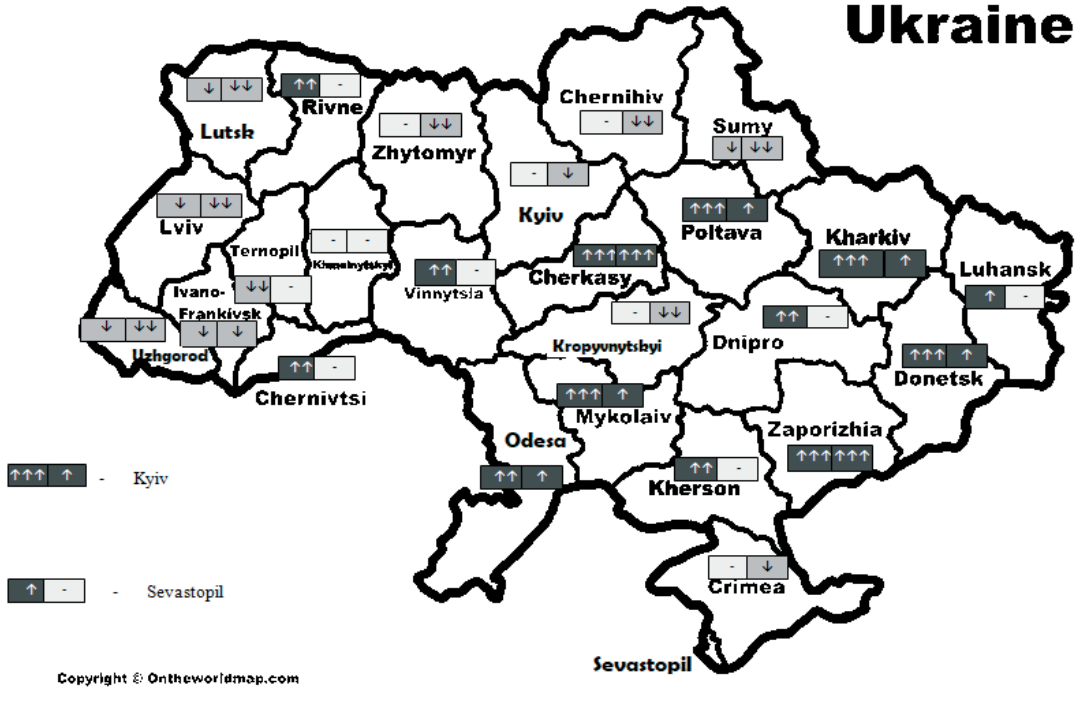
Thus, in relation to thyroid pathology, as well as in case of endocrine diseases in general, a statistically significant inverse relationship between the level and IR of the general (r_s= -0.687, p<0.001) and primary (r_s=-0.735, p<0.001) morbidity of adult population in 27 investigated AT of Ukraine was revealed.

Concerning certain nosological forms of thyroid pathology, during the period of monitoring, significant regional features were revealed both for the levels of morbidity indices and the direction of their changes in the period of study.

Thus, the levels of primary and general morbidity of diffuse goiter (DG) were the highest in the western (Zakarpattia, Rivne and Volyn regions – DG of degrees I and II–III, Chernivtsi – DG of degrees II–III, Lviv and Ivano-Frankivsk – DG of degree I) and northern (Kyiv, Sumy, Chernihiv) regions, and the lowest ones – in the eastern (Donetsk) and southern (Odesa, Zaporizhia, Kherson regions and Sevastopil) regions (Table 1).

During 14 years of the study, the general morbidity rate for diffuse goiter of degrees I (DG-I) and II–III (DG-II–III) in 11 and 6 regions respectively decreased, in 8 and 13 AT – increased, which was confirmed by statistically significant correlation coefficients. In 3 and 2 AT respectively, there was a tendency to increase (0.707>r>0.622, 0.05<p<0.1); in the rest of the AT there were no significant changes. Regarding the primary morbidity of diffuse goiter of different degrees, its level decreased in 16 AT (p<0.05), in one region – a tendency to decrease was evidenced; in the other 10 AT there were no significant changes. It should be noted that between the level and GR of both general and primary morbidity with diffuse goiter of degrees I and II–III there was a reliable inverse relationship (from r_s=-0.390 to r_s=-0.582; $r_{s\ tabl}$=0.381 at n=27 and p=0.05): the lower the level of incidence in the region, the greater the pace of its growth.

The highest levels of nodular goiter primary and general morbidity were registered in the northern regions (Kyiv city, Kyiv, Chernihiv and Sumy regions), Vinnytsia and Kherson regions (Table 1). At the same time, in those regions, as well as in Sevastopil, the highest levels of thyroid cancer morbidity incidence and prevalence were proved. The lowest levels of morbidity rates for both nodular goiter and thyroid cancers were registered in western



Notes: 1. Changes in ranges: «-» - less than ±25 %; ↑/↓ - 25-50 %; ↑↑/↓↓ - 50-100 %; ↑↑↑/↓↓↓ - more than 100%; GM | PM - general/primary morbidity.

2. ↑ – index increasing; ↓ - index decreasing; - - significant ghanges are absent.

Fig. 2. Dynamics of changes in the thyroid general and primary morbidity.

(Zakarpattia, Ivano-Frankivsk, Ternopil) and Luhansk regions; only nodular goiter – in Lviv and Odesa, only cancer – in Volyn and Rivne regions. In general, between the levels of nodular goiter primary and general morbidity, on the one hand, and cancer, on the other hand, there was a significant positive correlation (Table 2).

Regarding the dynamics, in the study period, the nodular goiter general morbidity in 25 of 27 AT increased, which was confirmed by a statistically significant positive correlation coefficient; in Zhytomyr region there was a tendency to increase ($0.707>r>0.622$, $0.05<p<0.1$) and only in Volyn region the index decreased ($r=-0.902$; $p<0.01$). Nodular goiter primary morbidity increased in 22 AT ($r>0.707$, $p<0.05$); in three – it did not change significantly, remaining steadily high in Kyiv and Sumy regions; and in Volyn and Zhytomyr – decreased ($r=-0.908$, $p<0.01$ and -0.748, $p<0.05$, respectively).

Regarding the overall incidence of thyroid cancer, in general in the period of 2000–2013 there was no statistically significant relationship in the period of monitoring between 25 out of 27 AT ($p>0.05$), only in Kyiv region the increase ($r=0.784$, $p<0.05$) took place, while in Chernivtsi – the decrease ($r=-0.716$, $p<0.05$) of the index.

The primary incidence of thyroid cancer in the period of investigation increased in 15 regions and the Crimea Autonomous Republic ($r>0.707$, $p<0.05$), while in 4 regions (Rivne, Zhytomyr, Kropyvnytskyi, and Odesa), there was a tendency to increase ($0.707>r>0.622$, $0.05<p<0.1$), in the rest of the AT the relationship between the level of the indices and the year of monitoring was not detected ($p>0.05$).

The highest levels of both indices of the incidence of adult thyroiditis in Ukraine were registered in Kyiv city, Kharkiv, Donetsk, Kyiv, Dnipropetrovsk and Kherson regions, and the lowest – in Ternopil, Volyn, Luhansk, Zakarpattia and Rivne regions (Table 1). In general, both thyroiditis morbidity indices correlated with the incidence of thyroid cancer, nodular goiter and hypothyroidism (Table 2).

During the study period, the overall incidence of thyroiditis, hypothyroidism and thyrotoxicosis increased in most (24, 20 and 26, respectively) of the studied AT, which was confirmed by a statistically significant positive coefficient of correlation. Regarding the primary morbidity of thyroiditis, hypothyroidism and thyrotoxicosis, the indices increased in 13, 17 and 11 respectively.

Discussion

The regional peculiarities of the levels and dynamics of changes in the incidence rates of the thyroid morbidity among adult population of Ukraine can be related to the priority environmental factors for each region. It is established that the western and northern regions of Ukraine are different from the rest of the low natural content in the soil of iodine. The northern (Kyiv, Chernihiv, Zhytomyr, Rivne) and Cherkasy regions are significantly contaminated by radioactive substances, including radioactive iodine isotopes (predominantly I-131), as a result of the Chernobyl accident. In the eastern region, Dnipropetrovsk and Zaporizhia regions, powerful industrial centers are concentrated, in which relatively high levels of environmental pollution by industrial toxicants, including heavy metals, are registered. The central and southern regions have developed agricultural production, which today widely uses chemical protection products for plants. The study of the environmental factors influence on the Ukrainian adult population thyroid morbidity will be a topical issue of our further research.

Conclusions

In the period from 2000 to 2013, high levels of adult population endocrinopathies, thyroid glands in general and diffuse goiter of varying degrees, general and primary morbidity have been recorded in the western and northern regions of Ukraine, low – in the central, eastern and southern regions.

Statistically significant ($p<0.001$) positive correlation between the level of prevalence and the level of incidence of endocrine pathology, diseases of thyroid gland as a whole, as well as individual nosology has been detected.

It has been established that endocrine diseases incidence rates of adult population correlate ($p<0.01$) with the prevalence and morbidity rates of diffuse goiter of various degrees, which is explained by a high specific gravity of the latter in the structure of endocrine diseases in general and thyroid pathology, in particular.

The factors that cause development of various diseases of thyroid gland as well as regional peculiarities of their development require further study to develop effective methods of protection and prevention.

** Kropyvnytskyi is a city in central Ukraine, and is the administrative center of the Kirovohrad Oblast. Between 1939 and 2016 it was called Kirovohrad.*

АНАЛІЗ ЗАХВОРЮВАНОСТІ ДОРОСЛОГО НАСЕЛЕННЯ УКРАЇНИ НА ПАТОЛОГІЮ ЩИТОПОДІБНОЇ ЗАЛОЗИ З 2000 ПО 2013 РОКИ

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НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ О. О. БОГОМОЛЬЦЯ, КИЇВ, УКРАЇНА

Вступ. *Патологія щитоподібної залози останнім часом привертає значну увагу фахівців клінічної та профілактичної медицини. Частково це пов'язано з тим, що щитоподібна залоза активно реагує на геохімічний стан і забруднення навколишнього середовища промисловими і сільськогосподарськими відходами з наступним поширенням певних патологічних процесів.*

Метою дослідження *було проаналізувати захворюваність дорослого населення України на захворювання щитовидної залози в період з 2000 по 2013 роки.*

Методи. *Використовувалися методи емпіричного та теоретичного дослідження наукової інформації: аналіз, синтез, індукція, дедукція та систематизація, а також епідеміологічні та статистичні методи. За допомогою програми Microsoft Office Excel (2007) та програми IBM SPSS StatisticsBase v.22 були проведені кореляційний та регресійний аналіз.*

Результати. *З 2000 по 2013 рр. зареєстровано високий рівень загальної та первинної захворюваності на ендокринопатії, дифузний токсичний та ендемічний зобу у дорослого населення західних і північних областей України, низький рівень – у центральних, східних і південних регіонах. Виявлено статистично значущу (p <0,001) позитивну кореляцію між рівнем поширеності та рівнем захворюваності ендокринною патологією, захворюваннями щитовидної залози, а також окремою нозологією.*

Висновки. *Регіональні особливості рівню та динаміки частоти захворюваності щитовидної залози серед дорослого населення України можуть бути пов'язані з екологічними факторами у кожному регіоні. Цей фактор потребує подальшого вивчення для розробки ефективних методів профілактики та захисту.*

КЛЮЧОВІ СЛОВА: **захворюваність; щитоподібна залоза; доросле населення, Україна.**

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CEREBROPROTECTION BY GERMANIUM COORDINATION COMPOUNDS IN EXPERIMENTAL ACUTE GLOBAL BRAIN ISCHEMIA

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Background. *Stroke is among the leading causes of death and disability worldwide with rising incidence among young people today. This is the third most common cause of disability-adjusted life-years worldwide*

Objective. *The present study evaluated the cerebroprotective action of coordination compounds of germanium with underlying global cerebral ischemia in rats.*

Methods. *Global cerebral infarction was induced by bilateral common carotid artery occlusion. For primary screening we used numerous bis(citrate) germanates (stannates) compounds, which contained different metals: OL1-8, and VITAGERM-1,2,3 and 4. All germanium complexes used were injected intraperitoneally (1 % aqueous solution at a dose of 50 and 100 mg/kg in 35 min after bilateral common carotid artery occlusion). Piracetam was used as a reference drug. Criteria of cerebroprotection efficacy survival of rats (%), ET50 (median effective time), observational Irwin's test.*

Results. *Almost all bis(citrate) germanates (stannates), which contained different metals, possessed anti-ischemic activity of different intensity. The exceptions were cobalt-containing OL-6 and OL-2 compounds. The most significant efficac of all investigated indices (which exceeded even reference drug) was evidenced for VITAGERM-1 – a coordination compound of germanium, diethylenetriaminepentaacetic acid and lithium.*

Conclusions. *Results of our experiments are the substitution for further more profound pharmacological investigation of VITAGERM-1 for stroke cerebroprotection and its implementation into clinics.*

KEY WORDS: **global brain ischemia; germanium coordination compounds; screening.**

Introduction

Today stroke is in the top 10 causes of death in the world. It maintains the position as the leading killer, and accounts for more than 6 million deaths annually [1]. In Ukraine, it is near 100 000 cases each year, and almost 40 000 of them are fatal [2].

Risk of stroke is rising in direct proportion to the patient's age. However, it is observed its rising incidence among young people up to 40 years old in age [2, 3]. Stroke affects men and women equally and causes major social and economic burdens to society: 80 % of survivors are disabled until the end of life [4]. This is the third most common cause of disability-adjusted life-years worldwide [4, 5]. More than 80 % of all strokes are ischemic and characterized by occlusion of brain vessel of thrombi or embolus.

The aim of the study is to find the effective cerebroprotector among original synthesized coordination compounds of germanium in the experimental model of acute global cerebral ischemia.

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Methods

Experiments were performed on white inbred rats, males and females, 180–220 g of body weight. Acute brain ischemia was modeled by bilateral occlusion of both common carotid arteries before its bifurcation to external and internal branches under thiopental general anesthesia.

All animals' procedures were carried out according to the rules and requirements of European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes as well as EU Directives 2010/63/EU.

For primary screening we used numerous bis(citrate)germanates (stannates) compounds, which contained different metals: magnesium bis(citrate) germanate (germacit), mangan bis(citrate) germanate (OL1), cobalt bis(citrate) germanate (OL2), nickel bis(citrate) germanate (OL3), zinc bis(citrate) germanate (OL4), mag-

nesium bis(citrate) stannate (OL5), cobalt bis(citrate) stannate (OL6), nickel bis(citrate) stannate (OL7), zinc bis(citrate) stannate (OL8), VITAGERM-1 (coordination compound of germanium, diethylenetriaminepentaacetic acid and lithium), VITAGERM-2 (coordination compound of germanium, diethylenetriaminepentaacetic acid and sodium), VITAGERM-3 (coordination compound of germanium, diethylenetriaminepentaacetic acid and potassium), VITAGERM-4 (coordination compound of germanium, diethylenetriaminepentaacetic acid and magnesium). All the compounds were primarily synthesized in the laboratory of Department of General Chemistry and Polymers, I. Mechnikov Odesa National University, headed by prof. I. Seifullina.

All used germanium complexes were administered to rats at the doses according to the results of our previous studies [6, 7]. We injected them intraperitoneally in the form of 1 % aqueous solution at a dose of 50 and 100 mg/kg in 35 min after bilateral common carotid artery occlusion.

As a comparator (reference) drug, Piracetam medication was used (Farmak, Ukraine, 20 % solution in 5 ml ampules). It was injected intraperitoneally at a dose of 350 mg/kg in 30 min after ischemia. The rats of control group (ischemia without any correction) got equivalent dose of saline.

Criteria of cerebroprotector activity of the studied compounds were: survival of rats (%) each hour during the first 12 hours, then on the 24th, 48th and 72nd hours of ischemia; monitoring the clinical signs for ET_{50} (median effective time – the time required for a half as organisms in a toxicity test to reveal the nonlethal response). It was evaluated to be $ET_{50}=f(N/2)$ by interpolation of data of lethality time to mathematical function $T=f(N)$, where T is the time of animals death, N – the number of animals. Neurological status was assessed by Irwin's test in the model of acute brain ischemia and administration of germanium compounds. The primary observation test was used for rodents to assess acute toxicity of a test agent and its effects on behavior and physiological function [8, 9].

Statistical analysis. Statistical analysis of animals' survival have been carried out using the nonparametric criterion Fisher's exact test and average time of lifespan by use of t-criterion of Students' test. All data were presented as M (mean) ± m (standard error). A probability level (p value) of less than 0.05 was considered to be statistically significant.

Results

The results obtained in the screening model of global brain ischemia are presented in the Table 1. Almost all bis(citrate) germanates (stannates), which contained different metals, possessed anti-ischemic activity of different intensity. The exception was the OL-6 compound, which comprised cobalt. Even at the dose of 50 mg per kg in this group during the first hour of occlusion of *aa. carotica communes* the rate of rats survival was only 28.5 % opposite to 100 % in the control group. In addition, the other cobalt-containing compound OL-2 proved very low efficacy for cerebroprotection.

As for the results in Table 1, the most significant efficacy on the 48th hour of the experiment was evidenced for VITAGERM-1 – a coordination compound of germanium, diethylenetriaminepentaacetic acid and lithium. The animals' survival in this group was 57.1 % compared to 100 % lethality in the control group. It is important that the anti-ischemic activity of VITAGERM-1 exceeded even the effect of reference drug Piracetam.

To estimate the obtained screening results more profoundly and detail, we suggested and used in our experiment the index of median effective time ET_{50}. According to the results presented in Table 1, ET_{50} for group of rats administered with VITAGERM-1 was 53.31±2.77. It was in 7.5, 3.0 times better compared to the control and reference group respectively.

At the same time to make an all-round comparison analysis of the results of screening series we assessed clinical signs of acute ischemia: neurological status of rats, which got the different types of germanium compounds. All data are presented in Table 2.

The rats with stroke, on the model we chose, experienced the severe clinical signs of global ischemia. The animals of control group after occlusion of both carotid arteries became less alert and more passive. It was proved by the indices of emotional condition: grooming decreased up to 0 points, vocalization increased up to 1.4. The tonus of limbs also diminished, ataxia signs increased up to 2.9 points. The character of these neurological changes testified quite a deep injury of brain cortex because of global ischemia. It resulted in the disruption of motor activity in the control group rats: suppression of reactions to touch and knock up to 1.5 and 1.9 points respectively. Brain ischemia also strengthened the tremor up to 2.3 points. The suppression of ptosis reaction in 2.1 times as well as increased

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Table 1. The survival dynamics (%) and the average time of death (ET_{50}) of rats with brain ischemia under the influence of bis(citrate) germanates (stannates) compounds with different metals (n=10)

Group of animal (n=10)	The dose, mg/kg	Rats' survival (%) in the certain period of time (hours)															P_1 P_2	ET_{50} (hours)	P_3 P_4
		1	2	3	4	5	6	7	8	9	10	11	12	24	48	72			
Intact rats	-	100	100	100	85.7	57.1	57.1	57.1	42.8	42.8	42.8	42.8	42.8	14.2	-	-	-	6.96 ±0.26	-
Reference group (piracetam)	**350**	100	100	100	100	85.7	71.4	71.4	71.4	71.4	57.1	57.1	57.1	42.8	42.8	42.8	- <0.05	16.12 ±0.79	<0.001 -
VITAGERM-1	**100**	**71.4**	**71.4**	**71.4**	**71.4**	**71.4**	**71.4**	**71.4**	**71.4**	**71.4**	**71.4**	**71.4**	**71.4**	**57.1**	**57.1**	**42.8**	**<0.05 <0.05**	**53.31 ±2.77**	**<0.001 <0.001**
magnesium bis (citrate) germanate (germacit)	**100**	100	100	85.7	85.7	85.7	85.7	85.7	85.7	85.7	85.7	85.7	71.4	71.4	28.5	28.5	>0.05 >0.05	43.79 ±2.36	<0.001 <0.001
mangan bis (citrate) germanate (OL1)	**100**	100	85.7	85.7	85.7	85.7	85.7	85.7	85.7	85.7	71.4	71.4	57.1	42.8	42.8	42.8	<0.05 <0.05	33.71 ±4.05	<0.001 <0.01
cobalt bis(citrate) germanate (OL2)	**50**	42.8	42.8	42.8	42.8	42.8	42.8	42.8	42.8	42.8	42.8	42.8	42.8	14.2	14.2	14.2	>0.05 >0.05	9.13 ±0.82	<0.05 <0.001
nickel bis (citrate) germanate (OL3)	**50**	100	28.5	28.5	28.5	28.5	28.5	28.5	28.5	28.5	28.5	28.5	28.5	-	-	-	>0.05 >0.05	5.92 ±0.47	<0.05 <0.001
zinc bis (citrate) germanate (OL4)	**50**	100	100	100	100	100	100	100	71.4	71.4	42.8	42.8	42.8	28.5	-	-	>0.05 >0.05	11.03 ±0.82	<0.01 <0.01
magnesium bis (citrate) stannate (OL5)	**100**	100	100	100	100	100	57.1	57.1	57.1	57.1	57.1	57.1	28.5	28.5	-	-	>0.05 >0.05	10.68 ±1.49	<0.05 <0.05
cobalt bis (citrate) stannate (OL6)	**50**	28.5	28.5	28.5	28.5	28.5	28.5	-	-	-	-	-	-	-	-	-	>0.05 <0.05	1.09 ±0.09	<0.001 <0.001
nickel bis(citrate) stannate (OL7)	**50**	100	100	85.7	14.2	14.2	14.2	14.2	14.2	14.2	14.2	14.2	14.2	-	-	-	>0.05 <0.05	4.31 ±0.31	<0.001 <0.001
zinc bis (citrate) stannate (OL8)	**100**	100	100	100	100	57.1	57.1	57.1	57.1	57.1	57.1	28.5	28.5	28.5	-	-	>0.05 >0.05	12.26 ±0.76	<0.001 <0.05
Vitagerm-2	**100**	100	100	100	57.1	57.1	28.5	28.5	14.2	14.2	14.2	14.2	14.2	14.2	14.2	14.2	>0.05 >0.05	5.19 ±0.17	<0.001 <0.001
Vitagerm-3	**100**	100	100	71.4	71.4	71.4	57.1	57.1	57.1	57.1	57.1	42.8	42.8	28.5	28.5	28.5	>0.05 >0.05	10.69 ±0.49	<0.001 <0.001
Vitagerm-4	**100**	100	71.4	71.4	57.1	57.1	42.8	28.5	28.5	28.5	28.5	14.2	14.2	14.2	-	-	>0.05 >0.05	5.14 ±0.32	<0.01 <0.001

Notes: Statistical significance was evaluated by Fisher exact test in 48 hours after arteries occlusion; p1, p3 – compa ed to the control group; p2, p4 – compared to Piracetam

Table 2.1. Neuropharmacological profile (Irwin's observation test) of bis(citrate)germanates (stannates) compounds, which contained different metals, in acute brain ischemia

Neuropharma-cological profile		Basal score	Statistical Data	Control	Piracetam	Bis(citrate) germanates (stannates) compounds with different metals									
						Vitagerm-1	Germacit	OL-1	OL-2	OL-4	OL-5	OL-8	Vitagerm-2	Vitagerm-3	Vitagerm-4
Behavioral profile	Alertness	4	M±m p1 p2	0.0±0.0	2.9±0.3 <0.001 -	3.0±0.3 <0.001 <0.05	2.6±0.2 <0.001 >0.05	2.7±0.4 <0.001 >0.05	1.7±0.5 <0.01 >0.05	2.1±0.2 <0.001 >0.05	2.2±0.2 <0.001 >0.05	2.3±0.5 <0.01 >0.05	1.5±0.3 <0.01 >0.05	2.6±0.4 <0.001 <0.05	1.6±0.3 <0.001 >0.05
	Passivity	0	M±m p1 p2	2.9±0.4	2.6±0.1 >0.05 -	1.2±0.1 <0.01 <0.001	1.5±0.3 >0.05 <0.05	1.4±0.3 <0.05 <0.01	2.0±0.3 >0.05 >0.05	2.3±0.3 >0.05 >0.05	2.4±0.3 >0.05 >0.05	1.9±0.3 >0.05 <0.01	1.9±0.4 >0.05 >0.05	1.6±0.3 >0.05 <0.05	1.1±0.3 <0.05 <0.01
Mood	Grooming	4	M±m p1 p2	0.0±0.0	1.5±0.2 <0.001 -	2.8±0.5 <0.01 >0.05	1.0±0.2 <0.01 >0.05	0.9±0.5 >0.05 >0.05	1.8±0.2 <0.001 >0.05	1.0±0.1 <0.001 >0.05	1.1±0.1 <0.001 >0.05	2.1±0.3 <0.001 >0.05	1.4±0.4 <0.01 >0.05	1.0±0.5 >0.05 >0.05	1.7±0.2 <0.001 >0.05
	Vocaliza-tion	0	M±m p1 p2	1.4±0.3	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -
Motor activity	Touch response	4	M±m p1 p2	1.5±0.3	2.1±0.2 >0.05 -	3.6±0.3 >0.05 <0.05	1.5±0.3 - >0.05	2.0±0.4 >0.05 >0.05	2.0±0.3 >0.05 >0.05	2.5±0.4 >0.05 >0.05	2.0±0.2 >0.05 >0.05	2.3±0.3 >0.05 >0.05	1.9±0.4 >0.05 >0.05	2.3±0.4 >0.05 >0.05	2.0±0.2 >0.05 >0.05
	Knock response	4	M±m p1 p2	1.9±0.2	2.9±0.3 <0.01 -	3.5±0.3 <0.01 >0.05	1.7±0.3 <0.05 <0.05	2.0±0.4 >0.05 >0.05	1.8±0.3 >0.05 >0.05	2.3±0.4 >0.05 >0.05	2.0±0.1 >0.05 >0.05	2.6±0.5 >0.05 >0.05	1.9±0.3 >0.05 >0.05	2.4±0.3 >0.05 >0.05	1.7±0.2 >0.05 <0.01

Notes: In Tables 2.1 and 2.2: p1 – statistical significance compa ed to the control, p2 – compared to the comparator (reference) drug.

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Table 2.2. Neuropharmacological profile (Irwin's observation test) of bis(citrate)germanates (stannates) compounds, which contained different metals, in cases of acute brain ischemia

Neuropharmacological profile		Basal score	Statistical Data	Control	Piracetam	Bis(citrate) germanates (stannates) compounds with different metals									
						Vitagerm-1	Germacit	OL-1	OL-2	OL-4	OL-5	OL-8	Vitagerm-2	Vitagerm-3	Vitagerm-4
Neurological profile	Limb tone	4	M±m p1 p2	2.5±0.2	2.2±0.3 <0.05 -	3.3±0.3 <0.05 >0.05	0.8±0.3 <0.001 <0.05	1.3±0.3 <0.01 >0.05	1.2±0.1 <0.001 >0.05	1.2±0.2 >0.05 -	1.6±0.2 >0.05 -	2.2±0.3 >0.05 >0.05	1.5±0.4 <0.05 >0.05	1.4±0.4 <0.05 >0.05	1.6±0.3 <0.01 >0.05
Motor coordination	Ataxia	0	M±m p1 p2	2.9±0.3	1.9±0.3 <0.01 -	0.0±0.0 <0.001 <0.001	0.0±0.0 <0.001 <0.001	0.0±0.0 <0.001 <0.001	0.0±0.0 <0.001 <0.001	2.0±0.3 <0.05 <0.05	2.4±0.3 >0.05 >0.05	0.0±0.0 <0.001 <0.001	0.0±0.0 <0.001 <0.001	0.0±0.0 <0.001 <0.001	0.0±0.0 <0.001 <0.001
CNS status	Tremor	0	M±m p1 p2	2.3±0.3	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	2.1±0.3 >0.05 <0.001	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -
Autonomic Profile	Ptosis	4	M±m p1 p2	1.9±0.2	0.9±0.2 <0.01 -	2.2±0.3 <0.05 <0.001	1.6±0.2 >0.05 >0.05	0.8±0.3 <0.05 >0.05	1.4±0.2 >0.05 >0.05	1.5±0.2 >0.05 <0.05	0.7±0.3 >0.05 >0.05	1.5±0.4 >0.05 >0.05	1.0±0.3 <0.05 -	0.9±0.3 <0.05 >0.05	1.1±0.2 >0.05 >0.05
	Urination	0	M±m p1 p2	1.4±0.1	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -	0.0±0.0 <0.001 -
	Defecation	0	M±m p1 p2	0.9±0.3	0.0±0.0 <0.05 -	0.0±0.0 >0.05 -	1.9±0.3 >0.05 <0.05	0.7±0.6 - >0.05	1.1±0.2 >0.05 <0.001	1.5±0.4 >0.05 <0.01	0.0±0.0 >0.05 -	1.6±0.2 >0.05 <0.001	0.8±0.3 >0.05 >0.05	0.9±0.3 - >0.05	0.8±0.2 >0.05 >0.05

frequency of urination and defecation was evidenced in comparison to the indices of healthy group.

The next stage of our experiment was to estimate the neurological and behavioral profile of rats under the influence of germanium compounds in global brain ischemia model. The most efficient compound was VITAGERM-1 again (Tables 2.1 and 2.2). The significant increase of alertness up to 3.0 points and decreasing of expressiveness of passivity by 41.0 % was fixed in this group compared to the control rats. This investigated compound increased the grooming and normalized the vocalization. Administration of VITAGERM-1 led to absence of ataxia and increase of extremities tonus up to 3.3 points that was nearly the same as the indices of the intact rats. Responses to touch and knock intensified also up to 3.6 and 3.5 points respectively in comparison to the control.

We noticed total positive effects of this germanium compounds in prevention of tremor, urination and defecation; the ptosis rate increased up to 2.2 points.

Discussion

The pathophysiology of stroke is complex, and involves inflammatory pathways, oxidative damage, apoptosis, energy deficiency etc. The main goal of treatment is to preserve tissues in ischemic penumbra, where perfusion is decreased but sufficient to stave off infarction, and save the neurons (neuroprotection) [4, 5].

Ischemic stroke and its consequences is a major public health problem. In spite of huge number of medications to treat it, we still have no perfect one according to the present requirements of safety and efficacy. That is why the search and investigation of newly synthesized organic and other compounds with capability for stroke protection is an actual challenge and target for chemists, pharmacologists, physicians and health professionals.

Numerous bis(citrate) germanates (stannates) compounds with wide spectrum of therapeutic activity are synthesized and investigated today. Our previous experiments proved its low toxicity to warm-blooded species [10, 11, 12]. Use of the rodent models is recommended and approved by different researches for screening and investigating of promising compounds to prevent and treat ischemic brain lesions [13]. In the screening series in the model of global brain ischemia (via occlusion of both common carotid arteries before its bifurcation), it was determined that almost all compounds had potency for stroke protection. Such criteria as survival and median effective time plus Irwin's test results were adequate to evaluate it [14, 15]. Piracetam was used as a reference drug [16].

Among all investigated bis(citrate) germanate (stannates) compounds, which contained different metals, the most potent anti-ischemic activity was possessed by coordination compound of germanium, diethylenetriaminepentaacetic acid and lithium coded as VITAGERM-1. It was a leader among the germanium compounds according to the analyses of the indices of survival and lifespan of rats from the moment of occlusion of coronary arteries.

Such positive effects were manifested by the highest level of survival and the most favorable clinical course of cerebrovascular blood circulation. Median effective time ET_{50} in this group exceeded in 7.5 and 3.0 times the index of control and reference group respectively. We noticed total positive effects of this germanium compounds in prevention of disorders of behavioral, neurological and autonomic profiles of rats. Therefore, we concluded the prominent improvement of clinical course of acute brain ischemia under the Vitagerm-1 impact.

We suppose that the presence of the cobalt in structure of OL-2 and OL-5 molecules is responsible for animals lethality during the first hour after the disorder development. Low efficacy of these germanium compounds could be explained by the fact that this metal is a cytochrome P450 inhibitor. The cytochromes P450 (CYPs) enzyme family are crucial in processes of detoxification of the organism, and suppression of its activity is one of the most possible causes of high lethality in cases of experimental global brain ischemia [17].

Conclusions

Almost all bis(citrate) germanates (stannates), which contained different metals, possessed anti-ischemic activity of different intensity. The exceptions were cobalt-containing OL-6 and OL-2 compounds. The most expressive efficacy for all investigated indices (which exceeded even the reference drug) proved for VITAGERM-1 – a coordination compound of germanium, diethylenetriaminepentaacetic acid and lithium.

The results of our experiments are suggested for the substitution for further deeper pharmacological investigation of VITAGERM-1 for stroke cerebroprotection, and its implementation into clinical practice.

ПОШУК ЦЕРЕБРОПРОЕКТОРІВ СЕРЕД КООРДИНАЦІЙНИХ СПОЛУК ГЕРМАНІЮ НА МОДЕЛІ ІШЕМІЧНОГО ІНСУЛЬТУ

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Вступ. *Інсульт залишається однією з провідних причин смертності та інвалідності в усьому світі, однак спостерігається зростання захворюваності серед молоді.*

Мета дослідження – *скринінгове дослідженння церебропротекторної дії координаційних сполук германію на моделі гострої ішемії у щурів.*

Методи. *Гостру ішемію моделювали двосторонньою оклюзією загальної сонної артерії. Для первинного скринінгу використовувалися сполуки біс(цитрато)германати (станати), що містили різні метали: OL1-8, VITAGERM-1,2,3 та 4. Усі використовувані комплекси германію вводили внутрішньочеревно (1% водного розчину при дозі 50 і 100 мг/кг через 35 хв після двосторонньої загальної оклюзії сонної артерії). Пірацетам використовували в якості референс препарату. Критерії ефективності церебропротекції: виживання щурів (%), ЕТ50 (середній час загибелі 50% тварин), тест Ірвіна.*

Результати. *Результати, отримані на скринінговій моделі тотальної ішемії головного мозку, показали, що практично всі різнометальні біс(цитрато) германати (станати) володіють антиішемічною активністю різної інтенсивності. Виняток становили сполуки, що містять кобальт OL-6 і OL-2. Найбільш значима ефективність всіх досліджених показників (які перевищували навіть еталонний препарат) була підтверджена для VITAGERM-1 – координаційного з'єднання германію, діетилентриамінпентаоцтової кислоти і літію.*

Висновки. *Результати наших експериментів слугують підгрунтям для подальшого вивчення сполуки VITAGERM-1 у якості церебропротектора та її впровадження в клінічну практику.*

КЛЮЧОВІ СЛОВА: **тотальна ішемія головного мозку; координаційні сполуки германію; скринінг.**

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CARRAGEENAN INDUCES CELL DEATH IN RATS BLOOD

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Background. *Because of its physical and chemical properties, carrageenan is fairly widely used. About 70 % of the carrageenan produced in the world is used in the food industry. Previous studies point to the development of oxidative stress in rats, by means of which carrageenan chronic enterocolitis was modeled.*

Objective. *The aim of our study was to investigate the level of apoptosis and necrosis in the suspension of leukocytes in rats using 0.5 % and 1.0 % solutions of carrageenan.*

Methods. *Annexin V (V) binding assays were performed using Annexin V Apoptosis Kit (Sigma Aldrich, USA), caspase rate in leukocyte-lymphocyte blood fractions was determined by spectrofotometry.*

Results. *It was established that in the experimental application of carrageenan, the percentage of leukocytes with signs of apoptosis in both experimental groups statistically significantly increased. It was detected by the increased activity of effector caspase-3 in 1 month after the experiment in 1.5 times in the 2nd group and in 2.8 times in the 3rd group vs control data that point to caspase-dependent apoptotic pathway in case of carrageenan usage in rats.*

Conclusions. *Oral use of carrageenan in rats was accompanied by the increase in the number of leukocytes with signs of apoptosis. The animals that consumed 1.0 % solution of carrageenan had more obvious increase in the activity of caspase-3 in serum relative to a group of rats consuming 0.5 % of carrageenan, proving the increase in the severity of apoptotic processes in intestine with the increase of the dose of carrageenan.*

KEY WORDS: **carrageenan, apoptosis, caspase-3, rat.**

Introduction

In world practice it is allowed to use about 500 different substances as nutritional supplements, not taking into account a huge number of different types of flavors and some varieties of combined supplements [1].

In the USA, the amount of nutritional supplements (NS) exceeds 1500, in the EU it reaches 1200, in Russia it is 415, in the Federal Republic of Germany – 350 [2]. By the beginning of 90s of the 20th century the use of nutritional supplements in Ukraine was limited in comparison with other countries of Europe and the USA [3], only 194 NS were permitted until 1994, and according to the Cabinet of Ministers Resolution in 2000, 221 NS were permitted. In addition, in the EU countries it is allowed to use more than 400 flavors in food production.

For food and in medical practice, there is a nutritional supplement called carrageen that is emitted by water extraction from marine algae [4]. This NS (E407) constitutes a family of polysaccharides (also known as Irish moss) contained in the red seaweed *Chondrus Crispus*, *Eucheuma* species, *Gigartina* species, and others. By chemical nature carrageenan is close to agarose and represents unbranched sulfated heteroglycans which molecules are made of residues of derivatives of D-galactopyranose with a strict alternation of α- (1,3) and β- (1,4) bonds between them, i.e. of repetitive disaccharide units including residues of β-D-galactopyranose and 3,6-anhydro-α-D-galactopyranose. Depending on the peculiarities of the structure of disaccharide repeating units, there are three main types of carrageenans, for which the letters of the Greek alphabet are used to define [1, 5, 6].

Because of its physical and chemical properties, carrageenan is a fairly widely used. About 70 % of the carrageenan produced in the world is used in the food industry: in the production of dairy products (chocolate milk, sherbets, farmer cheese, cheese paste, baby food), meat and fish products (canned food, sausage casings, jelly coatings), seasonings, nonalcoholic beverages, bakery (bread dough, fruit muffins, sugar icing) and confectionery [7].

Previous studies point to the development of oxidative stress in rats, by which carrageenan chronic enterocolitis was modeled [8]. The

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activation of lipid peroxidation is caused by direct stimulation of generation of active forms of oxygen by carrageenan, or indirectly, via the tumor-alpha necrosis factor [9].

The fundamental of carrageenan influence on the body is the development of oxidative stress as one of the mechanisms of damage of intestine as well as the major multiple organ lesions in heart, lungs and liver. Therefore, the aim of our study was to investigate the level of apoptosis and necrosis in the suspension of leukocytes in rats using 0.5 % and 1.0 % solutions of carrageenan.

Methods

The study was conducted on 36 mature white nonlinear male rats, which were kept on a standard diet at the vivarium of I. Horbachevsky Ternopil State Medical University. During the study we followed the principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The rats were divided into 1 control and 2 experimental groups: the 1st group – control (intact animals); the 2nd group comprised the animals that consumed 0.5 % solution of carrageenan, the 3rd group consisted of the animals that consumed 1.0 % solution of carrageenan. The 2nd and the 3rd groups of animals were provided with free access to 0.5 % solution of carrageenan and 1.0 % solution of carrageenan in drinking water for 1 month [10, 11].

Annexin V (V) binding assays were performed using Annexin V Apoptosis Kit (Sigma Aldrich, USA). Apoptotic cells of blood leukocyte suspension were identified by flow cytometry using flow cytometer Epics XL (Beckman Coulter, USA). To distinguish cells that had lost membrane integrity, propidium iodide (PI) was added to a final concentration of 10 mg/mL before the analysis. The results were presented as a percentage of the total number of cells as follows: live cells – not stained (V^-/PI^-), cells with early signs of apoptosis – stained with annexin (V^+/PI^-), cells with late signs of apoptosis – positive double fluorescence staining, cells with signs of necrosis – stained with propidium iodide (V^-/PI^+).

To determine caspase rate in leukocyte-lymphocyte blood fractions, 0.25 ml of buffer and 50 mcl of 2 mM DEVD-p-NA was added to 0.7 ml of the test liquid. It was incubated for 2 hours at 37 °C; the intensity of light absorbance was measured at 405 Nm, which was directly proportional to the product of hydrolysis of Acetyl-Asp-Glu-Val-Asp n-nitroanilide caspase – 3-n-nitroanilide [12].

Statistical analysis

The results were analyzed using Statistica 7.0 software and presented as mean with standard error of mean. The differences between all groups were determined using one-way ANOVA, followed by post hoc the Least Significant Difference test. A p-value <0.05 was considered statistically significant.

Results

It was established that with the experimental application of carrageenan, the percentage of leukocytes with signs of apoptosis in both experimental groups increased significantly (Table 1). Thus, the percentage of V^+/PI^--cells in the 2nd group increased in 1.9 times, and in group 3 – in 2.2 times vs the control indexes ($p<0.001$). The percentage of leukocytes with later signs of apoptosis increased significantly, with respect to control: in group 2 – in 8.9 times, in group 3 – in 22.3 times ($p<0.001$). It should be noted that the level of necrotic cells when introducing 0.5 % carrageenan did not significantly differ from the normal indices, while the use of 1.0 % solution of carrageenan in drinking water caused the increase of V^-/PI^+-cell in 1.7 times ($p<0.001$).

Caspases in general are important mediators in apoptosis, especially caspase-3, which is the main caspase effector that cleaves cell substrates. It was established that the activity of caspase-3 effector in 1 month of the experiment increased in 1.5 times in the 2nd group and in 2.8 times in the 3rd group vs control data that proved the caspase-dependent apoptotic pathway in case of carrageenan use for rats (Fig. 1).

Discussion

Caspase-3 is probably the best understood of the mammalian caspases in terms of its specificity and roles in apoptosis. Overall, recent progress has generally confirmed the notion of multiple, complex death pathways (some of which require caspase-3 in specific cell types) that converge on common events including cell shrinkage, blebbing, chromatin condensation and DNA. Two established ways of apoptosis include internal or mitochondrial, involving protein family Bcl–2, cytochrome C and caspase – 9 and external with the activation of caspase–8 linking a specific cell receptor Fas– and soluble tumor necrosis factor receptors on the cell

Table 1. Indicators of cell death in serum of the rats in experimental use of carrageenan Me (Q25-Q75)

Index	Control	2nd group	3rd группа
Alive leukocytes, %	95.76 (95.28; 96.77)	90.05* (89.26; 90.43)	83.87*# (82.62; 86.11)
Leukocytes with early signs of apoptosis, %	3.05 (2.20; 3.68)	5.89* (5.55; 6.20)	6.73*# (5.83; 7.55)
Leukocytes with late signs of apoptosis, %	0.36 (0.10; 0.60)	3.19* (2.78; 3.65)	8.02*# (6.75; 8.73)
Leukocytes with signs of necrosis, %	0.83 (0.65; 0.98)	0.86 (0.73; 0.98)	1.39*# (1.20; 1.61)

*Notes: * – the difference between the control and the experimental group is statistically significant (p<0.05-0.001)*
 # – the difference between the 2nd and the 3rd study groups is statistically significant (p<0.05)

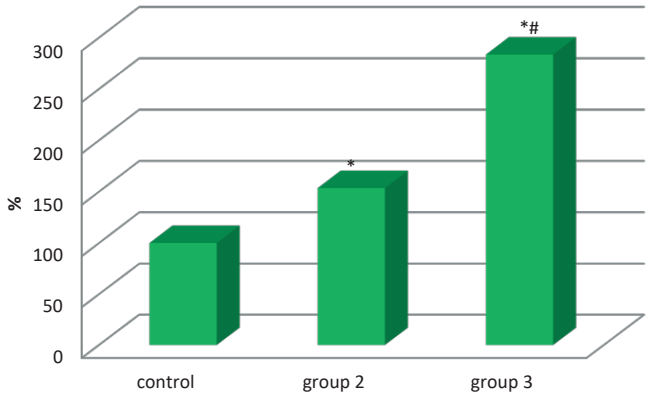

Fig. 1. Caspase-3 level in case of carrageenan intoxication (* – significant difference compared with the control group, # – significant difference compared with the experimental groups).

surface [13]. Caspase-3 is the most involved pathway which should be generated from its inactive protein (procaspase-3), caspase 3 is required for some apoptosis features (chromatin condensation, DNA damage and apoptotic body formation) and its part may take place before cell viability suppression starts [14]. Hridneva SV notes that in chronic enterocolitis endothelial functions are impaired, which manifests itself in the activation of free radical oxidation processes with underlying decrease in the activity of the antioxidant system that explains the excessive production of ROS [15] and cell death. The activity of caspase-3 increases with increased carrageenan concentration, that proves a more obvious enterocyte apoptosis with the increase in daily intake of carrageenan. At the same time, animals of the 3rd group have a more obvious increase in the activity of caspase-3 in serum, indicating the increase in the severity of apoptotic processes in intestine. The obtained data contradicts the results of some studies that prove that exposure of human intestinal epithelial cells to carrageenan in vitro does not lead to the activation of caspase-3, caspase-7 or increased percentage of fragmented DNA, suggesting no apoptotic alterations following carrageenan exposure [16]. Otherwise, the results of other studies evidence that carrageenan induced chronic gastroenterocolitis is accompanied by the decrease in the activity of PARP and elevation of MMP-2, MMP-9 and caspase-3 in blood serum of animals [17]. The results are controversial and require detailed consideration. Therefore, further research is warranted to elucidate the role of carrageenan in intestinal caspase-depending cell death that may help define novel nutritional strategies for hindering the development of gut diseases.

Conclusions

Oral use of carrageenan in rats was accompanied by the increase of the number of leukocytes with signs of apoptosis: $V+/PI^-$- cells in the 2nd group increased in 1.9 times, and in the 3rd group – in 2.2 times, $V+/PI^+$-cells increased in 8.9 and 22.3 times, compared with the control ($p<0.001$); the percentage of leukocytes with later signs of apoptosis was significantly increased too ($p<0.001$). Animals that consumed 1.0 % solution of carrageenan had more obvious increase in the activity of caspase-3 in serum relative to the group of rats consuming 0.5 % carrageenan, proving the increase in the severity of apoptotic processes in intestine with the increase in the dose of carrageenan.

КАРАГІНАН СПРИЧИНЯЄ ЗАГИБЕЛЬ КЛІТИН КРОВІ ЩУРІВ

О. М. Копаниця, М. І. Марущак, І. Я. Криницька
ТЕРНОПІЛЬСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Завдяки своїм фізико-хімічним властивостям карагінан досить широко застосовується, а близько 70% усього виробленого в світі продукту використовується в харчовій промисловості. Попередні дослідження вказують на те, що введення карагінану при моделюванні хронічного ентероколіту викликає розвиток оксидативного стресу.*

Метою дослідження *стало вивчення рівня апоптозу та некрозу в суспензії лейкоцитів у щурів при застосуванні 0,5% і 1,0% розчинів карагенану.*

Методи. *Аналіз зв'язування анексину V (V) проводили з використанням набору Sigma Aldrich, США; рівень каспази у суспензії лейкоцитів та лімфоцитів крові щурів визначали спектрофотометрично.*

Результати. *Встановлено, що при застосуванні карагінану відсоток лейкоцитів з ознаками апоптозу в обох експериментальних групах статистично достовірно збільшувався. Активність каспази-3 зростала в 1,5 рази у 2-й групі і в 2,8 рази в 3-й групі у порівнянні з контрольними даними, які вказують на каспазо-залежний шлях апоптозу клітин при введенні карагінану.*

Висновки. *Пероральне застосування карагінану в щурів супроводжувалося збільшенням кількості лейкоцитів з ознаками апоптозу. Тварини, які споживали 1,0% розчину карагінану, мали більш значуще збільшення активності каспази-3 в сироватці відповідно до групи щурів, які споживали 0,5% каррагінану, що доводить дозо-залежний ефект апоптотичних процесів у кишечнику.*

КЛЮЧОВІ СЛОВА: **карагінан; апоптоз; каспаза-3; щури.**

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CYTOKINES PROFILE IN EXPERIMENTAL CONTACT ALLERGIC DERMATITIS AND USE OF NANOENCAPSULATED AGENTS

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Background. *Inflammation, oxidative and nitro-oxidative stress are the essentials of the pathogenesis of contact allergic dermatitis as well as cytokines imbalance.*

Objective. *The concentration of TNF-α, IL-1β, IL-4 and IL-10 in blood serum of rats with nickel-induced contact allergic dermatitis was evaluated to determine whether it correlated with the use of free and nanoencapsulated preparations of betamethasone, superoxide dismutase (SOD) and potent highly selective inhibitor of iNOS (1400W).*

Methods. *To induce contact dermatitis (CD), 5 % nickel sulfate was used for 12 days. Experiments were performed on white inbred male rats, 180–220 g of body mass. All rats were divided into 10 groups (n=10). Group I – the control one; II – the animals with CD; III – the rats with CD treated with empty polymeric chitosan nanoparticles; groups IV–VI – the rats with CD treated with free SOD, 1400W and betamethasone; groups VII-IX – the rats administered with nanoencapsulated SOD, 1400W and betamethasone; X – CD + nano-composition of all agents.*

Results. *The statistically higher serum concentrations of TNF-α, IL-1β and decrease of IL-4 and IL-10 in experimental contact dermatitis is proved in comparison with the healthy rats. Mono-treatment with betamethasone, SOD and 1400W is efficient, but the use of nanoparticles loaded with these preparations surpasses its effects. The use of the combination of all nanoencapsulated medicines is the most effective.*

Conclusions. *Chitosan nanoparticles loaded with topical anti-inflammatory glucocorticoid, and inhibitors of oxidative and nitro-oxidative stress is a promising method for treatment of allergic contact dermatitis and can be recommended for further research and use in clinics.*

KEY WORDS: **contact nickel dermatitis; cytokines; nanoparticles; betamethasone; SOD; 1400W.**

Introduction

Contact dermatitis (CD) frequency has been rising recently [1, 2]. Its two types are differentiated: triggered by antigens as allergic contact dermatitis and by obligatory cutaneous irritants – irritant contact dermatitis (non-immunologic driven reaction) [3]. All types of dermatitis (atopic, psoriatic, allergic, etc.) are challenge for health care system because of chronic and relapsing character. It is forth reason for doctor's visiting among all skin disorders [4]. Today the first choice and the mainstay of dermatitis drug therapy are topical glucocorticoids (TGs): betamethasone, dexamethasone, fluocinolone, triamcinolone, etc. In spite of their high efficacy a lot of side effects (systemic and local) are common: skin atrophy, acneiform eruption, striae, and telangiectasia, as well as hypothalamic–pituitary-adrenal axis suppression, glaucoma development, high risks of thrombosis and others [5, 6].

One more serious challenge for dermatitis treatment is transdermal delivery of active components. Skin is a prime barrier against a lot of environmental physical, chemical, and biological stressors (ultraviolet irradiation, bacteria, viruses, allergens, etc.). *Stratum corneum*, claudins and occludins of tight junctions are crucial in defense, but also impede the absorption of topical medicines [7]. Nanoencapsulated medications have been developed as a vehicle into the deeper skin layers and demonstrated its benefits [8–10]. Nanotechnology through the reduced particle size improves the absorption and concentration of the drug in the target tissue, its pharmacokinetic parameters and long-term release of the medication at the target site [11].

It is established at present that even regular use of TGs does not prevent the relapse of CD and does not provide the appropriate control of its severity [12]. Cytokines are crucial in pathogenesis of different types of dermatitis (allergic, atopic, etc.). Contact allergic dermatitis

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is mediated via T lymphocytes. Contacting the allergens epithelial cells may produce mediators, which intensify the cascade of immune response [13]. The combination of sensitized T cell plus antigen releases cytokines cascade and lead to inflammation [14].

The objective of the research is to study the cytokines profile in experimental allergic contact dermatitis and its correction with nanoencapsulated steroid anti-inflammatory agent, antioxidant and iNOS inhibitor.

Methods

Animals and experimental design. Experiments were performed on white inbred male rats 180–220 g of body mass. All rats were divided into 10 groups, 10 animals in the each one. group I – the control one involved the animals sensitized only with solid lanoline base; group II – the animals with contact dermatitis (CD) induced by sensitization with 5 % $NiSO_4$ dissolved in the base; group III – the rats with CD treated with empty polymeric chitosan nanoparticles; groups IV–VI involved the rats with CD treated with free SOD, 1400W and betamethasone accordingly; groups VII–IX – the rats, which were administered with nano-encapsulated SOD, 1400W and betamethasone; group X – CD + nano-composition of SOD, betamethasone and 1400W.

To induce the contact dermatitis the hair on the dorsal area, 4x4 cm in size, was removed and the cleaned skin area was administered with 4 g of solid lanoline composition containing 5 % nickel sulfate for 12 days. All correctors were used for one week after the development of CD twice a day.

We used Sigma-Aldrich (USA) betamethasone 17,21-dipropionate, PEGylated superoxide dismutase (SOD) and N-([3-(Aminomethyl) phenyl]methyl)ethanimidamide dihydrochloride (1400W)-potent highly selective inhibitor of iNOS. Chitosan nanoparticles loaded with above-mentioned preparations were prepared according to the method described by Hussain Z., et al. [15]. Chitosan solution (25 mL, 0.2 % w/v, prepared in 1 % v/v acetic acid, pH 5.0) was incubated with Betamethasone solution (1 mg/mL in a 30:70 mixture of ethanol/water) or 1400W (1 mg/mL) or SOD solution (10 mg/mL) stirring for 30 minutes. Loaded nanoparticles were spontaneously formed by adding 10 mL of pentasodium tripolyphosphate solution (0.1 % w/v, in distilled water) dropwise with constant stirring at 700 rpm. The nanoencapsulated preparations were harvested by ultracentrifugation (28,000 rpm) for 30 minutes and subsequently resuspended. The size of nanoparticles was determined using the morphometric program Video-Test–5.0, Kappa ImageBase, and it was 40–100 nm.

All procedures for the animals were performed according to the rules and requirements of European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Directive 2010/63/EU and were approved by local TSMU Ethic Committee. The rats were euthanized under the ketamine anesthesia on the 20th day of examination. Amounts of cytokines in blood serum were evaluated by ELISA test (RayBiotech Inc., Norcross, GA, USA). The concentrations of TNF-α, IL-1β, the Th2-specific cytokines IL-4 and IL-10 were analyzed.

Statistical analysis. The data are presented as mean±SE standard error. Statistical analysis was performed by the Statistica 10.0 (StatSoft Inc., USA) program. The distribution of indices was estimated using Shapiro-Wilk normality test. The significance of the results was determined by the Mann-Whitney U-test and ANOVA-test. A probability level of less than 0.05 was considered as statistically significant.

Results

It has been established that in CD, induced by nickel sulfate, blood serum concentration of

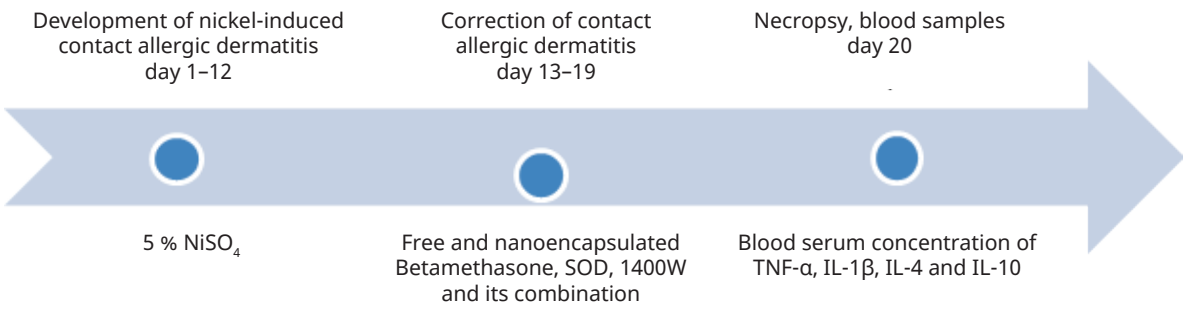

Fig. 1. Schematic diagram of experimental design.

pro-inflammatory cytokines TNF-α and IL-1β significantly increased in 5.5 and 3.7 times compared to the intact rats (Table 1). Anti-inflammatory Th2-specific cytokines IL-10 and IL-4 concentration was significantly lower, in 2.3 times and 2.4 times respectively.

Table 1. Blood serum concentration of cytokines in experimental contact nickel dermatitis, M±m, n=10

Groups / Inter-leukins	Intact rats	CD	CD + empty nano-particles	CD + beta-metha-sone	CD + nano-beta-methasone	CD + SOD	CD + nano-SOD	CD + 1400W	CD + nano-1400W	CD + nano-combi-nation of beta-methasone, SOD and 1400W
TNF-α, pg/ml	7.48 ±0.56	41.16 ±4.10*	40.54 ±3.98*	26.98 ±2.30 *,**	20.94 ±1.16 *,**, #[1]	35.48 ±1.95 *,**	30.22 ±2.02 *,**, #[2]	36.22 ±1.78 *,**	30.12 ±1.56 *,**, #[3]	12.88±1.28 *,**,γ[123]
IL-1β, pg/ml	10.46 ±0.88	37.98 ±2.42*	35.88 ±3.36*	24.77 ±1.75 *,**	19.35 ±1.28 *,**,#[1]	31,32 ±1.54*, **	26.45 ±1.54 *,**, #[2]	32.16 ±1.98 *	26.66 ±1.38 *,**, #[3]	14.48±1.34 *,**,γ[123]
IL-10, pg/ml	12.05 ±1.08	5.24 ±0.42*	5.15 ±0.42*	7.54 ±0.52 *,**	8.96 ±0.32 *,**,#[1]	6.88 ±0.50 *,**	7.88 ± 0.64 *,**	6.32 ±0.44*	8.48 ±0.46 *,**, #[3]	10.33±0.48 **,γ[123]
IL-4, pg/ml	19.26 ±1.88	8.02 ±0.78*	7.68 ±0.66*	11.43 ±0.45 *,**	13.16 ±0.58 *,**	10.04 ±1.00*	11.25 ±0.78 *,**	11.14 ±0.32 *,**	13.26 ±0.44 *,**,#[3]	15.68±0.77 **,γ[123]

*Notes: statistical significance p<0.05 compared to: *intact rats; **CD rats; #1 – rats with CD treated with free form of beta-methasone, #2 – free form of SOD, #3 – free form of 1400W, γ[1] – nanoencapsulated betamethasone, γ[2] – nanoencapsulated SOD, γ[3] – nanoencapsulated 1400W.*

The use of empty nanoparticles did not cause any significant changes in the indices. Use of each nanoencapsulated agent was more efficient to restore the cytokines imbalance in comparison with free form usage. But the most prominent effect was caused by the combination of all three nanoencapsulated correctors.

Betamethasone is a topical corticosteroid of high potency. Its free form caused positive changes in cytokines profile: the decrease in pro-inflammatory TNF-α and IL-1β concentration by 34.5 and 34.8 %; and increase of anti-inflammatory IL-10 and IL-4 – by 43.9 and 42.5 % was evidenced. But nanoencapsulated TG was more effective and surpassed its free form efficacy.

TNF-α and IL-1β rates were statistically significantly lower in this group in comparison with CD group by 49.1 and 49.05 %; in comparison with its free form by 22.4 and 21.9 %.

Also the increase in anti-inflammatory interleukins IL-10 and IL-4 concentration was evidenced in group CD + nano-betamethasone: by 71.0 and 64.1 % in comparison with pathology; and by 18.8 % for IL-10 in comparison with free form of TG.

The same tendency was for free and nanoencapsulated forms of SOD. The use of free SOD caused decreased concertation of TNF-α and IL-1β by 11.4 and 17.5 %; and increased rates of IL-10 and IL-4 by 31.3 and 25.2 % accordingly. Nanoencapsulated SOD surpassed the activity of its free form in decreasing of concentration of pro-inflammatory cytokines. The rate of TNF-α was statistically significantly lower by 17.2 %, IL-1β – by 15.5 %. But there was a positive tendency only in correction of anti-inflammatory interleukins indices.

Highly selective inhibitor of iNOS was also quite efficient. The concentration of TNF-α decreased by 9.6 % in use of its free form; and by 26.8 and 29.8 % – of nanoencapsulated 1400W accordingly. The last rates were statistically significantly improved by 19.1 % for TNF-α and 17.0 % – for IL-1β in comparison with CD + 1400W group. IL-4 concentration increased by 38.9 % in use of free 1400W; and by 65.3 % – of nanoencapsulated one in comparison with control pathology (the last index was higher by 19.0 % compared to CD + 1400W group). Value of IL-10 was statistically significantly different only in case of use of nano-form of 1400W by

61.8 % in the control group and by 34.2 % in CD + 1400W group.

The best results were evidenced in the group CD + nano-combination of betamethasone, SOD and 1400W. The combination of all nanoencapsulated agents was the most efficient in restoring of cytokines balance in comparison with mono-use of each corrector (table 1).

Discussion

Among a lot of allergens, nickel is one of the most common metals responsible for allergic CD. Its regular contacting for hypersensitive persons leads to a delayed-type hypersensitivity reaction and up to 20 % of the general population may be affected in Europe [16–18]. High frequency of positive nickel patch tests and high serum nickel concertation are also observed in people with intrinsic atopic dermatitis [19].

Pathogenesis of allergic contact dermatitis includes sensitization (or induction) and elicitation (or efferent) phases, which are innate (opposite to contact irritant dermatitis) [20]. It is established at present that cytokines are crucial in inflammation, including immune-involved dermatitis [21–23].

A lot of skin cells are able to produce cytokines [24] (Table 2). Dermal dendritic cells are crucial in the process of activating naive T cells; after contacting the allergen Langerhans cells start to synthesize IL-1β mRNA and to release the protein; then, keratinocytes are activated and release TFN-α and GM-CSF [25].

Table 2. Cytokines developed by epidermal cells

Epidermal cells	Cytokine (constitutive or inducible expression)
Keratinocytes	IL-1α, IL-1β, IL-1RA, IL-3 (mouse), IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-18, TNF-α, G-CSF, GM-CSF, MCSF, Gro, MIP-2 (mouse), IP-10, RANTES, MCP-1, TGF-α, TGF-β
Langerhans cells	IL-1α, IL-1β, IL-6, IL-15, IL-18, TNF-α, Gro, MIP-2, MIP-1α, TGF-β
Melanocytes	IL-1α, IL-1β, IL-6, IL-7, IL-8, IL-10, IL-12, TNF-α, G-CSF, GM-CSF, MCSF, Gro, MIP-2 (mouse), RANTES, MCP-1, TGF-α, TGF-β

Skin hypersensitivity reactions are the result of imbalance between a variety of types of T-cell responses and inflammatory mediators, including T-helper (Th) 2 cytokines and also T-helper 1 cells [26]. A Th1/Th2 hypothesis suggests that imbalance among these cells toward Th2 is the key to allergic reaction intensifying [22, 27]. The Th1 cells release of pro-inflammatory cytokines includes IL-1α, IL-1β, IL-8, IL-13, TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF). They increase the vascular permeability and cause swelling and redness associated with inflammation and immunologic reactions in skin exposed to irritants, also affect the proliferation and differentiation of keratinocytes and mediators of cellular infiltration [3, 21, 22].

The key regulators are TFN-α and IL-1β. These pleiotropic cytokines are responsible for inflammation, apoptosis and necrosis of cells, phagocytic and cytotoxic activities [28].

IL-10 is an anti-inflammatory interleukin produced mainly by monocytes, T cells, macrophages, and dendritic cells. Mast cells can also produce IL-10, which limits the rate of leukocyte infiltration, inflammation, and skin disorders such as contact dermatitis [21]. IL-4 and IL-10 suppress Th1 immune response. IL-10 deficiency is crucial in maintaining of CD clinical signs [29].

The pro-inflammatory cytokines TNF-α and IL-1β and the Th1/Th2 cytokines IFNγ and IL-4 are involved in both the induction and elicitation of cutaneous immune response and are modulated by glucocorticoids, that is also proved by the results of our study. Glucocorticoids decrease TNF-α and IL-1β production and shift the Th1/Th2 ratio in a Th2 direction by augmenting production of IL-4 [16, 30]. Mechanisms of such imbalance are associated with activation of oxidative stress, which is realized by generation of reactive oxygen and nitric species (ROS and RNS) and depletion of antioxidant defense. 'Vicious circle' is running here: low antioxidant indices intensify the release of cytokines and inflammation processes that promotes the generation of ROS and RNS again.

Use of nano-encapsulated medications is emerging as potential therapeutics for a wide variety of diseases [22]. They have been successfully used for delivery of hydrophobic and hydrophilic small molecule drugs and biomacromolecules, nucleic acids of various sizes and structures. It is very important for skin diseases treatment to go through the *stratum corneum* to target tissues and subcellular compartments and nanoparticles are quite

successive for that. Nano-form of preparations, especially its combination was the most powerful to alleviate experimental contact dermatitis clinical sings and intensity of oxidative and nitro-oxidative stress [31, 32].

Our previous findings proved the importance of oxidative and nitro-oxidative stress activation in pathogenesis of CD. The last one is aggravated by cytokines. Nitric oxide synthase (NOS) is an enzyme that catalyzes the synthesis of NO and L-citrulline using oxygen and L-arginine as substrates. And its isoform iNOS is induced by the cytokines TNF-a, IL-1 [33].

So, modulation of NO-synthase (NOS) activity, use of antioxidants and free radical scavengers are important and prospect part of complex treatment for different types of CD (plus common recommendations for allergens avoiding, emollients and others) [2, 33, 35]. The perspective results of combined treatment with betamethasone-, SOD (superoxide dismutase) and 1400W (highly selective inhibitor of inducible nitric-oxide synthase, iNOS)-loaded nanoparticles were obtained. Such combination of the potent anti-inflammatory steroid agent, the powerful antioxidant and iNOS-inhibitor in nanoencapsulated form was more effective in experimental contact dermatitis than use of their free forms [31, 36].

Conclusions

Experimental contact dermatitis induced by 5 % nickel sulfate is accompanied by pathological cytokines imbalance. Mono-treatment with betamethasone, SOD (superoxide dismutase) and 1400W (highly selective inhibitor of inducible nitric-oxide synthase, iNOS) was efficient, but the use of nanoparticles loaded with these agents surpassed its effects. The use of combination of all nanoencapsulated medicines was the most effective.

Chitosan nanoparticles loaded with topical anti-inflammatory glucocorticoid, and inhibitors of oxidative and nitro-oxidative stress is a promising method for treatment of allergic contact dermatitis and can be recommended for further research and use in clinics.

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ЦИТОКІНОВИЙ ПРОФІЛЬ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ КОНТАКТНОМУ ДЕРМАТИТІ ТА ВИКОРИСТАННІ НАНОІНКАПСУЛЬОВАНИХ ПРЕПАРАТІВ

І. І. Худан-Цільо, О. О. Шевчук, М. М. Корда

ТЕРНОПІЛЬСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Запалення, оксидативний та нітрооксидативний стрес є основою патогенезу контактного алергічного дерматиту та розвитку дисбалансу цитокінів.*

Мета дослідження *полягала у вивченні змін цитокінів TNF-α, IL-1β, IL-4 і IL-10 в сироватці крові щурів з контактним нікель-індукованим дерматитом при використанні вільних і наноінкапсульованих препаратів бетаметазону, супероксиддисмутази (СОД) і потужного високоселективного інгібітора iNOS (1400W).*

Методи. *Для моделювання контактного дерматиту (КД) 5% сульфат нікелю наносили на шкіру щурам протягом 12 днів. Білі щури-самці масою тіла 180–220 г було рандомізовано на 10 груп (n = 10): I група - контрольна; II - тварини з КД; III - щури з КД, які отримували полімерні хітозанові наночастинки; IV – VI групи - щури з КД, які отримували вільні форми СОД, 1400W та бетаметазону відповідно; VII-IX групи - щури, яким вводили наноСОД, нано-1400W і нано-бетаметазон; X група - КД + нанокомпозиція всіх препартів корекції.*

Результати дослідження. *Доведено, що при експериментальному контактному дерматиті достовірно зростає рівень прозапальних цитокінів TNF-α, IL-1β у сироватці та спостерігається зниження IL-4 і IL-10 у порівнянні зі здоровими щурами. Монотерапія препаратами бетаметазону, СОД і 1400W є ефективною, однак використання нанаінкапсульованих форм перевищує результати монозастосування кожного препарату. Найбільш ефективним є використання поєднання всіх наноінкапсульованих лікарських засобів.*

Висновки. *Хітозанові наночастинки з інкапсульованими препаратами топічного протизапального глюкокортикоїда та інгібіторів оксидативного і нітрооксидативного стресу є перспективним методом лікування контактного дерматиту і можуть бути рекомендовані для подальшого вивчення з впровадженням у клінічну практику.*

КЛЮЧОВІ СЛОВА: **контактний нікелевий дерматит; цитокіни; наночастинки; бетаметазон; СОД; 1400W.**

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OPPORTUNISTIC BACTERIA IN AGROECOSYSTEMS OF UKRAINE

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Background. *Recently, the number of diseases caused by opportunistic bacteria has been increasing all over the world. Opportunistic microorganisms are characterized by ubiquitous proliferation, flexibility in adaptation to the conditions of the environment, lack of specific relationship with the macroorganism. Phytopathogenic bacteria are also able to infect both plants and animals.*

Objective. *The purpose of the research was to detect the Pantoea agglomerans and Pseudomonas fluorescens bacteria in various ecological niches and establish their virulence.*

Methods. *Classical microbiological, biochemical, serological methods were used in the research. The identification of bacteria was carried out according to their phenotypic properties.*

Results. *It has been established that opportunistic bacteria species P. agglomerans and P. fluorescens are present in wheat agrophytocenoses. The bacteria isolated from the agrophytocenoses are virulent for wheat, rye and weed plants. Antibodies to opportunistic bacteria, which are spread in agrophytocenoses of cereals crops, have been found in the blood of healthy rabbits.*

Conclusions. *Thus, we have established that virulent strains of opportunistic bacteria P. agglomerans and P. fluorescens are spread in agrophytocenoses of cereals. The presence of antibodies to these bacteria in the blood of healthy rabbits proves that opportunistic bacteria from plant material get into animals and humans. Knowledge of biology and the spread of opportunistic pathogens in agrophytocenoses is necessary for prevention of infections that these bacteria cause in humans.*

KEY WORDS: **opportunistic bacteria; *Pantoea agglomerans*; human health.**

Introduction

Recently, the number of diseases caused by opportunistic bacteria has been increasing all over the world. Opportunistic microorganisms are characterized by ubiquitous proliferation, flexibility in adaptation to the conditions of the environment, lack of specific relationship with the macroorganism. Such bacteria are characterized by the ability to cause nonspecific toxic infections in weakened people and animals.

It has been established that strains of certain species of microorganisms can cause damage to plants, insects, animals and humans [1]. This phenomenon is known as polybiotrophy [2], which is particularly spread among opportunistic microorganisms. For example, conditionally pathogenic bacteria for humans *Pseudomonas aeruginosa* cause an internal of putrefaction onion during storage [3]. Strains *P. aeruginosa*, isolated from sick people, under experimental conditions affect plants, nematodes and insects [4]. Phytopathogenic bacteria are also able to infect both plants and animals [1, 2]. Bacteria of the genus *Erwinia,* which are well known exclusively as pathogenic to plants, are often isolated in pathological processes in humans and animals [5]. In this case isolates isolated from humans and animals are pathogenic to plants [2]. The causative agent of vascular bacteriosis of *Erwinia toxica* cucumbers, in intraperitoneal administration to mice, leads to sepsis in animals. Infected with these bacteria fruits of cucumbers cause poisoning in people [6].

A high degree of similarity established by DNA hybridization analyses and phenotypic data between strains of *Erwinia herbicola*, *Enterobacter agglomerans* and *Erwinia milletiae* led Gavini *et al*. [7] to unite them as a single species, namely *Pantoea agglomerans* (Beijerinck 1888) comb. nov. *P. agglomerans* is widespread in numerous diverse natural habitats and is particularly associated with many different plants as a common epiphyte and endophyte [8]. Additionally, it has been also isolated from seeds, water, humans (e.g., wounds, blood,

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urine and internal organs) as well as from animals [7, 9-12].

Pantoea species are ubiquitous in nature and occasionally are associated with infections caused by contaminated clinical material. Hence, *Pantoea agglomerans* is considered as an opportunistic pathogen of humans [13].

The *Pseudomonas fluorescens* bacteria, under conditions favorable to their development, can cause diseases of agricultural crops [14]. For some human diseases, antibodies to *P. fluorescens* lipopolysaccharide have been identified in the patients' blood.

Therefore, the purpose of the research was to detect the *Pantoea agglomerans* and *Pseudomonas fluorescens* bacteria in various ecological niches and establish their virulence.

Methods

Materials for research were the plants with symptoms of damage of rye, wheat and weeds that grew in wheat agrocenoses. Bacteriological analysis and isolation of bacteria were carried out using generally accepted methods [15]. Virulent properties were investigated on the host: plant, aggressiveness of the pathogen was determined by a 4-point scale. The biological properties were investigated by the methods described by Klement et al [15]. The bacteria were identified by comparing their properties with the characteristics of strains collection, and according to the Bergey's Manual of Systematic Bacteriology [16].

Results

We have found out that yellow-pigmented bacteria, flat or with a conical center, are opaque, wavy edges; and the oxidase-positive gray-colored isolates with wavy edges were isolated from all investigated plant materials.

It was established that a part of the investigated isolates of bacteria was avirulent for plants. In all other cases, the isolated bacteria caused diseases of rye, wheat and weeds.

The yellow pigmented bacteria are polymorphic, short rods, single arranged, sometimes in pairs in the form of short chains. Bacteria are mobile, Gram negative, spores are not formed, oxidase negative. Facultative anaerobes (Table 1). On the meat-peptone broth they grow with the formation of uniform turbidity, ring, film and sediment. The bacteria are utilized glucose (anaerobic), reduce nitrates, acidify litmus serum. Strains differ in the use of rafinose, sorbitol and inulin. All investigated strains do not use dulcitol, cause coagulation or peptonization of milk.

The isolates obtained from the affected plants according to the physiological and

Table 1. Physiological and biochemical properties of isolates

Test	Yellow-pigmented isolates	*P.agglomerans* [16]	Unpigmented isolates	*P.fluorescens* [16]
Gram's staining	–	–	–	–
Motility	+	+	+	+
Nitrate reduction	+	+	–	–
Oxidase	–	n/i	+	+
Formation of H_2S	–/+	n/i	–	n/i
Formation of indole	–	–	–	n/i
Gelatinase	+/-	+	+	n/i
OF-test	Facultative anaerob	Facultative anaerob	Aerob	Aerob
Utilization: D-glucose, L-arabinose, D-mannitol, D- xylose	+	+	+	+
Fructose, galactose	+	+	+	n/i
Salicin	+	+	–	–
Lactose	+	d	–	–
Inositol	+	+	+/–	+
Raphinose	+/–	d	+/–	n/i
Dulcitol	–	–	+/–	n/i
Sorbitol	+/–	–	+/–	+
Inulin	+/–	n/i		

Note: n/i – not investigated.

biochemical properties are similar to each other and do not differ from the characteristics of the described *P. agglomerans* species [16]. According to this they were identified as *P. agglomerans*.

Gray colored oxidase-negative bacteria grow on the meat-peptone broth, form a film, ring and precipitate, and use glucose (aerobic), mannitol, xylose, fructose, arabinose, and do not utilize lactose, inulin, salicin, as a single source of carbon.

Most of the isolates utilize raffinose, maltose and dulcitol. All isolates hydrolyze gelatin, alkalinize the litmus serum, and do not reduce nitrates (Table 1).The isolates of bacteria obtained from the affected cereals and weeds on the morphological and cultural-biochemical properties did not differ from the described strains of *P. fluorescens* and from the *P. fluorescens* characteristic given in Bergey's Manual of Systematic Bacteriology [16].

All bacterial strains tested were virulent for wheat, rye and weed plants (Table 2). It was established that the strains of the isolated bacteria are more aggressive on weeds than on agricultural crops.

Consequently, pathogenic bacteria for plants of the species *Pantoea agglomerans* and *Pseudomonas fluorescens* are able to come into contact with humans and animals and cause their diseases. This is evidenced by the fact that we detected antigens to the strains *Pantoea agglomerans* and *Pseudomonas syringae* pv. *syringae* in the serum of non-immunized rabbits (true phytopathogen) (Table 3).

Thus, the study of phytopathogenic and opportunistic bacteria is important not only for the development of plant protection methods, but also from the point of view of studying their effects on human health, since these bacteria are widespread in nature and can be ingested by humans.

Discussion

Our research has found that rye, wheat and weeds that grow in the agroecosystem of wheat are affected by opportunistic bacteria *Pantoea agglomerans* and *Pseudomonas fluorescens*. Our previous studies proved that strains of *P. agglomerans* and *P. fluorescens* were more commonly found in agricultural crops as epiphytes and did not cause plant diseases [17]. With the change of environmental factors and the influence of agronomic techniques, these opportunistic bacteria in recent years increasingly acquire virulent properties. For example, the bacteria of *P. agglomerans* were isolated from the affected locales of cotton bolls collec_ ted in a field in the USA and were able to cause comparable disease symptoms in greenhouse grown cotton fruit [18].

P. agglomerans, gram negative bacteria of *Enterobacteriaceae* family, were isolated from feculent material, plants and soil. Soft tissue and bone-joint infections due to *P. agglomerans* following penetrating trauma by vegetation and bacteraemia in association with intravenous fluid, total parenteral nutrition, blood products and anesthetic agent contamination were reported [19]. Some authors on the basis of their studies suggested that, independent of their origin, all *P. agglomerans* strains might possess indistinguishable virulence potential [13]. *P. agglomerans* was also proved to be an antibiotics producer [20].

Some researchers reported on isolation of *P. agglomerans* in two cases of septic monoarthritis after plant thorn and wood sliver injuries [21]. This indicated the transfer of *P. agglomerans* from the infected plant material to humans.

Table 2. Virulence properties

Species	Aggressiveness (marks) on plant:		
	Rye	Wheat	Weed
Pantoea agglomerans	1–2	1–3	2–3
Pseudomonas fluorescens	1–2	2	2–3

Table 3. Results of agglutination reaction

Species, strains	Titres of agglutination reaction with sera of non-immunized rabbits	
	Serum 1	Serum 2
Pantoea agglomerans, 116	50	100
Pseudomonas syringae pv. *syringae, NCPPB* 281	200	200
Erwinia amylovora, 2024	0	0

When studying the effect of bacteria of the genus *Klebsiella* (*K. pneumoniae, K. hinoscleromatis, K. ozaenae*), which, like the *Erwinia* phytopathogenic genus, belonged to the *Enterobacteriaceae* family, on leaves of potatoes, horse beans, beans, cabbage, cucumber, pumpkin and apple fruits Jonathan found out that four strains of *K. pneumoniae* were capable to affect horse beans and potatoes. The phytopathogenic properties of *Klebsiell*a species did not correlate with their ability to produce pectinases [22].

At the same time, phytopathogenic properties can be presented by bacteria, which are traditionally pathogens of animals and humans. It is proved that 15% of the strains of bacteria of genera *Escherichia*, *Citrobacter*, *Enterobacter*, *Proteus*, *Pseudomonas*, isolated from urological patients, have phytopathogenic properties. The most pronounced these properties were on fruits of tomatoes [23].

Among the species of the genus *Pseudomonas*, which produce pigments (*P. aeruginosa*, *P. fluorescens*, *P. aureofaciens*), it is proved that the strains have phytotoxic and entomocidal properties. The greatest number of such strains is found among the bacteria of *P.fluorescens* species. The cultural fluid of these strains suppresses the germination of seeds of radish, lettuce and, to a lesser extent, wheat, and also causes the death of 100% of mosquito larvae. Entomopathogenic strains of *P. fluorescens* have antagonistic effects on some saprophytic bacteria (*Bacillus subtilis*, *B. megaterium*, *Sarcina lutea*, *Escherichia coli*, *Mycobacterium* sp.). Thus, the toxins of pigmented strains of the genus *Pseudomonas* are not narrowly specific and affect a wide range of organisms [24]. In some human diseases antibodies to the lipopolysaccharide of *P. fluorescens* 7769, which were isolated from affected rye tissues, were identified [25].

The adaptation of *P. agglomerans* to diverse microenvironments might suggest that this species maintain high genetic plasticity. *P. agglomerans* appears to be readily accessible to horizontal gene transfer driven by plasmids and other mobile elements [26], a trait that may explain its flexibility in adapting to different life styles.

Some researchers noted that pathogenic microorganisms had a lot in common in mechanisms of pathogenicity, regardless of which macroorganism they were infected with [1, 27, 28].

Conclusions

Thus, in the agrophytocenosis of wheat, one of the most widespread agricultural crops in Ukraine, there are virulent strains of opportunistic bacteria of *P. agglomerans* and *P. fluorescens* species. Present agroecosystems, which are overloaded with chemical pollutants, create conditions for increasing the aggressiveness of opportunistic bacteria. It has been established that in the blood of healthy rabbits antibodies to opportunistic bacteria, which are spread in agrophytocenoses, are present. It proves the intake by animals of opportunistic bacteria together with plant food. Since opportunistic bacteria can cause the infections processes in humans, animals, insects and plants, the control of their spread and investigation of peculiarities of virulent strains circulation is necessary.

ОПОРТУНІСТИЧНІ БАКТЕРІЇ В АГРОЕКОСИСТЕМАХ УКРАЇНИ

Л. М.Буценко, Л. А. Пасічник

ІНСТИТУТ МІКРОБІОЛОГІЇ І ВІРУСОЛОГІЇ ІМЕНІ Д. К. ЗАБОЛОТНОГО НАН УКРАЇНИ, КИЇВ, УКРАЇНА

Вступ. *Останнім часом кількість захворювань, спричинених опортуністичними бактеріями, зростає в усьому світі. Опортуністичні мікроорганізми характеризуються значними темпами проліферації, гнучкістю в адаптації до умов навколишнього середовища, відсутністю специфічних зв'язків з макроорганізмом. Фітопатогенні бактерії також здатні інфікувати як рослини, так і тварини.*

Метою дослідження *було виявлення бактерій Pantoea agglomerans і Pseudomonas fluorescens в різних екологічних нішах і встановлення їх вірулентності.*

Методи дослідження. *У дослідженні використовувалися класичні мікробіологічні, біохімічні та серологічні методи. Ідентифікацію бактерій було здійснено за їх фенотиповими властивостями.*

Результати дослідження. *Встановлено, що в агрофітоценозах пшениці присутні опортуністичні бактерії виду P. agglomerans і P. fluorescens. Виділені бактерії вірулентні для рослин пшениці, жита та бур'янів. Антитіла до опортуністичних бактерій, які поширюються в агрофітоценозах зернових культур, виявлені в крові здорових кроликів.*

Висновки. *Встановлено, що вірулентні штами опортуністичних бактерій P. agglomerans та P. fluorescens поширюються в агрофітоценозах зернових культур. Наявність антитіл до цих бактерій у крові здорових кроликів доводить, що опортуністичні бактерії з рослинного матеріалу потрапляють в організми тварин і людини. Знання біології та поширення опортуністичних патогенів в агрофітоценозах необхідні для профілактики інфекцій, що викликаються цими бактеріями в організмі людини.*

КЛЮЧОВІ СЛОВА: **опортуністичні бактерії; *Pantoea agglomerans*; здоров'я людини.**

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THE ALTERATION OF PRO- AND ANTI-INFLAMMATORY CYTOKINES IN ADRENALINE-CALCIUM INDUCED MYOCARDIAL DAMAGE AND ITS CORRECTION WITH QUERCETIN IN RATS

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Background. *Cardiac insufficiency is one of the main causes of morbidity and mortality in the world.*
Objective. *The aim of the study was to determine the content of interleukins in male rats with adrenaline-calcium model of myocardial injury (ACM) and under correction by quercetin.*
Methods. *143 Wistar male-rats aged 5-6 months were used in the experiments. The changes of interleukins (IL-1beta, IL-2, IL-4, IL-6, IL-10, TNF-alpha) content in blood serum in the development of heart injury by adrenaline and calcium (AC) and use of quercetin for protection were studied. The period of investigation was in 1, 2, 24 hours, 3, 7, 14, 21, 28 days.*
Results. *In ACM model of myocardial injury TNF-alpha increased, IL-2, IL-4 decreased at each period of investigation. IL-1beta increased in 2 and 3 days after the injury, at all other periods (except in 1 hour and 21 days) those indices were decreased. Also we observed similar wave changes of IL-6 and IL-10 content.*
Injections of quercetin for 7 days caused a significant decrease of IL-2, IL-4, IL-10, and increase of IL-6. In 7 days of quercetin administration after AC-injury, decreased TNF-alpha was observed as well as increased IL-4, IL-6, and IL-1beta rates. In 14 days, the increase of TNF-alpha, normalization of IL-10 to control was evidenced. In 28 days, increased activation of IL-1beta, decreased content of TNF-alpha was fixed.
Conclusions. *The development of adrenaline-calcium heart damage is accompanied by bright cytokine wavelike reaction at different time points. Quercetin causes normalization of interleukins level.*
KEY WORDS: **heart damage; rats; adrenaline-calcium model; quercetin; interleukins.**

Introduction

Cardiac insufficiency is one of the main causes of morbidity and mortality in the world. One of the causes of its development is myocardial infarction, when cardiomyocytes die due to prolonged ischemia. Myocardial infarction is a dynamic process, which is accompanied by transition of reversible changes to irreversible ischemic damage and completes in replacement of the diminished part of myocardium by a fibrous scar. Development of fibrosis is a dysfunctional process in which myofibroblasts, the main fibrous cellular elements, are metabolically active and are able to produce and upregulate cytokines; they also have contractile properties [1]. Myocardial infarction leads to rapid necrosis of cardiac myocytes. To achieve tissue integrity and function, inflammatory cells are activated, including monocytes/macrophages. Impaired monocyte/macrophage function is a not established new pathophysiological mechanism for left ventricular thrombus development after myocardial infarction [2]. Myocardial damage in myocardial infarction takes place mainly due to ischemic necrosis and inflammatory mechanisms while apoptosis is the main mechanism of cell death in 'reperfusion injury' in addition to limited ischemic necrosis [3].

In the diseased heart, cardiomyocytes are lost to necrotic cell death, and phenotypically transformed fibroblast-like cells, termed 'myofibroblasts', are activated to initiate a 'reparative' fibrosis. The structural integrity of myocardium is preserved by this scar tissue, although the expense of its remodeled structure, which increases tissue stiffness and propensity to arrhythmias. A persisting population of activated myofibroblasts turns this fibrous tissue into a living 'secretome' that generates angio_ tensin II and its type 1 receptor, fibrogenic growth factors, all of which collectively act as a signal-transducer-effector signaling pathway to type I collagen synthesis and, therefore, fibrosis. Persistent myofibroblasts, and the resultant fibrous tissue they produce, cause progressive adverse myocardial remodeling, a

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pathological hallmark of the failing heart irrespective of its etiologic origin [4].

Cardiomyocyte necrosis activates an inflammatory response that serves to clear the injured myocardium from dead cells, and stimulates repair, but may also extend injury. Recently the studies in the literature have identified interleukin-1a and RNA released by necrotic cardiomyocytes as key danger signals that trigger the inflammatory response following infarction. Interleukin-1 promotes activation of a proinflammatory phenotype in leukocytes and fibroblasts, and delays myofibroblast transdifferentiation. Inhibitory lymphocytes are crucial in negative regulation of postinfarction inflammatory response by modulating macrophage and fibroblast phenotype. Cardiac macrophages exhibit significant heterogeneity and phenotypic plasticity and may manage the reparative response following infarction. In adult mice- animals replacement of resident macrophage populations with monocyte-derived macrophages may induce inflammation while inhibiting cardiac regeneration [5].

Taking into account that the main pathogenic links are the development of inflammation, oxidative and nitric explosion, spasm of coronary vessels, necrosis, fibrosis, the study of the effectiveness of cardioprotective activities for the treatment of the basic pathogenesis is necessary. These medications include quercetin, which has antioxidant, anti-spasm, anti-inflammatory, antisclerotic properties [6].

Therefore, the aim of our study was to determine the content of interleukins in male rats with adrenalin-calcium model of myocardial injury (ACM) and to correct the pathological process by quercetin.

Methods

The experiments were performed on 143 albino Wistar male rats 5-6 months of age. The animals were divided into 14 groups (Table 1). Each group comprised 10 animals. The rats were once intramuscularly administered with a 0.18% solution of adrenaline hydrotartrate at a dose of 0.5 mg/kg of body weight (Pharmaceutical firm "Darnitsa", Ukraine) and intraperitoneally with 5% solution of calcium gluconate ("Dniprofarm", Ukraine) at a dose of 1 ml/100 g of body weight. For the correction the solution of quercetin at a dose of 200 mg/kg of body weight was injected intraperitoneally once a day for 7 days after adrenaline and calcium injections.

Experiments were performed in a special room in the morning at 18-22 °C, relative humidity 40-60% and illumination 250 lux. The regulations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Resolution of the First National Congress on Bioethics (Kyiv, 2001) and the Order of the Ministry of Health of Ukraine № 690 dated September 23, 2009, were strictly followed.

The rats were euthanized by total heart bloodletting and previous thiopental sodium anesthesia (60 mg·kg^{-1} of body weight intraperitoneally). Blood samples were taken for the research. Concentration of interleukins IL-1beta, IL-2, IL-4, IL-6, IL-10, tumor necrosis factor alpha (TNF-alpha) was determined in blood serum by the method of immunoenzyme analysis using a set of reagents produced by ZAO vector-Best (Russia).

Table 1. Division of animals by groups

Group	Quantity of animals	
	Total	Mortality
Control	10	–
In 1 hour after ACM	10	–
In 2 hours after ACM	10	–
In 24 hours after ACM	10	–
In 3 days after ACM	10	–
In 7 days after ACM	10	–
In 14 days after ACM	10	–
In 21 days after ACM	10	–
In 28 days after ACM	12	16.67 %
Quercetin	10	–
In 7 days after ACM + quercetin	10	–
In 14 days after ACM + quercetin	10	–
In 21 days after ACM + quercetin	10	–
In 28 days after ACM + quercetin	11	8.33 %

A statistical analysis of digital data was performed by means of Excel (Microsoft, USA) and STATISTICA 6.0 (Statsoft, USA). Statistical significance of the differences between independent indices was estimated by Student t-test at normal distribution and by nonparametric methods in other cases.

Results

In 1 hour after adrenaline and calcium injection, the increase of TNF-alpha in 7.56 times ($p<0.001$), decrease of IL-4 by 35.63% ($p<0.001$) and of H-10 by 46.08% ($p<0.001$) were determined (Table 2).

In 2 hours IL-1 beta was increased by 45.69 % ($p<0.001$) and TNF-alpha – in 5.9 times ($p<0.001$); IL-2 was decreased by 18.24% ($p<0.002$) and IL-6 – by 48.57% ($p<0.001$). The increase of IL-1 beta by 45.82% ($p<0.01$), of IL-10 in 2.1 times ($p<0.001$), the decrease of IL-6 by 47.55% ($p<0.001$) were determined compared with the previous study.

In 1 day, compared to the control, there was a decrease of IL-1beta by 38.58% ($p<0.001$), IL-2 – by 30.40% ($p<0.001$), IL-4 – by 23.45% ($p<0.001$), and increase of IL-6 in 46.68% ($p<0.01$), TNF-alpha – in 5.21 times ($p<0.001$), and compared to the previous study, there was a decrease of IL-1beta by 57.84% ($p<0.001$), IL-2 – by 14.88% ($p<0.01$), IL-10 – by 23.37% ($p<0.01$), increase of IL-6 in 2.85 times ($p<0.001$).

In 3 days, compared to the control, the level of IL-1beta was higher by 21.94% ($p<0.001$), TNF-alpha – in 7.06 times ($p<0.01$), IL-10 – by 37.16% ($p<0.001$), and IL-2 was lower by 56.08% ($p<0.001$), IL-4 – by 39.66% ($p<0.001$), IL-6 – by 34.67% ($p<0.01$), and compared with the previous period, IL-1beta was increased by 22.06% ($p<0.001$), IL-10 – in 2.547 times ($p<0.001$), IL-2 was decreased by 83.20% ($p<0.02$), IL-6 – by 33.38% ($p<0.001$), IL-4 – by 6.26% ($p<0.001$).

In 7 days compared to the control the increase of TNF-alpha in 17.88 times ($p<0.001$) was observed, as well as the decrease of IL-1 beta by 45.81% ($p<0.001$), IL-2 – by 47.97% ($p<0.001$), IL-4 – by 20.54% ($p<0.001$), and compared with the previous period of study, IL-1beta was decreased by 55.57% ($p<0.001$), IL-10 – by 16.57% ($p<0.001$), IL-6 was increased by 70.81% ($p<0.01$), TNF-alpha – in 2.53 times ($p<0.001$), IL-4 – by 31.69% ($p<0.001$).

In 14 days compared to the control the increase of TNF-alpha in 19.69 times ($p<0.001$) was evidenced, as well as the decrease of IL-1 beta by 40.45% ($p<0.001$), IL-2 – by 37.84% ($p<0.001$), IL-4 – by 21.08% ($p<0.001$), IL-10 – by 39.67% ($p<0.002$), and compared with the previous study period – the decrease of IL-10 by 47.28% ($p<0.001$).

In 21 days compared with the control, the increase of TNF-alpha in 2.1 times ($p<0.001$), IL-6 – by 72.91% ($p<0.001$), the decrease of IL-4 by 28.06% ($p<0.001$), IL-2 – by 22.30% ($p<0.02$), and compared with a previous period of study – the decrease of TNF-alpha by 89.87% ($p<0.001$), increase of IL-6 by 70.73% ($p<0.001$) and IL-10 – by 78.85% ($p<0.001$) were present.

In 28 days the increase of TNF-alpha in 25.89 times ($p<0.001$), IL-6 – by 36.94% ($p<0.02$), the decrease of IL-1 beta by 45.38% ($p<0.001$), IL-2 – by 35.13% ($p<0.001$), IL-4 – by 19.52% ($p<0.001$), and compared to the previous period of study, the increase in TNF-alpha in 11.87 times ($p<0.001$), and the decrease of IL-1beta by 39.43% ($p<0.05$) were evidenced.

Injections of quercetin for 7 days caused a significant decrease of IL-2 by 62.84% ($p<0.001$), IL-4 – by 32.32% ($p<0.001$), IL-10 – by 51.92% ($p<0.001$), and increase of IL-6 in 2.7 times ($p<0.001$).

In 7 days after the injury, quercetin correction proved increased level of IL-1beta in 2.61 times ($p<0.001$), IL-4 – by 35% ($p<0.001$), IL-6 – in 2.27 times ($p<0.001$), and decrease of TNF-alpha in 32.99 times ($p<0.001$). In this period, IL-2, IL-4, IL-10 levels did not significantly differ from the control ones, and IL-1beta was increased by 41.6% ($p<0.001$), IL-2 – by 29.73% ($p<0.001$), IL-6 – in 2.54 times ($p<0.001$). IL-1 beta level was also higher by 60% ($p<0.01$), TNF-alpha – in 9.26 times ($p<0.001$), IL-4 – by 58.5% ($p<0.002$), IL-10 – in 2.69 times ($p<0.001$) compared to the quercetin level.

In 14 days after the injury, the quercetin correction proved higher level of TNF-alpha by 55.14% ($p<0.01$) and IL-10 by 84.76% ($p<0.001$). In this period, only IL-10 level did not significantly differ from the control ones, IL-6 was increased by 49.59% ($p<0.05$), TNF-alpha – in 32.1 times ($p<0.001$), IL-1beta was decreased by 28.5% ($p<0.01$), IL-2 – by 28.38% ($p<0.001$), IL-4 – by 23.7% ($p<0.001$), and compared to a previous period of study IL-1 beta was decreased by 49.5% ($p<0.001$), IL-6 – by 40.97% ($p<0.002$), and IL-4 – by 28.87% ($p<0.05$), TNF-alpha was increased in 2.68 times ($p<0.001$). IL-2 was also higher in 1.93 times ($p<0.001$), TNF-alpha – in 24.8 times ($p<0.001$), IL-10 – in 2.32 times ($p<0.001$) and IL-6 was lower by 44.7% ($p<0.01$) compared to the quercetin level.

In 21 days after the injury, the quercetin correction proved higher level of H-6 by 51.42% ($p<0.002$), TNF-alpha in 9.59 times ($p<0.001$), lower level of H-2 by 50.43% ($p<0.001$). In this period, only IL-1 beta level did not significantly differ from the control, H-6 was higher in 2.62 times ($p<0.001$), TNF-alpha – in 20.1 times ($p<0.001$), IL-10 – by 20.07% ($p<0.001$), IL-2 was

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Table 2. Changes in interleukin indices in rats' blood with adrenalin-calcium myocardial damage and its correction by quercetin, n=10, M±m

Group	Index					
	IL-1beta, pg/ml	IL-2, x10^{-3}, pg/ml	IL-6, pg/ml	TNF-alpha, pg/ml	IL-4, pg/ml	IL-10, pg/ml
Control	66.30±2.34	1.48±0.07	0.78±0.04	0.054±0.002	2.16±0.02	10.25±0.81
In 1 hour after ACM	66.24±5.09	3.87±1.56	0.77±0.01	0.408±0.087*	1.39±0.13*	5.53±0.42*
In 2 hours after ACM	96.59±7.91*,#	1.21±0.03*	0.40±0.04*,#	0.318±0.078*	1.83±0.17	11.63±0.87#
In 24 hours after ACM	40.72±5.50*,#	1.03±0.06*,#	1.15±0.12*,#	0.281±0.039*	1.65±0.07*	8.91±0.43#
In 3 days after ACM	80.85±0.14*,#	0.65±0.13*,#	0.51±0.09*,#	0.381±0.120*	1.30±0.04*,#	14.06±0.54*,#
In 7 days after ACM	35.93±5.54*,#	0.77±0.06*	0.87±0.08#	0.966±0.081*,#	1.71±0.03*,#	11.73±0.41#
In 14 days after ACM	39.48±4.82*	0.92±0.12*	0.79±0.09	1.117±0.193*	1.70±0.11*	6.18±0.92*,#
In 21 days after ACM	59.79±9.76	1.15±0.12*	1.35±0.12*,#	0.113±0.016*,#	1.55±0.06*	11.06±1.03#
In 28 days after ACM	36.21±3.07*,#	0.96±0.04*	1.07±0.10*	1.341±0.226*,#	1.74±0.11*	10.96±0.55
Quercetin	58.67±11.16	0.55±0.04*	2.12±0.33*	0.070±0.011	1.46±0.03*	4.93±0.26*
In 7 days after ACM + quercetin	93.88±3.03*,**,&	1.04±0.24	1.99±0.18*,&	0.647±0.107*,**,&	2.31±0.26**,&	13.27±2.12**
In 14 days after ACM + quercetin	47.40±5.63*,#	1.06±0.07*,**	1.17±0.17*,**,#	1.734±0.099*,**,#,&	1.65±0.09*,#	11.43±1.04**,&
In 21 days after ACM + quercetin	60.98±5.73	0.57±0.04*,#,&	2.05±0.08*,#,&	1.085±0.157*,**,#,&	1.37±0.07*,#	12.31±0.11*,**
In 28 days after ACM + quercetin	81.73±2.56*,#,&	0.86±0.06*,**,#	1.30±0.06*,**,#	0.479±0.163*,**,#,&	1.56±0.06*,#	9.89±0.33**,#

*Notes: * – statistically significant indices as compared with the controls;*
*** – statistically significant indices as compared with the quercetin;*
 # – statistically significant indices as compared with the previous period of investigation;
& – statistically significant indices as compared with the results without correction.

lower by 61.49% (p<0.001), IL-4 – by 36.5% (p<0.001), and compared with a previous period of study the decrease of IL-2 by 46.23% (p<0.001), TNF-alpha – by 37.4% (p<0.001), IL-4 – by 16.77% (p<0.05), the increase of IL-6 by 74.91% (p<0.002) were present. TNF-alpha was also higher in 15.53 times (p<0.001) and IL-10 – in 2.5 times (p<0.001) compared to the quercetin level.

In 28 days after the injury, the quercetin correction proved higher level of IL-1 beta in 2.26 times (p<0.001), lower level of TNF-alpha by 64.38% (p<0.01). In this period, only IL-10 did not significantly differ from the control level, IL-1 beta was higher by 23.28% (p<0.001), IL-6 – by 65.62% (p<0.001), TNF-alpha – in 8.87 times (p<0.01), IL-2 was lower by 41.89% (p<0.001), IL-4 – by 27.48% (p<0.001), and compared with a previous period of study the decrease of IL-6 by 36.74% (p<0.002), TNF-alpha by 55.88% (p<0.001), IL-10 by 19.68% (p<0.001), the increase of IL-1 beta by 34.04% (p<0.001), IL-2 by 50.88% (p<0.001), IL-4 by 14.19% (p<0.05) were present. IL-2 level was also higher by 56.36% (p<0.001), TNF-alpha – in 6.85 times (p<0.05) IL-10 – in 2 times (p<0.001), and IL-6 was lower by 38.82% (p<0.05) compared to the quercetin level.

Discussion

In first hour a direct damage of heart muscle by adrenaline was present, since the proinflammatory cytokine TNF-alpha was a mediator of cardiac pathology. TNF-alpha was synthesized by activated macrophages, and also stimulated them, promoted cytotoxic, immunomodulating, anti-inflammatory action. Decreases in IL-10 and IL-4 may be associated with the early period of damaging effects of adrenaline and calcium when the increase of one anti-inflammatory cytokine was sufficient with protection.

In two hours a high TNF-alpha content still was present. It could cause damage to endothelial cells, increase of microvascular permeability, activate the hemostasis system with DIC syndrome development. Normalizing the concentration of IL-4 and IL-10 may evidence development of immune response with involvement of T- and B-lymphocytes. That was evidenced by the increase of IL-1beta. The decrease of IL-2 and IL-6 proved immunosuppressive effect.

In day 1 high values of TNF-alpha and IL-6, in the presence of decreased IL-4, may evidence the activation of coagulation hemostasis with the development of blood clots. The increase of IL-6 also evidenced development of hypoxia, which may take place due to necrosis of cardiomyocytes.

The increase of IL-1beta and TNF-alpha in day 3 could be caused by more severe hypoxia and ischemia, products of fibrin degradation. The increase of IL-10 proved its protective influence. in day 7, compared to the previous period, IL-6 normalization may evidence the worsening of hypoxia. TNF-alpha increase and decrease of H-10 only confirmed this assertion. That was observed in 14 days also.

In 21 and 28 days the same changes as in a 24 hour period were present: high level of TNF-alpha and IL-6 in the presence of decreased IL-4. It may prove the secondary activation of coagulation hemostasis by means of alteration.

In the infarcted myocardium, necrotic cardiomyocytes release danger signals activating an intense inflammatory reaction that serves to clear the wound from dead cells and matrix debris, but may also extend injury. Dead cardiomyocytes release IL-1α that may function as a crucial alarm in triggering the post-infarction inflammatory reaction. IL-1β is markedly up-regulated in the infarcted myocardium; activation of inflammasome in both cardiomyocytes and interstitial cells results in release of bioactive IL-ip in the infarcted area. Binding of IL-1 to the type 1 receptor triggers an inflammatory cascade, inducing recruitment of proinflammatory leukocytes and stimulating a matrix-degrading program in fibroblasts, while delaying myofibroblast conversion. IL-1 mediates dilative remodeling following infarction and may influence on the pathogenesis of postinfarction heart failure [7].

Injection of quercetin in the experiments increased contents of IL-6, suppressing the formation of IL-2, IL-4 and IL-10. Adrenaline and calcium lesion of heart in which quercetin was used had a favorable course. Thus, in 7 days, the decreased activation of TNF-alpha was observed, and increased – of IL-4, IL-6, and IL-1 beta. In 14 days, the increased activation of TNF-alpha, normalization of H-10 to the control level was evidenced. The increased content of IL-6, decreased – of IL-1 beta, IL-2, IL-4 were present. In 21 days, the increased content of TNF-alpha, IL-6 was evidenced. IL-10 level did not differ from the control, IL-2 content was decreased. In 28 days, the increased activation of IL-1beta, decreased content of TNF-alpha was observed.

IL-10 improved myocardial function after acute global I/R and suppressed inflammation of STAT3 pathway [8]. Also the profibrotic effect of IL-10 autocrine loop promoted macrophages to secret osteopontin and TGFp. These mediators activated cardiac fibroblasts to produce collagen that caused cardiac fibrosis and increased cardiac stiffness [9]. Increased IL-6 content was associated with a cardiovascular disease and protected cells against apoptosis whilst being important for normal metabolism [10].

The obtained data has proved the inclusion of pro- and anti-inflammatory mechanisms of protection against damage, when used with the protective effect of quercetin. Quercetin in cases of more severe fibrosis with underlying injury of ACM of rats prevents cardiac necrosis.

Conclusions

The development of adrenaline-calcium heart damage is accompanied by bright cytokine wavelike reaction at different time points. In early time a direct damage of heart muscle by adrenaline and calcium is present. Then can be development of DIC syndrome, immune response, hypoxia, and ischemia. At day 3 increases the protective influence – anti-inflammatory interleukins level. Then evidence the worsening of hypoxia, secondary activation of coagulation hemostasis by means of alteration. Quercetin causes normalization of interleukins level.

ЗМІНИ ПРОЗАПАЛЬНИХ ТА ПРОТИЗАПАЛЬНИХ ЦИТОКИНІВ НА МОДЕЛІ АДРЕНАЛІНОВО-КАЛЬЦІЄВОГО УРАЖЕННЯ МІОКАРДА У ЩУРІВ ТА КОРЕКЦІЇ КВЕРЦЕТИНОМ

О. В. Денефіль, А. М. Мусієнко

ТЕРНОПІЛЬСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Серцева недостатність є однією з основних причин захворюваності та смертності у світі.*

Метою дослідження *було визначення коливань інтерлейкінів на моделі адреналіново-кальцієвого ураження міокарда та його корекції кверцетином.*

Методи. *Досліди проведені на 143 щурах-самцях лінії Вістар віком 5-6 місяців. Було досліджено зміни вмісту інтерлейкінів IL-1beta, IL-2, IL-4, IL-6, IL-10, TNF-альфа у сироватці крові при розвитку ураження серця та застосування кверцетину. Дослідження проводили у 1-у, 2-у, 24-у години, на 3-ю, 7-у, 14-у, 21-у та 28-у доби.*

Результати. *Адреналіно-кальцієва модель ураження міокарда характеризувалася підвищенням рівня TNF-альфа; а вміст IL-2 та IL-4 поступово достовірно знижувався. Рівень IL-1бета зростав на 2-у і 3-ю доби після формування ураження серцевого м'яза, у інших періодах часу досліджень (за винятком 1 години і 21-ї доби) ці показники знижувалися. Спостерігалися також хвилеподібні коливання вмісту IL-6 і IL-10.*

Ін'єкції кверцетину протягом 7 днів зумовили достовірне зниження IL-2, IL-4, IL-10 та підвищення вмісту IL-6. Також спостерігалося зниження рівня TNF-альфа, та підвищення вмісту IL-4, IL-6 та IL-1. Через 14 днів все ще спостерігалося підвищення рівня TNF-альфа та нормалізація показників IL-10. На 28-у добу зафіксовано підвищений вміст IL-1beta та зниження рівня TNF-альфа.

Висновки. *Розвиток адреналіно-кальцієвого ураження серця супроводжується яскравою хвилеподібною реакцією цитокінів у різні досліджувані проміжки часу час. Кверцетин викликає нормалізацію рівня інтерлейкінів.*

КЛЮЧОВІ СЛОВА: **адреналіново-кальцієва модель ураження міокарду; щури; кверцетин; інтерлейкіни.**

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CONVENTIONAL MINIMUM IN COPYRIGHT PROTECTION (THE BERNE CONVENTION)

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Background. *Intellectual property rights are present in our everyday lives to a huge extent. Law of intellectual property is generally governed by national law, with general principles set out in international treaties. Copyrights strictly protect only the expression of ideas, not the underlying ideas, procedures, methods of operation, or mathematical concepts themselves. Berne Convention was first signed in 1886 and to this day is one of the most important international treaties concerning copyrights and moral rights.*

Objective. *This paper aims to shortly explain the basic rights and privileges provided to the authors by the Berne Convention in its present version, i.e. Paris Act of July 24, 1971, amended on September 28, 1979.*

Results. *Berne Convention provides a „conventional minimum", meaning that all members must provide at least the rights granted by the Berne Convention to the authors. However, each member can grant more rights to the authors. In article 7 Berne Convention regulates the term of protection of copyrights, which is the life of the author and fifty years after her death. Moral rights, provided in Article 6bis, were added in 1928 and grant the author a right to claim authorship of the work and the right of respect. Article 10 of the Berne Convention provides "certain free uses of works".*

Conclusions. *The freedoms granted include possibilities of making quotations and of using the work of someone else to illustrate for teaching purposes. However, in both cases, an indication of the source of the work is required.*

KEY WORDS: **copyrights; moral rights; the Berne Convention; fair use.**

Introduction

Intellectual property rights are present in our everyday lives to a great extent. Law of intellectual property is generally governed by national law, with general principles set out in international treaties, and by international and regional organizations such as World Trade Organization, World Intellectual Property Organization (WIPO), or European Union. The basic rights of authors are set out in the *Berne Convention for the Protection of Literary and Artistic Works* (*the Berne Convention*), which will be the baseline regarding the rights of an author, the protected works, as well as the general understanding of copyrights and moral rights of authors.

One of the most important issues regarding copyrights is that they strictly protect only the expression of ideas, not the underlying ideas, procedures, methods of operation, or mathematical concepts themselves [1]. This basically means that when one describes a method used in her experiment, only the published description is copyrighted, not the method used to obtain the results (the method could be patented, if it fulfills the patent requirements). Similarly, only the way in which the results are presented in a paper are protected by copyrights. Second important issue concerning copyrights is the fact that generally an author does not have to apply to be granted copyrights and moral rights (according to the *Berne Convention*, as well as in the majority of jurisdictions). Nevertheless, most countries provide a registration system in one form or another. Such systems are usually voluntary and help with licensing and transferring copyrights and in solving various disputes [2].

The Berne Convention was first signed in 1886 and to this day is one of the most important international treaties concerning copyrights and moral rights. Not only was the *Berne Convention* the first international treaty relating to copyrights, but as of April 13, 2017, it has 173 members and hence applies almost all around the world. None of the international treaties provide a formal definition of copyrights, but generally such rights can be said to be 'a set of exclusive rights granted by a sovereign state to

1 Copyright. Official website of WIPO, accessed on 24th October 2017: http://www.wipo.int/copyright/en/ .

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2 Ibidem.

an author of an original work, for a limited period of time, and within a limited area'. *The Berne Convention* leaves the issue of fixing the work into a tangible medium up to the national legislation of member countries [3].

This paper will shortly explain the basic rights and privileges provided to the authors by the *Berne Convention* in its present version, i.e. Paris Act of July 24, 1971, amended on September 28, 1979.

Author's Exclusive Rights – Conventional Minimum

As stated above, intellectual property rights are granted at national level, rather than at the international one. Therefore, the *Berne Convention* provides only a so called 'conventional minimum'. All the members must provide at least the rights granted by the *Berne Convention* to authors. However, each member can grant more rights to the authors who seek to protect their works within that member's territory. From the rights granted to the authors by the conventional minimum, several are of greater importance with relation to publishing research and scholar papers. Provided that the author is a citizen of a member to the *Berne Convention*, they must be granted as a minimum the following copyrights: right of translation [4]; right of reproduction [5]; rights of adaptation, arrangement and alteration [6]; as well as moral rights [7]. There are several other copyrights set out in the *Berne Convention* that relate to public recitation of literary works, concerning dramatic and musical works, as well as broadcasting [8].

The first of analyzed copyrights, the right of translation, gives the author an exclusive right to translate their original work or to give permission for someone else to translate it. The right of reproduction allows the author to grant the right to copy, reproduce, and distribute such copies of their work. Moreover, the scope of this right granted by the *Berne Convention* is very broad as it contains not only currently available means of reproduction and distribution but also those not yet invented [9]. Finally, the right of adaptation gives the author an exclusive right to change the form of their work, i.e. make a derivative work, which can be both an infringement of the primary work (if done by someone other than the author and without their consent) and an original work itself [10].

In article 7 of the *Berne Convention* the term of protection of copyrights is regulated. Generally, copyrights are protected during the life of the author and fifty years after their death. As stated above, the members of the *Berne Convention* may provide more protection to the authors than is guaranteed in the convention but cannot breach the conventional minimum. Hence, in many member countries the term of copyright protection is longer than the conventional fifty years, i.e. in Poland, Ukraine, USA and many other countries the copyright term was elongated to the life of the author and seventy years after their death [11]. In cases of joint authorship, the copyright is protected during the lives of all authors and fifty years after their deaths. Here however, the fifty years starts to run after the death of the last surviving co-author [12].

Copyrights and Moral Rights

In common law, copyrights tend to cover only the economic rights of the author. On the other hand, in continental law the term 'copyright' includes both economic and moral rights alike. Hence, the need to unify the protection of the authors and their rights was urgent. This was achieved by including the moral rights of authors into the conventional minimum of the *Berne Convention*. Moral rights, provided in Article 6bis, were added in 1928 and grant the author (i) a right to claim authorship of the work and (ii) the right of respect. Moral rights are granted to the author "independently of the author's economic rights" and exist even after the author transfers their economic rights [13]. Moreover, moral rights are maintained at least until the expiration of author's economic rights. They can also be exercised after the death of the author by "persons or institutions authorized by the legislation of the country where protection is claimed" [14].

The first of the moral rights granted by the *Berne Convention*, the right to claim authorship, can be exercised in many various ways. Firstly,

3 Berne Convention for the Protection of Literary and Artistic Works, Article 2, paragraph 2, official website of WIPO, accessed on 24th October 2017: http://www.wipo.int/treaties/en/text.jsp?file_id=283698 .
4 Ibidem Article 8.
5 Ibidem, Article 9.
6 Ibidem, Article 12.
7 Ibidem, Article 6bis.
8 Ibidem, Articles: 11, 11bis, 11ter, 14, 14bis, 14ter
9 Guide to the Berne Convention for the protection of literary and artistic works (Paris Act, 1971), WIPO, Geneva, 1978, p. 54.
10 Ibidem, p. 76.
11 For more information on copyright term in various countries visit: https://en.wikipedia.org/wiki/List_of_countries%27_copyright_lengths (accessed on 25th October 2017)
12 Berne Convention, Article 7bis.
13 Guide to the Berne Convention, p. 42.
14 Berne Convention..., article 6bis, paragraph 2.

the author may place their name on their work and its copies, hence claiming the authorship of the work. Secondly, the author may publish their work under a pseudonym or even anonymously. Moreover, the author can change their mind and change their pseudonym or quit their secrecy. Finally, the author can use their right in a negative way by refusing their name to be put on a work that is not theirs. The right to claim authorship is a significant issue when considering fair use and publishing the work.

The second moral right is the right of respect or, alternatively, the right to integrity of the work. According to Article 6*bis* of the *Berne Convention*, the author can "object to any distortion, mutilation or other modification of, or other derogatory action in relation to, the said work, which would be prejudicial to their honor or reputation" [15]. This right prevents anyone from changing the work in any way without the consent of the author, even if the author transferred their economic rights. Therefore, a publisher or an editor may not delete any part from the work of the author, unless they have the author's informed and explicit consent. Even though a work is usually proofread before publication, the changes should be sent to the author for approval. Depending on the jurisdiction in which copyright protection is sought, the courts may allow some changes to be made when the work is being adapted into a different medium [16].

One of the most important differences between the economic and moral rights of the author is the issue of transferability. Economic rights are freely transferable, while in many countries moral rights are inalienable – they stay with the author, even after licensing or transferring copyrights, and after the death of the author. This means that the author can license or sell their rights of translation, reproduction, or alteration (among others) to someone else, but they may not be able to do such a thing with their moral rights [17]. With respect to their moral rights, the author can agree not to exercise them. In many jurisdictions such a waiver must be in writing to be binding.

Certain Free Uses of Works of Others

On one hand, as authors we would like to be protected for our works, on the other – as scholars we are aware of the necessity of accessing the works of others as well as for ours to be accessed by other researchers and authors. Hence, in Article 10 of the *Berne Convention* provides 'certain free uses of works'. The freedoms granted include possibilities of making quotations and of using the work of someone elseto illustrate for teaching purposes. However, in both cases indication of the source of the work is absolutely required.

The Berne Convention permits quotations of someone else's work under certain limitations. Firstly, the work must have been made lawfully available to the general public, i.e. published by the author, with the author's consent, or by means of compulsory license before it can be quoted [18]. Secondly, the quotation must be "compatible with fair practice" [19]. As this concept is not defined in the *Berne Convention*, what is or is not fair is left for the national courts to decide. Generally, the courts will consider, among other factors, the proportion between the size of the quote and the work in which it is used [20]. Thirdly, the purpose of the quotation is also analyzed. *The Berne Convention* does not specify the purposes for which the quote may be used, stating only that the extent of the quote should "not exceed that justified by the purpose" [21]. Most courts assume that the purpose of the quote should be educational, scientific, analytical, or parodist. When utilizing this 'free use', the author must remember that their own work cannot be overwhelmed by the quotes. In practice it means that quotes should be put into an original work to support the ideas of the author. The resulting original work of the author cannot be a compilation of quotes, with few commentaries in between them. Rather, it should be an original work of the publishing author supported by quotes of more established researchers.

"Use of works by way of illustration for teaching" is present in copyright laws of most countries. Plainly put, the teacher may reproduce a work of someone else during her class to compare, contrast, and analyze this work. Nevertheless, the teacher must still abide by the limitations provided for quotations, i.e. the extent of the reproduction during the class must be "justified by the purpose" and the use must be "compatible with fair practice" [22]. It is worth mentioning, that the word 'teaching' includes all levels of education, i.e. "educational

15 Ibidem, article 6bis, paragraph 1.
16 Cotter, Thomas F., Pragmatism, economics, and the droit moral, 76 N.C. L. Rev. 1, 1997, https://cyber.harvard.edu/metaschool/fisher/integrity/Links/Articles/cotter.html (accessed on 26th October 2017).
17 Guide to the Berne Convention..., p. 42.
18 Ibidem, p.58.
19 Berne Convention..., article 10, paragraph 1.
20 Guide to the Berne Convention..., p.59.
21 Berne Convention..., article 10, paragraph 1.
22 Berne Convention..., article 10, paragraph 2.

institutes, municipal and state schools and private schools" [23]. Generally speaking, a professor may reproduce the work of someone else during their lecture, for example by showing a reproduction of a picture or a painting, or a fragment of a movie. Moreover, the teacher may make copies of such works (photocopies of reproductions of pictures, articles, or a page from a textbook) to be analyzed during the class, provided it is 'justified by the purpose'. Note, that you absolutely cannot photocopy and distribute more of someone else's work than is necessary to teach the class. The professor must clearly indicate the author and the source of each and every work used by the way of illustration for teaching, be it a graph, table, photo, or a sentence from someone else's work. Note, that even if the professor modifies another's work, they still must provide the source of the original work and indicate the implemented changes.

From the practical point of view, right to quote is very close in nature to the 'use of works by way of illustration for teaching' – both allow us to use the work of someone else to some extent. However, the most important purpose of copyrights is to protect economic interests of the author. Hence, in a commercial lecture we use the right to quote with the purpose being scientific or analytical rather than educational. As indicated above, with that come certain restrictions which are stricter than in case of the second fair use – in most cases the author may quote someone else's work in their commercial lecture (presentation), but most likely will be prohibited from distributing photocopies of the part of the quoted work used in the lecture.

No matter under which paragraph of Article 10 one uses someone else's work, the source and the name of the author must absolutely be mentioned [24]. Hence, article 10 paragraph 3 of the *Berne Convention* restates one of the moral rights of authors. The requirement to indicate the name of the author (when possible) supports the notion of the right to claim authorship. One should also note that the indication of the source may infringe the cited author's second moral right. Misrepresentation of the cited author's ideas or putting their words out of context can breach their right to integrity of their work.

Conclusions

As stated in the beginning of this article, the protection of copyrights, both economic and moral rights of the authors, is within the national law. Therefore, the protections afforded to authors in public international law should be considered a set of minimum rights and privileges. *The Berne Convention* does not provide for an enforcement body or a court to solve the disputes that arise under copyright laws. On the contrary, in many Articles the convention leaves the means of redress for copyright infringement to national legislation and courts of the country where protection is sought.

For scholars and researchers there are some practical points that should be kept in mind. Firstly, always properly indicate the source of your quote or even paraphrase, customarily including (at least) the name of the author, the title of their work, the publisher or the title of the journal, and the year of publication. There are several styles of citation – which one to use generally depends on the publisher or the university. One important tip regarding the style of citation: it should be uniform across the paper. Secondly, no matter the situation, always, always read what you agree to. When scholars submit their papers for publishing, there usually are *terms of use*, *privacy policy*, *disclaimers*, *waivers* or alike to sign or mark with annotation "I have read and agree to the following (...)". In fine print there can be hidden various waivers of copyrights, for example stating that the author licenses (or transfers) their copyrights to the publisher, or that they will not submit the paper to be published in another journal while the publication is pending in the current one or simply ever. Moreover, there can also be statements indicating that the author will not enforce their moral rights regarding this paper. As mentioned above, in many jurisdictions moral rights are inalienable – no one can demand that the author transfers or disclaims their moral rights. In such a case, only the enforcement of moral rights (in a court of law) can be disclaimed when the moral rights are infringed. Infringement can happen for example by not putting the name of the author on the work or changing the work in any way without the author's consent.

To summarize, whatever waivers or statements might be included in *terms of use* or publishing contract be sure to read it carefully before agreeing or signing. To be honest, it is a good idea to read a document thoroughly before signing it, whatever the circumstances.

23 Guide to the Berne Convention..., p. 60.
24 Berne Convention..., article 10, paragraph 3.

КОНВЕНЦІЙНИЙ МІНІМУМ ЗАХИСТУ АВТОРСЬКОГО ПРАВА (КОНВЕНЦІЯ БЕРНЕ)

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Вступ. *В нашому повсякденному житті, значною мірою, використовуються права інтелектуальної власності. Як правило, закон інтелектуальної власності регулюється національним законодавством, а загальні принципи викладені в міжнародних договорах. Авторські права суворо захищають лише вираження ідей, а не основні ідеї, процедури, методи роботи або самі математичні поняття. Бернська конвенція була вперше підписана в 1886 році і до цього дня є одним з найважливіших міжнародних договорів про авторські та моральні права.*

Мета дослідження *полягає у короткому поясненні основних прав і привілеїв, наданих авторам Бернською конвенцією у її нинішній редакції, Паризький акт від 24 липня 1971 року, змінений 28 вересня 1979 року.*

Результати. *Бернська конвенція передбачає «гарантований мінімум», що означає, що авторам повинні гарантуватися щонайменше права, надані Бернською конвенцією. Однак, їм також може надаватися більше прав. У статті 7 Бернської конвенції регулюється термін захисту авторських прав, який є життям автора і п'ятдесят років після його смерті. Моральні права, передбачені статтею 6bis, були додані в 1928 році, і надають авторові право вимагати авторські права на власний твір/наукову роботу та право на повагу. Стаття 10 Бернської конвенції передбачає "безсумнівне вільне використання творів".*

Висновки. *Надані свободи включають в себе можливості цитування і використання наукових доробків інших авторів в навчальних цілях. Проте в обох випадках обов'язково повинно вказуватися першоджерело.*

КЛЮЧОВІ СЛОВА: **авторські права; моральні права; Бернська конвенція; використання прав.**

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2. Berne Convention for the Protection of Literary and Artistic Works. September 9, 1886, as amended on September 28, 1979. Official website of WIPO. 2017: http://www.wipo.int/treaties/en/text.jsp?file_id=283698.

3. Guide to the Berne Convention for the protection of literary and artistic works (Paris Act, 1971), WIPO. Geneva. 1978.

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