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ESTIMATION OF INFLUENCE OF THE THERAPEUTIC – PROPHYLACTIC COMPLEX ON THE IMMUNE SYSTEM INDICES OF EXPERIMENTAL ANIMALS WITH SIMULATED GINGIVITIS

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The territory of Lviv region is significantly different from other territories of Ukraine in terms of its natural and ecological characteristics. This is the only biogeochemical terrain within which separate biogeochemical territories are distinguished according to their differences in natural geochemical conditions [1; 2]. Multicomponent conditions of the geological environment, combin ed with anthropogenic load, create a different degree of environmental tension and affect the health of children, including dental morbidity [3-5].

The ecological situation in Lviv region is determined by the activities of oil-extracting, mining, chemical, paper and pulp industries. According to the data [1;6] on the total level of environmental pollution, the territory of Lviv region is regarded as a contaminated one. Pollution of soils with mineral fertilizers is high, with pesticides are dangerous, and pollution of atmospheric air with carbon monoxide, nitrogen dioxide, organic compounds, and metals is increased. In addition to the unfavorable ecological situation, the climate and weather conditions for the population in Lviv region are regarded as moderately uncomfortable.

Children, due to the age immaturity of their protective and adaptive mechanisms, are particularly sensitive to negative environmental factors, that's why the health of the younger generation can be considered as the main indicator of the state of the environment [7].

A large number of studies have shown that children living in unfavorable environment conditions have a high level of major dental diseases. Of particular concern is a high prevalence of periodontal diseases, as well as of caries in children. [8;9].

Therefore, the study of the complex impact of adverse environmental conditions, as well as natural deficiency of iodine and fluoride, on the occurrence of periodontal diseases in children and the development of measures to prevent them, makes the study relevant.

The objective of our work is to study the efficacy of the offered therapeutic-prophylactic complex (TPC) in rats with simulated gingivitis under the influence of individual xenobiotics, as well as iodine and fluoride deficiency by means of the evaluation of immunological parameters of their blood.

Methods

For experimental studies, we used white rats, all groups of which were kept under the same conditions. The experimental gingivitis in rats was modeled by the transfer of animals aged 30±5 days on the peroxide model of gingivitis, by adding to the normal ration of peroxygen sunflower oil at a dose of 1 ml per animal during 3 weeks [10]. The experiment on 100 rats of herd breeding, with an average weight of 54±5 g, females and males equally, was performed. Depending on the modeling of anthropogenic environmental conditions, the animals were divided into 5 groups of 20 animals in each: 1 (control) − intact rats kept on normal vivarium diet; 2 − rats, with simulated gingivitis; 3 − rats on peroxidation model of gingivitis, with adding to the water of heavy metals based on their molecular weight (CdCl$_2$=0.010 mg/l; Pb(NO$_3$)$_2$=0.36 mg/l) [10;11]; 4 − rats on peroxidation model of gingivitis + heavy metals + iodine and fluoride deficiency. The iodine deficiency in rats was induced by adding to the water mercazole, based on 50 mg/kg of weight per day during 3 weeks [11]; 5 − rats with simulated gingivitis + heavy metals + iodine and fluoride deficiency + TPC ("Kinder Biovital Lecithin" gel – 40 mg / kg," Laminaria" – 200 mg / kg," Ascorutin" – 0.25 mg / kg," Apple Pectin" – 200 mg / kg).

The diet of vivarium consisted of the following components (ration for 1 day): grain of barley - 9 g, wheat grain - 8 g, wheat bread - 2 g, oat flakes and meat-and-bone meal - 0.5 g each, powdered milk – 0.7 g, wheat flour – 1.0 g, beet and carrots – 1.8 g each, cabbage – 0.4 g, sunflower oil – 0.04 g, salt – 0.1 g, egg shell – 0.015 g.

The inorganic toxicants (Cd, Pb) selected for the experiment corresponded to their saturation of the environment and were added to water taking into account their molecular weight (CdCl$_2$ = 0.010 mg/l; Pb (NO$_3$)$_2$ = 0.36 mg/l).

The iodine deficiency in the rat's body was caused by the addition of merkazole to water, at the dose of 50 mg/kg of body weight. Fluorine deficiency in the body of animals was caused by keeping them on a low-calorie diet, with the exception of products containing a lot of fluoride. The low calorie-diet included: wheat bread - 2 g, oat flakes – 0.5 g, chalk – 0.015 g, sunflower oil – 0.04 g.

From the moment of transferring rats to the experimental model of gingivitis they were kept on the low-calorie diet, toxins and mercazole were added to their water and they were given the corrective treatment-prophylactic complex (TPC).

The drugs were administered to animals daily, in the morning in the form of aqueous suspension ("Apple Pectin", "Ascorutin"); "Laminaria" and "Kinder Biovital Lecithin, Gel" were added to the low-calorie diet.

Withdrawal from the experiment and collection of blood were performed under general anesthesia after 21 days from the beginning of the experiment. Determination of immunoglobulins G, A, and M in blood serum was carried out by the method of radial immunodiffusion in agar according to G. Manchini et al. using diagnostic kits of the firm "Microgene" [12]. The count of leukocytes in the peripheral blood of animals was determined by means of the Goryaev's chamber. The studies were carried out with observance of the general rules and provisions of the European convention for the protection of vertebrate animals used for research and other scientific purposes (Strasbourg, 1986) and "General ethical principles of animal experimentation" (Kyiv, 2001). The obtained results were worked out statistically.

Results and their discussion

As a result of our research we found that in intact animals of group 1 the lowest level of leukocytes in the

peripheral blood – 7.40 ± 0.29 • 10 ^{9} / l was determined. Under the conditions of modeling of gingivitis, in animals of group 2, the level of leukocytes in the peripheral blood had increased and was 13.24 ± 0.28 • 10^9 / l (p<0.01). In rats of group 3, where the simulated gingivitis was combined with the influence of heavy metals, the concentration of leukocytes in peripheral blood had reached the level of 18.46 ± 1.18 • 10^9 / l, which was significantly higher than those in groups 1 and 2 (p < 0.01, P$_1$ <0.01).

The highest level of leukocytes in the peripheral blood was obtained in group 4, in which the rats with simulated gingivitis were also exposed to heavy metals, under fluoride and iodine deficiency, (24.79 ± 1.26 10 ^{9} / l, p <0,01, p$_1$ <0.01).

It is noteworthy, that in group 5, in which rats with simulated gingivitis were exposed to heavy metals, under iodine and fluoride deficiency with simultaneous application of the therapeutic-prophylactic complex, a significant decrease of leukocytes level in peripheral blood (9, 18 ± 0.28 pg / ml) was determined. The obtained result was 1.4 times lower than that in group 2 of rats with simulated gingivitis (p$_1$ <0.01), but remained 1.2 times higher than that of intact animals in group 1 (p <0.01). At the same time, the level of leukocytes in rats of group 5 was 2.0 times higher than that in rats of group 3 (p$_2$ <0.01) and 2.7 times higher than that in group 4 (p$_3$ <0.01) .

It was found that in the group of intact rats the level of IgG in blood serum was 4.12±0.06 g / l (Table 1). In animals with simulated gingivitis (group 2) IgG level has decreased to 3.15±0.29 g / l, which was significantly lower than that in group 1 (p<0.01). In group 3 a further decrease of IgG level up to 2.28±0.31 g / l was registered, which was significantly lower than that in group 1 (p<0.01) and in group 2 (P$_1$> 0.05). The lowest level of IgG in blood serum was found in group 4 – 1.58±0.21 g/l,

which was significantly lower in comparison with IgG levels in group 1 and 2 (respectively p <0.01 and p$_1$<0.05). Due to application of the TPC the level of IgG in blood serum of rats in 5 group (2.93±0.19 g / l) was a bit higher, than in groups 3 and 4, although it was by 28.88% lower than that in group 1 (p <0.01), and by 6.98% lower than that in 2 group (p$_1$ <0.01).

After application of the TPC to the rats OF group 5, IgG concentration in their blood serum was 28.50% higher than that of the animals in group 3 (p$_2$ <0.05) and 85.44% higher than that of the animals in group 4 (p$_3$<0.01).

It was found that in the presence of simulated gingivitis and the impact of environmental adverse factors levels of IgA in blood serum of rats had been decreasing. In intact animals of group 1 the IgA level was 1.16±0.01 g / l, while in rats with simulated gingivitis (group 2) it haS decreased to 0.87±0.03 g / l (p <0.01). Under the influence of heavy metals and in the presence of simulated gingivitis (group 3) IgA concentration in serum was 0.74±0.04 g / l, which was less than that in group 1 (p <0.01) and group 2 (p$_1$> 0.05). The lowest IgA level (0.46±0.03 g / l) was found in group 4, where in the presence of simulated gingivitis the experimental animals were exposed to heavy metals, as well as to iodine and fluoride deficiency (p <0.01, p$_1$ <0.05).

We have determined that after application of the TPC the concentration of IgA in the 5 experimental group has increased to 1.08±0.03 g / l, which was 24.13% higher than that of animals in group 2 (p$_1$ <0.01), but by 6.9% lower than that in intact animals (group 1) (p <0.05). It should be noted that the concentration of IgA in blood serum of animals in group 5 was by 45.94% higher compared with the data in group 3 (p$_2$ <0.01) and by 134.78% higher than that in group 4 (p$_3$<0.01).

Table 1
Immune system indices in blood serum of experimental animals

Experimental groups	Leukocytes, 10^9/l	IgG, g / l	IgA, g / l	IgM, g / l
intact rats (1 group)	7,40±0,29	4.12±0.06	1,16±0,01	3,23±0,02
model of gingivitis (2 group)	13,24±0,28*	3.15±0.29**	0,87±0,03**	2,61±0,17**
model of gingivitis + heavy metals (3 group)	18,46±1,18*,°	2.28±0.31**,°	0,74±0,04**,°	2,18±0,19**,°
model of gingivitis + heavy metals + deficit of iodine + deficit of fluoride (4 group)	24,79±1,26*,°	1.58±0.21**,°	0,46±0,03**,°	1,28±0,18**,°°
model of gingivitis + heavy metals + deficit of iodine + deficit of fluoride + TPC (5 group)	9,18±0,28*,°,•,▫	2.93±0.19**,°°,•,▫▫	1,08±0,03*,°°,••,▫▫	3,17±0,19*,°,••,▫▫

Notes. Probability of error in comparison with: * p <0.05,
**p <0.01; ° p$_1$ <0.05, °° p$_1$ <0.01; • p$_2$< 0.05, •• p$_2$ < 0.01; ▫ p$_3$ < 0.05, ▫▫ p$_3$ < 0.01.

Changes in IgM concentration in blood serum of experimental animals were characterized by a similar trend. The reduction of IgM level from 3.23±0.12 g / l in intact animals to 2.61±0.17 g / l in rats with simulated gingivitis was statistically significant (p <0.01). The lowest levels of IgM were determined in 3 and 4 experimental groups in which the IgM concentration in the blood serum of animals were 2.18±0.19 g / l (p <0.01, p$_1$> 0.05) and 1.28±0.18 g / l (p <0.01, p$_1$ <0.01).

Due to application of the proposed TPC in the 5 experimental group of animals the IgM level in blood serum has increased to 3.17±0.9 g / l, which exceeded the data in animals with simulated gingivitis (group 2) by 21.43% (p$_1$<0.05), but was by 2,47% lower in relation to the data of intact animals (group 1) (p< 0.05). The concentration of IgM in serum of experimental rats in group 5 was by 45.41% higher than in group 3 (p$_2$ <0.01) and by 147.65% higher than in group 4 (p$_3$ <0.01).

Conclusions. The provided animal experiment has

shown that the use of ecotoxicants in combination with iodine and fluoride deficiency was accompanied by a significant decrease of concentration in blood serum of IgA, IgM by 2.5 times and IgG by 2.6 times, as well as by increase in the number of leukocytes by 3.4 times. The use of the therapeutic-prophylactic measures in experimental animals improved their metabolic parameters, which was accompanied by a decrease in blood serum of the level of leukocytes by 44.23%, as well as an increase of concentration of IgG by 7.30%, IgA by 19.45%, IgM by 17.67%.

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ВПЛИВ ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНОГО КОМПЛЕКСУ НА ІМУНОЛОГІЧНІ ПОКАЗНИКИ ЩУРІВ НА ТЛІ ЕКСПЕРИМЕНТАЛЬНОГО ГІНГІВІТУ

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Резюме

Для оцінки впливу важких металів, йод-, фтордефіциту на стан тканин пародонта щурів проведено експериментальні дослідження на 100 білих тваринах, які були поділені на 4 групи по 20 тварин у кожній. До 1 групи ввійшли інтактні тварини; 2 – тварини з гінгівітом; 3 – тварини, що зазнавали впливу важких металів з додаванням до води кадмію та свинцю на тлі моделі гінгівіту; 4 – тварини з гінгівітом на фоні впливу важких металів, йоддефіциту, фтордефіциту; 5 – тварини з гінгівітом, які отримували важкі метали, фтор-, йоддефіцит та лікувально-профілактичний комплекс. Аналіз імунологічного статусу тварин оцінювали за вмістом імуноглобулінів у сироватці крові. Вміст імуноглобулінів G, A, M визначали методом радіальної імунодифузії в агарі за G. Manchini et. al. з використанням діагностикумів фірми НПО «Мікроген». Вміст лейкоцитів у периферійній крові тварин визначали шляхом підрахунку у камері Горяєва.

Встановлено, що застосування важких металів у комбінації з йод-, фтордефіцитом супроводжується зниженням у сироватці крові імуноглобулінів А, М у 2,5 рази, а G у 2,6 рази на тлі підвищення кількості лейкоцитів у 3,4 рази. Застосування лікувально – профілактичних заходів в експериментальних тварин покращило метаболічні показники, що супроводжувалось зниженням вмісту лейкоцитів на 44,23% на тлі підвищення Іg G на 7,30%, Іg A на 19,45%, Іg M на 17,67%.

Ключові слова: гінгівіт; щури; сироватка крові; токсичні фактори.

Резюме

Для оценки влияния тяжелых металлов, йод, фтордефицита на состояние тканей пародонта крыс проведены экспериментальные исследования на 100 белых животных, которые были разделены на 4 группы по 20 животных в каждой. К 1 группе вошли интактные животные; 2 - животные с гингивитом; 3 - животные, подвергавшиеся воздействию тяжелых металлов с добавлением к воде кадмия и свинца на фоне модели гингивита; 4 - животные с гингивитом на фоне влияния тяжелых металлов, йоддефицита, фтордефицита; 5 - животные с гингивитом, получавшие тяжелые металлы, фтор, йоддефицита и лечебно-профилактический комплекс. Анализ иммунологического статуса животных оценивали по содержанию иммуноглобулинов в сыворотке крови. Содержание иммуноглобулинов G, A, M определяли методом радиальной иммунодиффузии в агаре по G. Manchini et. al. с использованием диагностикумов фирмы НПО «Микроген». Содержание лейкоцитов в периферической крови животных определяли путем подсчета в камере Горяева.

Установлено, что применение тяжелых металлов в сочетании с йод, фтордефицитом сопровождается снижением в сыворотке крови иммуноглобулинов А, М в 2,5 раза, а G в 2,6 раза на фоне повышения количества лейкоцитов в 3,4 раза. Применение лечебно - профилактических мероприятий у экспериментальных животных улучшило метаболические показатели, сопровождалось снижением содержания лейкоцитов на 44,23% на фоне повышения Іg G на 7,30%, Іg A на 19,45%, Іg M на 17,67%.

Ключевые слова: гингивит; крысы; сыворотка крови; токсические факторы.

Summary

In order to assess the influence of heavy metals, iodine and fluoride deficiency on the status of periodontal tissues in rats experimental studies were carried out on 100 white rats, which were divided into 5 groups of 20 animals in each. 1-st group included intact rats; 2-nd - rats with gingivitis; 3-rd - rats with gingivitis exposed to heavy metals by means of adding cadmium and lead to drinking water; 4-th - animals with gingivitis exposed to heavy metals, as well as iodine and fluoride deficiency; 5-th - animals with gingivitis exposed to heavy metals, under iodine and fluoride deficiency, receiving therapeutic-prophylactic complex. Immunological status of animals was evaluated by means of measuring the level of

immunoglobulins in serum. Immunoglobulins (Ig) G, A, and M were determined by their radial immunodiffusion in agar according to G. Manchini et al. using diagnostic kits of the firm "Microgene". The count of leukocytes in the peripheral blood of animals was determined by means of the Goryaev's chamber.

It was found that under influence of heavy metals in combination with iodine and fluoride deficiency the levels of immunoglobulins A and M in blood serum of rats were decreased by 2.5 times, and Ig G – by 2.6 times. The application of the therapeutic-prophylactic complex in 5-th group of rats significantly corrected immunological status of their blood, which was characterized by a significant increase in levels of immunoglobulins.

Key words: gingivitis; rats; blood serum; toxic factors.