EFFICIENCY OF LOCAL THERAPY WITH THE USE OF A NEW PREPARATION FOR ORAL CARE AT PERIODONTITIS IN THE COURSE OF HYPERPEPTIC GASTRITIS AFTER TOBACCO SMOKE INTOXICATION

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ABSTRACT

Introduction: Periodontal diseases, arising against a background of stomach pathology at tobacco addiction remain an acute problem of modern dentistry.

The aim of the work is an experimental study of efficiency of application of the new preparation for oral care during treatment of periodontitis simulated against a background of hyperpeptic gastritis under conditions of intoxication by tobacco smoke.

Materials and methods: At the first stage all experimental animals were divided into 4 groups: I — intact, II — with simulated periodontitis, III — with simulated periodontitis against a background of simulated hyperpeptic gastritis, IV — with simulated periodontitis against a background of hyperpeptic gastritis under conditions of tobacco smoking. Biochemical researches at periodontitis in rats were conducted for determination of influencing stomach pathology and tobacco smoke. On the II stage efficiency of local therapy was studied with the use of the new preparation for oral care and a comparator agent.

Results: At experimental periodontitis against a background of hyperpeptic gastritis under conditions of smoking the considerable changes in periodontal tissues typical for the inflammatory process develop. The local therapy at rats with the use of the new preparation resulted in accelerating removing harmful influence of damaging factors and restoring the state of periodontal tissues, than in case of application of the comparator agent.

Conclusions: The efficiency of the new preparation consists in normalizing influence on processes of lipid peroxidation, inflammation and activation of the protective systems of oral cavity during periodontitis which arises up against a background of hyperpeptic gastritis.

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Introduction

The periodontal diseases remain one of the most actual problems of dentistry in connection with the wide prevalence, chronization and multifactor nature. Often the periodontal diseases arise as concomitant at pathology of gastrointestinal tract (GIT) because of influencing endogenous and exogenous factors, the extent of which depends on the form, severity, duration of the basic disease and is conditioned by the morphofunctional unity of the digestive channel. A rise of acidity of gastric juice is often accompanied by salivation, pallor, edema and inflammation of oral mucosa (OM) [1]. The clinical-laboratory researches in

smokers with generalized periodontitis defined the negative influencing of smoking on tissues of oral cavity, which are the site of primary contact with the toxic and carcinogenic matters of tobacco and tobacco smoke [2].

However, features of pathogenesis of periodontal diseases, arising against a background of concomitant GIT pathology, mechanisms lying in the basis of influencing changes in GIT on the pathobiochimical reaction of inflammatory process in periodontal tissues, the possibilities of medical treatment of such comcomitant pathology taking into account the harmful habit of smoking at patients need the concrete study now [3].

Therefore, search and study of efficiency of new facilities of local therapy of periodontal diseases, as concomitant pathology of hyperpeptic gastritis under conditions of smoking we consider to be actual and perspective for the practical dentistry.

THE AIM

The aim of work is an experimental study of efficiency of local application of the new preparation for oral care in case of medical treatment of periodontitis simulated against a background of hyperpeptic gastritis under conditions of intoxication by the tobacco smoke.

Materials and methods

Researches have been conducted on 72 Vistar line rats-males, 1–1.5 months of age, weight 180–220 g, which were in the terms of vivarium of the Odessa National Medical University on the standard mixed feed stuff for laboratory rats. In accordance with the set tasks of experiment the researches were conducted in 2 stages. At the first stage the rats were divided into 4 groups (by 10 animals in each). The first group consisted of intact rats (the control). The second group of rats were periodontitis simulated. The third group consisted of the rats who after simulation of hyperpeptic gastritis were simulated periodontitis. The fourth group consisted rats which against a background of simulated hyperpeptic gastritis were simulated periodontitis under conditions of dosed action of tobacco smoke. After conducting the first series of experiment on determination of influence of concomitant pathology of stomach and tobacco smoke on the metabolic violations in oral mucosa (OM) tissues and blood serum of rats at simulation of periodontitis, they studied efficiency of local treatment with the use of the new preparatiom on the basis of biologically active substances, bee products and other natural compounds with antiinflammatory, antimicrobial, antioxidant effects [4] and comparator agent — Propolis extract gel. Experimental animals were distributed also into 4 groups: 1 — intact (the control) 2 — rats with the simulated model of periodontitis against a background of hyperpeptic gastritis under conditions of tobacco smoke influence; 3 — the basic one treated by the new preparation consisted of rats with simulated periodontitis against a background of hyperpeptic gastritis under

conditions of intoxication by the tobacco smoke; 4 — group of comparison consisted of rats with the model of periodontitis the same as in the 2, 3 groups, treated with Propolis extract gel.

Damage of gastroduodenal area at rats was induced by addition of ammonium acetate 2 g/l to the drinking-water during 10 days, in 3 days after that they were administered per os 0.4 ml suspension of Helicobacter pylori 5.108 CFU/ml twice per day during 7 days [5]. Hyperpeptic gastritis was simulated by one-time introduction of 5% solution of acetic acid at the rate of 4 ml/kg of weight through the probe 5 days before the experiment beginning. For the control they conducted intragastric pH-metry under intra-abdominal anesthesia of thiopental sodium in dose 20 mg/kg of weight of rat by introduction to the gastric cavity at supramedian laparotomy of glass electrode (EL-40) with the help of pH-meter ("pH-340"). The level of basal acidity during simulated hyperpeptic gastritis was 1.80–2.00.
The rats of the 3rd and 4th groups after simulation of hyperpeptic gastritis and 2nd group at the first day of experiment in the first series of tests under the thiopental anesthesia (20 mg/kg) were simulated periodontitis by imposition of ligature on the central incisor. The essence of the model consists in formation of retention point for the dental plaque, which initiates development of inflammation and destruction of periodontal tissues [6]. The rats of the 4th group in the first series of tests and 2nd, 3rd and 4th groups of the second series created the terms of tobacco smoking.

For smoking terms simulation they used a plastic impermeable chamber with volume of 28 l with three different compartments, in which under pressure by motor they delivered tobacco smoke from 15 cigarettes (with 1.0 mg nicotine and 10 mg tar level) through opening inward during 30 minutes, daily, during 15 days. Simultaneously 7 animals were in the chamber. During fumigation they observed behavioral reactions of rats: at the beginning of the tobacco smoke delivery to the chamber rat were disturbed, looking for a place for normal breathing, in 10 minutes they calmed down and fell asleep. After the end of inhalation by the tobacco smoke and fresh air supply, the rats activated, began breathing often, in 15 minutes came round.

The animals were removed from the experiment within few stages. Euthanasia of rats of 1st–4th groups of the first series was carried out immediately after the last procedure of tobacco smoke inhalation (on the 15th day) under the thiopental anesthesia (20 mg/kg) by the total bloodletting from heart. All animals, treated after periodontitis simulation against a background of hyperpeptic gastritis under conditions of smoking, killing was partly conducted on the 8th day and 14th day after the treatment onset.

They conducted sampling of blood, bioptates of gingiva for the biochemical researches. In the gingival homogenate supernatants and blood serum they determined a level of end product of lipid peroxidation – malonic dialdehide (MDA) by thiobarbituric method [7], the state of antioxidant defence (AOD) was estimated by catalase activity [8], level of inflammation by activity of elastase [9], index of nonspecific

defence by lysozyme activity [10]. According to correlation of catalase activity in MDA concentration they calculated the antioxidant-prooxidant index (API).

Quantitative estimation of concentration of pro-inflammatory cytokines (interleukine 6 (IL-6) and anti-inflammatory (interleukine 10 (IL-10) in the blood serum they conducted by the method of enzyme-linked immunosorbent assay on the immunoenzymometric analyzer RT-2100C by test-systems. The results of reaction were determined on spectrophotometer at wave-length 450 nm. By the calibration plot they calculated concentrations of cytokines in picograms on 1 ml. During conducting researches they use general principles of experiments on animals, approved on National Congress on Bioethics (Kiev, Ukraine, 2001) and coordinated with points of European Convention about vertebrates protection, used for the experimental and other scientific purposes (Strasburg, France, 1985). The statistical processing of obtained data were conducted by the "Statistica 6.0" with the use of Student t-test. The changes were considered reliable at $p \leq 0.05$.

Results

The experimental animals before simulating pathological states had intact gingival mucosa, without visible pathological changes, gingival bleeding was not revealed during probing. All rats after ligature induced periodontitis already on the third day had signs of clinical symptoms of inflammation of periodontal tissues, namely, hyperemia, edema, gingival bleeding in area of incisors. Gingival inflammation was determined in 5 days and in area of molars, so generalisation of inflammatory process in periodontal tissues took place. The animals who were simulated hyperpeptic gastritis became weak, ate little, the inflammatory phenomena of OM were fixed — hyperemia and edema. After the simulated periodontitis these animals on the 2nd day had a distinct picture of gingival inflammation as edema and marginal edge hyperemia, gingivitis.
The simulated periodontitis resulted in growth of MDA level in gingival tissues, which indicated to intensification of lipid peroxidation at the lowered activity AOD (the activity of catalase decreased) in periodontal tissues. The most low indexes of activity of catalase in gingiva (4.96 ± 0.39 mkat/kg) and the most high level MDA (19.70 ± 1.30 mmol/kg) are marked in the 4th group of animals, which 2.3 times exceeds the given index of intact animals ($p<0.05$) and 1.3 times — in rats of the 2nd group with periodontitis (table I).

In gingival tissues of rats with the hyperpeptic gastritis, which were under influence of tobacco smoke against a background of the periodontitis elastase activity became 1.35 times more than intact animals ($p<0,05$), without intoxication by the tobacco smoke the examined index grew by 25% less ($p<0.05$). The concomitant hyperpeptic gastritis substantially affected the extent of violations of oral tissues metabolism in animals with the induced inflammation of periodontal tissues, strengthening phenomena of oxidative stress, inhibiting functional state of the AOD system, which caused the damages of biological membranes,

structural-functional changes OM with the elements of inflammation. Elastase activity in gingival tissues increased in rats with periodontitis against a background of hyperpeptic gastritis 1.26 times as compared with intact animals, exceeding the value in rats without the concomitant pathology. Simultaneously there was a decline of local resistance of oral tissues in rats of 3rd and 4th groups, about what testified activity of lysozyme in the gingival homogenates on 36.3% and 44.3% less as compared with intact animals.

Simulated periodontitis itself caused the changes of biochemical values in the blood serum: elastase activity increased 1.22 times, MDA level 1.15 times ($p<0.05$), catalase activity decreased 18.2% in relation to the examined indexes in intact animals (table II). Simultaneously in the blood serum of animals with periodontitis proinflammatory interleukin IL-6 level increased 1.8 times and the antiinflammatory interleukin IL-10 level decreased 1.3 times as compared with the control group. Development of periodontitis against a background of concomitant GIT pathology was accompanied by the still more high indexes of elastase activity, MDA level, the proinflammatory interleukin IL-6 level with a definite dynamics of decrease of catalase AOD enzyme activity and antiinflammatory interleukin IL-10 level.

The conducted researches showed that a 15-day injury by the tobacco smoke of rats during simulated periodontitis against a background of hyperpeptic gastritis resulted in the most pronounced changes of inflammation markers and cytokine regulation in the blood serum of experimental animals: the elastase activity increased 1.3 times, MDA level — 1.25 times, IL-6 level — 3.6 times with decrease of catalase activity by 41% and antiinflammatory interleukin IL-10 level — 2.3 times as compared with the control group.

A new mucosal apigel (honey bee gel) on the basis of biologically active substances, bee products and other natural compounds application in the local therapy of periodontitis simulated against a background of hyperpeptic gastritis under conditions of intoxication by the tobacco smoke promoted reduction of damage affects influence on the oral cavity of animals and improved renewal of tissue condition. After the local application of apigel state of periodontal tissues improved already in 5 days after the beginning of treatment, but in case of application of comparator agent Propolis extract gel — only in 10 days. The results of conducted biochemical researches showed that the new preparation considerably lowered the markers of inflammation in gingival tissues. On the 8th day after the treatment with the new gel the most animals (86%) were revealed normalization of indexes of the antioxidant-prooxidant system, markers of inflammation in gingival tissues.

During conducting applications with Propolis extract gel the positive effect was revealed only in 38% rats on the 8th day after the beginning of application, but the rest of animals (62%) had metabolic violations, which were removed mainly by the end of research (tables III, IV).

Discussion

Summing up results of experimental researches, it is possible to establish that at rats in case of simulated periodontitis against a background of hyperpeptic gastritis under conditions of intoxication by the tobacco smoke the activity of the prooxidant system increased and activity of the antioxidant system decreased, elastase activity increased in case of decrease of nonspecific defence in periodontal tissues. New gel on the basis of biologically active substances, bee products and other natural compounds usage as applications in rats with periodontitis against a background of hyperpeptic gastritis after intoxication by the tobacco smoke considerably decreased the processes of inflammation in periodontal tissues, making affect on normalization of processes of lipid peroxidation, inflammation and activation of the protective systems of oral cavity. The results obtained in the experiment indicate to necessity of studying influence of a developed preparation on the parameters of nonspecific resistance and immune reactivity in the oral cavity at periodontitis against a background of concomitant GIT pathology and making indication for its usage in the complex therapy of dental diseases.

Conclusion

1. During experimental periodontitis against a background of hyperpeptic gastritis under conditions of intoxication by the tobacco smoke the changes in periodontal tissues typical for the inflammatory process develop: the activity of lipid peroxidation increases and activity of the antioxidant system decreases, markers of inflammation increase while nonspecific defense decreases. The disbalance develops in the blood serum in the POL-AOD system with growth of endogenous intoxication and violation of the cytokine regulation.

2. Local therapy of periodontitis in rats with the use of the new apigel which is developed by us resulted in correction of definite metabolic violations in the homogenates of gingiva and blood serum, accelerating removal of harmful influence of damage affects and restoring the state of periodontal tissues than using agent of comparison Propolis extract gel.

3. The results of researches give reason to recommend the local application of the new apigel on the basis of biologically active substances, bee products and other natural compounds for prevention of inflammatory processes in tissues of oral cavity and creation of optimal terms for the removal of the structural-functional violations caused by the endogenous and exogenous factors of risk.

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Table I. Changes of biochemical values in gingival tissues at rats in case of simulating periodontitis against of background of hyperpeptic gastritis.

Groups of animals	MDA level, mmol/kg	Elastase, mckat/kg	Catalase, mckat/kg	Lysozyme, U/kg
Intact (control), n=10	8.42±0.34	34.0±2.00	7.18±0.33	276±24
Periodontitis, n=10	14.70±0.62	40.0±3.00	6.74±0.41	188±14
P	<0.05	>0.05	>0.05	<0.05
Hyperpeptic gastritis+periodontitis, n=10	17.80±1.20	43.0±3.00	5.86±0.48	176±22
P	<0.05	<0.05	<0.05	<0.05
P1	<0.05	>0.05	>0.05	>0.05
Hyperpeptic gastritis+periodontitis+tobacco smoke, n=10	19.70±1.30	46.0±4.00	4.96±0.39	154±22
P	<0.05	<0.05	<0.05	<0.05
P1	<0.05	>0.05	<0.05	>0.05
P2	>0.05	>0.05	>0.05	>0.05

Notes: P — probability in relation to the control group;

P1 — probability in relation to the group of periodontitis;
P2 — probability of distinctions between 3rd and 4th groups.

Table II. Changes of biochemical values in the blood serum of rats at simulated periodontitis against a background of hyperpeptic gastritis.

				Cytokines
Groups of animals	Elastase, mckat/L	MDA level, mcmol/L	Catalase, mkat/L	IL-6, pg/ml
Intact (control), n=10	184.6±6.7	1.02±0.02	0.22±0.005	0.32±0.08
Periodontitis, n=10	226.3±5.6	1.18±0.03	0.18±0.004	0.58±0.05
P	<0.05	<0.05	<0.05	<0.05
Hyperpeptic gastritis+ periodontitis, n=10	234.6±4.8	1.23±0.04	0.15±0.005	0.96±0.10
P	<0.05	<0.05	<0.05	<0.05
P1	>0.05	>0.05	<0.05	<0.05
Hyperpeptic gastritis+periodontitis+tobacco smoke, n=10	242.4±6.8	1.28±0.04	0.13±0.006	1.18±0.14
P	<0.05	<0.05	<0.05	<0.05
P1	<0.05	<0.05	<0.05	<0.05
P2	>0.05	>0.05	<0.05	>0.05

Notes: P — probability in relation to the control group;

P1 — probability in relation to the group with periodontitis;
P2 — probability of distinctions between 3rd and 4th groups.

Table III. Influence of local treatment on the biochemical values of blood serum in rats at simulated periodontitis against a background of hyperpeptic gastritis under conditions of smoking (M ± m).

Groups of animals	Catalase,	Cytokines

	MDA level, mcmol/L	Elastase, mckat/L	mckat/L	IL-6, pg/ml	IL pg
Intact (control), n=10	1.02±0.02	184.6±6.7	0.22±0.005	0.32±0.08	1.
Hyperpeptic gastritis+ periodontitis+ tobacco smoke, before treatment, n=10	1.28±0.04	242.4±6.8	0.13±0.006	1.18±0.24	0.
P	<0.05	<0.05	<0.05	<0.05	<0
The basic group, n=10	1.06±0.03	192.3±5.2	0.24±0.006	0.40±0.08	1.
P	>0.05	>0.05	>0.05	>0.05	>0
P1	<0.05	<0.05	<0.05	<0.05	<0
The group of comparison, n=10	1.16±0.04	214.8±6.4	0.19±0.007	0.62±0.11	0.
P	<0.05	<0.05	<0.05	<0.05	<0
P1	<0.05	<0.05	<0.05	<0.05	<0
P2	<0.05	<0.05	<0.05	>0.05	<0

Notes: P — probability in relation to the control group;

P1 — probability in relation to parameters before treatment;

P2 — probability of distinctions between the basic group and group of comparison.

Table IV. Correction of metabolic violations in periodontal tissues of rats at local treatment of simulated periodontitis against a background of hyperpeptic gastritis (M ± m).

Groups of animals	MDA level, mcmol/kg	Elastase, mckat/kg	Catalase, mckat/kg	Lysozyme, U/kg
Intact (control), n=10	8.42±0.34	34.0±2.0	7.18±0.33	276±24

Hyperpeptic gastritis+periodontitis+tobacco smoke, before treatment, n=10	19.7±1.30	46.0±4.0	4.96±0.39	154±34
P	<0.05	<0.05	<0.05	<0.05
The basic group, n=10	9.87±0.48	37.0±2.0	6.88±0.58	218±28
P	<0.05	>0.05	>0.05	>0.05
The group of comparison, n=10	13.31±0.74	40.0±3.0	6.23±0.42	197±22
P	<0.05	>0.05	<0.05	<0.05
P1	<0.05	>0.05	<0.05	>0.05
P2	<0.05	>0.05	>0.05	>0.05

Notes: P — probability in relation to the control group;

P1 — probability in relation to parameters before the treatment;

P2 — probability of distinctions between the basic group and group of comparison.