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EFFECT OF THE NEW DENTAL ELIXIR ON THE CONDITION OF RATS' PERIODONTAL TISSUES WITH SIMULATED METABOLIC SYNDROME

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Biochemical changes in the blood serum, liver and gingival tissue were examined during simulation of the metabolic syndrome of alimentary genesis in experiment on 28 white male rats of reproductive age with an average body weight (280 ± 14) g. It was found that keeping animals for ten weeks on a diet high in saturated fats and carbohydrates led to increase in body weight and in level of biochemical markers: insulin resistance, hyperglycemia, inflammation, both at the level of the body and locally in the periodontal tissues. Local application of the new dental elixir in the form of applications to rats gums against the background of metabolic syndrome reduced the level of triglycerides, had an anti-inflammatory and antioxidant effect, reducing contamination by pathogenic microflora (urease activity), lipid peroxidation activity (malonic dialdehyde content), activating locally in the periodontal tissues the antioxidant defense enzyme and nonspecific immunity – lysozyme.

Key words: metabolic syndrome of gingival tissue, inflammation, treatment, dental elixir.

Л.С. Кравченко, О.Л. Аппельханс, А.Є. Поляков, О.В. Пасечник, С.В. Щербаков ВПЛИВ НОВОГО ЗУБНОГО ЕЛІКСИРУ НА СТАН ТКАНИН ПАРОДОНТУ ЩУРІВ З ВІДТВОРЕНИМ МЕТАБОЛІЧНИМ СИНДРОМОМ

В експерименті на 28 білих щурах-самцях репродуктивного віку, середньою масою тіла 280 ± 14 г охарактеризовані біохімічні зміни у сироватці крові, печінці і ясеневій тканині при відтворенні метаболічного синдрому аліментарного генезу. Було встановлено, що утримання тварин протягом десяти тижнів на раціоні з високим вмістом насичених жирів і вуглеводів призводило до збільшення маси тіла та підвищення вмісту біохімічних маркерів інсулінорезистентності, гіперглікемії, запалення, як на рівні організму, так і локально в тканинах пародонту. Місцеве застосування новоствореного зубного еліксиру у вигляді аплікацій на ясна щурів на тлі метаболічного синдрому знижувало рівень тригліцеридів, надавало протизапальну і антиоксидантну дію, знижуючи обсіменіння патогенною мікрофлорою (активність уреаз), активність перекисного окислення ліпідів (вміст малондеальдегіду), активуючи локально в тканинах пародонту фермент антиоксидантного захисту каталазу і неспецифічного імунітету лізоциму.

Ключові слова: метаболічний синдром, тканини ясни, запалення, лікування, зубний еліксир.

The work is a fragment of the research project "Development of new therapeutic and prophylactic agents and pathogenetic justification of their use in inflammatory periodontal diseases against the background of metabolic syndrome", state registration No. 0120U002197.

The influence of pathogenetic changes, that accompany pathological conditions with metabolic disorders, on the formation of inflammatory diseases in the oral cavity, the peculiarities of their course are currently insufficiently studied. It is known that the development of inflammation on the periodontium tissues, the mucous membrane of the oral cavity is associated with systemic processes of an organism, which is characterized by inflammatory manifestations [1]. One of such condition is the metabolic syndrome (MS), which is characterized by various triggers of the disorders cascade (fat, carbohydrate metabolism, vascular endothelial status) with known components: insulin resistance, visceral obesity, the dyslipidemia syndrome, impaired glucose tolerance [2, 6].

Last decade, a number of data appeared indicating a significant effect of MS on the pathological processes in the oral cavity. In particular, MS is considered to be an underlying condition of inflammatory and dystrophic-inflammatory processes in the the oral cavity tissues. The low efficiency of prevention and treatment of inflammatory diseases of periodontal tissues, oral mucosa (OM) with comorbid conditions is explained by underestimation of MS disorders, which effect their occurrence and clinical course [5, 10, 11]. In this regard, the study of the periodontal tissues condition changes peculiarities that occur during MS development, and the definition of a local method of treatment is of scientific interest.

The purpose of the work was to study the changes in the periodontal tissues of rats with simulation of MS of alimentary genesis and to determine a possibility of their correction with the new dental elixir.

Materials and methods. The experiment was performed on Wistar rats 2–2.5 months of age weighing (280 ± 12) g. All the animals were divided into three groups. Group I – intact group consisted of 8 rats. Animals in this group received a standard vivarium diet and had free access to drinking water. The rats of group II were simulated MS by introducing 20% visceral fat into the diet, and 10% fructose solution instead of drinking water. The duration of MS simulation was 70 days. The introduction of these substances into the diet was explained by a possibility of creating disorders of carbohydrates and lipids metabolism of alimentary nature in animals typical for MS signs [12, 13]. The rats of group III were simulated MS like in the group II, after which the newly created tooth elixir based on bee products [7] was applied topically into the gums daily by 0.5 ml/rat with a tampon, applying at the oral cavity bilaterally from inside of the cheek at the incisors near the transitory fold of the upper alveolar ridge during 5–7 minutes. The treatment lasted 15 days. The rats of groups II and III were observed for 15 days after MS simulation, after that the animals were removed from the experiment under thiopental anesthesia (40 mg/kg) by opening the main vessels, collected blood from which serum was obtained, cutted the gums, isolated the liver after previous chilled saline perfusion. In the blood serum levels of triglycerides (TG) were determined using the enzymatic colorimetric test [3], total cholesterol (Ch), Ch in high-density lipoprotein (HDL), glucose, alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST).

In the blood serum, the liver tissues, gums, the level of lipid peroxidation (LPO) was determined by the content of malonic dialdehyde (MDA) by thiobarbituric method [14], and the state of the antioxidant system by catalase activity. Biochemical markers of inflammation were determined in rats' gingival tissues: elastase activity [4], urease activity [4], MDA content [14], insulin resistance – triglycerides level [4], antioxidant protection – catalase activity, antioxidant-prooxidant index (API) [4], non-specific immunity – lysozyme activity [8].

The research was guided by the national “General Ethical Principles of Animal Experiments” (Ukraine, 2001), which come to an agreement with the provisions of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg, 1986).

Statistical processing of digital data was performed using the software STATISTICA 6.0. The values of the arithmetic mean (M) and the mean error (m) were calculated for all indicators. The significance of the difference in values was determined using Student's t-test. The changes were considered statistically significant at $p < 0.05$.

Results of the study and their discussion. The results of the study showed that a diet high in visceral fat and fructose in drinking water for 70 days led to a significant increase in body weight as compared to the intact rats. When measuring the circumference of the middle part of the body of the experimental rats, it was determined that the animals of group II got thicker by 38.7% (from (12.4 ± 0.3) cm to (17.2 ± 0.5) cm), group III – by 36.4% (from (13.2 ± 0.4) cm to (18.0 ± 0.3) cm), while the intact rats got thicker on average by 16.4% (from (12.2 ± 0.2) cm to (14.2 ± 0.3) cm). The diet provoked obesity in rats: weight gain was 2.3 times greater than in normal animals (table 1).

Table 1

Weight gain of rats with metabolic syndrome development (M±m)

Rat groups	Indices	
	Weight gain, g	
Intact, n=8	36.4 ± 2.2	
Metabolic syndrome, n=8	82.3 ± 3.8	
p	=0.001	

Note: p – is the probability of differences from the intact group.

Analyzing the data, it should be noted that this diet led to carbohydrate and fat metabolism disorders, resulting in biochemical parameters changes in the blood serum, liver tissue and oral mucosa (tables 2, 3).

Table 2

Changes in the biochemical parameters of the blood serum of rats in metabolic syndrome simulation (M±m)

Groups of animals	
Indices	Intact, n=8
Cholesterol, mmol/l p	4.96±0.04
Triglycerides, mmol/l p	1.38±0.02
HDL, mmol/l p	4.26±0.14
Glucose, mmol/l p	2.78±0.05
ALT, □kat/l p	0.30±0.02
AST, □kat/l p	0.42±0.01

Note: p – is the probability of differences from the intact group.

A significantly higher concentration of cholesterol and triglycerides as compared with the intact group was observed in the blood serum of the experimental groups after 10 weeks of the diet. At the same time, cholesterol level in HDL in MS simulation was reduced 3 times ($p=0.001$) (Table 2). MS simulation revealed a 2.4-fold increase in glucose levels ($p=0.001$), increase in transaminases activity of in the blood serum: ALT activity increased 2.1 times ($p=0.001$), ACT activity – 1.9 times ($p=0.001$) relative to data in the intact group, which proved functional disorders in the liver of rats.

Under MS simulation lipid peroxidation processes increased in the blood serum, liver tissues and oral cavity, which was proved by increase in MDA level in the blood serum 2 times, in the liver – 1.8 times, in OM – 1.3 times ($p=0.001$). On the body level there was a slight reduction of the activity of antioxidant protection by a decrease in the activity of the enzyme of antioxidant system-catalase in the blood serum, gingival tissue, liver by an average 7–8% ($p>0.05$) (Table 3).

Table 3

MDA level and the activity of the antioxidant enzyme catalase in the blood serum and tissues of OM, liver in MS simulation in rats (M=m)

Indices	
Groups of animals	MDA
Intact, n=8	
Blood serum	2.87±0.70
Oral mucosa	4.64±0.50
Liver	3.86±0.11
Metabolic syndrome simulation, n=10	
Blood serum P	5.96±0.40 =0.001
Oral mucosa P	6.16±0.50 =0.05
Liver P	7.12±0.60 =0.001

Note: P is the probability of differences from the intact group.

The oral mucosa of animals with simulated MS was pallor with moderate edema. In some cases pathological changes localized predominantly at the area of visual gingival pockets looking like inflammatory foci.

MS simulation causes violation of metabolic processes in the tissues of the gums of rats: an increase in elastase activity by 63.5% ($p=0.001$), increase in MDA level by 45.8% ($p=0.001$), increase in urease activity by 65.8% ($p=0.001$), the content of triglycerides by 133.4% ($p=0.001$) with reduction in catalase activity by 15.6% ($p=0.001$) and lysozyme activity by 57.0% ($p=0.001$).

Our observation and analysis of the obtained data show that MS simulation provokes quantitative increase of opportunistic and pathogenic microflora in the gingival tissues of rats against the background of reduced nonspecific immunity, reduced antioxidant activity and inflammation. Local treatment with the new dental elixir led to reduction of pathogenic microflora development in gingival tissue (urease activity reduction 1.3 times) ($p<0.05$) and the inflammatory process (decrease in elastase activity 1.3 times ($p=0.001$), MDA level – 1.2 times ($p<0.05$) with increasing activity of the protective antioxidant system (catalase activity increased 1.2 times) ($p<0.05$) and non-specific protection (lysozyme activity increased 1.5 times) ($p=0.001$). According to our observations, with MS simulation insulin resistance of gingival tissues increased almost 2.3 times ($p=0.001$). Usage of the new hygienic agent resulted in reduction of triglycerides content on average from 10.46±0.9 mmol/g to 8.60±0.30 mmol/g ($p=0.001$) Antioxidant-prooxidant index in MS was 6.1±0.3 vs.

10.5±0.2 in intact animals. Tooth gel application in rats of group III contributed to the increase of API to 8.7±0.3 (p=0.001).

Table 4

Changes in markers of inflammation, insulin resistance, microbiocenosis and antioxidant and nonspecific protection in the gums of rats in MS development and local treatment with the new dental elixir (M±m)

Indices	Groups of animals	
	Intact (Group I), n=8	Metabolic syndrome (Group II), n=10
Elastase activity, μ -cat/kg p p 1	23.6±1.1	38.6±1.8 0.001
MDA content, mmol/kg p p 1	8.64±0.40	12.60±0.90 0.001
Urease activity, μ -cat/kg p P 1	0.82±0.12	1.36±0.10 0.001
Catalase activity, mcat/kg P P 1	9.12±0.10	7.70±0.40 0.001
Lysozyme activity, units/kg p P 1	386±19	168±12 0.001
Triglycerides content, mmol/g P 1	4.48±0.80	10.46±0.90 0.001
API p P 1	10.5±0.2	6.1±0.3 0.001

Notes: p – the probability of differences from the intact group; p 1 – the probability of differences between 2 and 3 groups of animals.

Therefore, with MS simulation against the background of metabolic disorders inflammatory processes occur in the tissues of the oral cavity, which are characterized by increased activity of inflammation markers and decrease in nonspecific resistance. The use of the new dental elixir based on bee products and adaptogens of plant origin leads to improvement of inflammatory markers activity level, removes contamination with pathogenic microflora and increases non-specific immunity and antioxidant protection of periodontal tissues.

Thus, it was found that a long-term high-calorie diet in rats leads to metabolic disorders typical for MS in the body and homeostasis system dysfunction: hypercholesterolemia, hyperglycemia, hypertriglyceridemia. Biochemical studies revealed in rats during MS reproduction increased activity of inflammatory markers and reduced nonspecific resistance in gum tissue. Changes in the body's defenses, local changes in the tissues of the gums create favorable conditions for the reproduction of pathogenic bacteria with the development of inflammatory processes in the oral mucosa, periodontium. The similar conclusions were reached by researchers in experiments on rats with the MS modified model [9, 13], which proved the MS negative effect in animals on the periodontal condition associated with a significant deterioration in the body's reactivity to pathogenic microbial invasions, particularly in the periodontal tissues.

Under conditions of experimental metabolic syndrome on a diet high in saturated fats and simple carbohydrates against the background of visceral obesity, hyperglycemia, increase in total cholesterol, hyperuricemia, lowering of cholesterol in high-density lipoproteins, alveolar process, a partial degradation of collagen (oxyproline) glycosaminoglycans atrophy and intercellular matrix failure of periodontal connective tissue, oral mucosa and bone tissue took place [2, 12].

The use of the metabolic disorders model in animals from the scientists' point of view reflects the main signs of metabolic syndrome in humans and can be used to study pathological processes occurring under this pathology, as well as to develop methods of prevention and treatment.

Topical application of the new dental elixir based on bee products and plant origin adaptogens leads to normalization of the level of inflammatory markers activity, eliminates contamination with pathogenic microflora and increases nonspecific immunity and antioxidant protection of periodontal tissues. In experiments on the MS model in rats, we identified the similar features of inflammation markers occurrence and the course of free radical processes in reducing antiradical protection in oral tissues and their normalization when using the oral ointment based on natural components [15]. The obtained results experimentally substantiate the possibility of using the new dental elixir in the complex therapy of patients with metabolic syndrome with the prospect of prevention or correction of inflammatory complications in the oral tissues, periodontium.

Conclusions

1. Under conditions of MS simulation with a diet high in saturated fat and simple carbohydrates in rats, body weight increases, disorders in the body take place, which are manifested by an increase in serum

concentrations of triglycerides (1.57 times), cholesterol (1.25 times), glucose (2.4 times), ALT (2.1 times), AST (1.8 times). High-density lipoproteins level decrease 3 times as compared with the intact group.

2. MS simulation in rats leads to increase in MDA level in the blood serum 2 times, in the liver – 1.8 times, in the oral mucosa – 1.3 times and a decrease in catalase activity, which revealed lipid peroxidation activation and dysfunction of the antioxidant system.

3. Local application of the new dental elixir for treatment of the inflammatory processes of gingival tissues against a background of MS simulation has a positive effect on the biochemical parameters of periodontal tissues, significantly reducing triglycerides level, providing anti-inflammatory and antioxidant effects, activating locally functional activity of enzymes of antioxidant defense, non-specific resistance.

4. The use of the new oral hygiene product in experimental metabolic syndrome improved metabolism, reduced inflammation, presenting periodontal protective properties, which allows to offer it for clinical testing in patients with periodontitis against a background of metabolic syndrome.

Prospects for further research include further studies of metabolic and immunological changes in periodontal tissues, alveolar bone in the experiment under the conditions of metabolic syndrome simulation and testing the new oral hygiene product are perspective, which will inhibit risk factors for comorbid pathology manifestation and expand the complex of treatment and prevention measures.

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