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ORIGINAL PAPER

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A new local therapy of periodontitis in the course of stomach pathology and tobacco smoke intoxication

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ABSTRACT

Introduction. Inflammatory periodontal diseases, arising against a background of stomach pathology from tobacco addiction remain an acute problem of modern dentistry.

Aim. The experimental assessment of a new local treatment efficiency during therapy of simulated periodontitis with hyperacid gastritis and the tobacco smoke intoxication.

Material and methods. The work was conducted in 2 stages. At the first stage, all experimental animals were divided into 4 groups: I — intact, II — with simulated periodontitis, III — with simulated periodontitis and hyperacid gastritis, IV — with simulated periodontitis with hyperacid gastritis and tobacco smoking. The local therapy efficiency was evaluated with the use of a new preparation for oral care and a comparison product conducted at the 2nd stage in rats with simulated periodontitis with hyperacid gastritis and tobacco smoking.

Results. Experimental periodontitis with hyperacid gastritis and tobacco smoking provokes considerable changes in the periodontal tissues typical for the inflammatory process: lipid peroxidation activity rises and antioxidant system activity reduces. A local therapy in rats resulted in correction of detected metabolic disorders, improving removal of the damaging factors harmful influence and restoring the periodontal tissues condition.

Conclusion. The medical efficiency of a new gel normalizes the influence of lipid peroxidation processes, inflammation and the oral cavity protective system activation during periodontitis which arises up against a background of the concomitant pathology of stomach – hyperacid gastritis.

Keywords. periodontitis, gastritis, smoking

Introduction

Periodontal disease remain one of the most actual problems of dentistry in connection with the wide prevalence, chronization and its multifactorial nature.[1] The modern ideas about the etiopathogenesis of chronic generalized periodontitis define it as the result of the interaction between microbial factors and the host.[2,3] Modern epidemiological data indicate a high prevalence of chronic

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generalized periodontitis associated with the influence of both endogenous and exogenous factors. Among exogenous factors, tobacco smoking plays a leading role in the chronic generalized periodontitis etiopathogenesis.[4] There is a close functional connection between the concomitant pathology of the gastrointestinal tract and inflammatory periodontal diseases, which aggravates the concomitant pathology course. Often the periodontal diseases arise as a concomitant with gastrointestinal tract (GIT) pathology because of the endogenous and the exogenous factors influence, the extent of which depends on the form, severity, duration of the basic disease and conditioned by the morphofunctional integrity of the digestive channel.[5] The changes in the oral cavity with the presence of chronic gastritis depend on the state of secretion and acid-forming function of stomach. A rise of gastric juice acidity is often accompanied by salivation, pallor, edema, and inflammation of the oral mucosa (OM).[6] In addition, modern data testifies to the negative influence of *Helicobacter pylori* on the periodontal diseases course.[7] Clinical laboratory experiments in smokers with generalized periodontitis revealed the negative influence of tobacco smoking on the oral cavity tissues, which are the site of primary contact with the toxic and carcinogenic matters of tobacco and tobacco smoke.[8]

Pathogenesis of periodontal disease arising against a background of GIT pathology require a more profound investigation.[9] The modern therapy of this combined pathology taking into account the adverse habits is not effective.

Therefore, we consider searching for and studying the efficiency of new ways of local therapy of periodontal disease as a concomitant pathology of hyperacid gastritis and tobacco smoking for practical dentistry. Hygiene based on honey products with bactericidal and bacteriostatic properties without manifestations of microbial resistance have been recently used in the complex treatment of inflammatory periodontal diseases.[10-17] However, the lack of information about the honey products, in particular, of propolis and wax in the treatment of associated periodontal diseases and the gastrointestinal tract against the background of tobacco smoking was the impetus to searching, developing and studying the efficiency of a new local therapy.

Aim

The experimental assessment of a new local treatment efficiency during therapy of simulated periodontitis with hyperacid gastritis and the tobacco smoke intoxication.

Material and methods

Experiments were conducted on 60 Vistar line rats-males, 1–1.5 months of age, weighting 180–220 g under conditions of the Odessa National Medical University animal facility on a standard diet for laboratory rats. In accordance with the set tasks, the experiments were conducted in 2 stages. In the first stage, the rats were divided into 4 groups. The first group (I) consisted of healthy rats (control, n=10). The second group (II) of rats were subjected to simulated periodontitis (n=10). The third group (III) consisted of the rats who after simulation of hyperacid gastritis were subjected to simulated periodontitis (n=10). The fourth group (IV) consisted rats subjected to a dosed tobacco smoke intoxication and simulated hyperacid gastritis and simulated periodontitis (n=30). After conducting the first series of experiments to investigate the concomitant stomach pathology and the tobacco smoke influence on the metabolic disorders in rat OM tissues with simulated periodontitis, the efficiency of local treatment using a new preparation on the basis of honey products and other biologically active matters with anti-inflammatory, antimicrobial, antioxidant effects – a gel made of honey products (Apigel) and a comparison product – a Propolis extract gel was studied.[18-19] The fourth group (IV) of rats were divided into 3 subgroups (Table 2): the 1st — rats with the simulated periodontitis with hyperacid gastritis and tobacco smoke influence (untreated subgroup, n=10); the 2nd — (the basic one) consisted of rats with simulated periodontitis with hyperacid gastritis and the tobacco smoke intoxication the treated by a new preparation (n=10); the 3rd — (a comparison group) consisted of the rats with simulated periodontitis like in the 1st and 2nd subgroups, subjected to local treatment with a Propolis extract gel (n=10).

Gastroduodenal area damage in rats was induced by addition of ammonium acetate 2 g/L to drinking-water during 10 days, after the third day they were administered 0.4 ml *Helicobacter pylori* suspension 5.108 CFU/mL twice per day during 7 days by a special dispenser.[20-25] Hyperacid gastritis was simulated by a one-time introduction of 5% solution of acetic acid at the rate of 4 mL/kg of weight through the probe 5 days before beginning clinical investigation. For the control, we conducted intragastric pH-metry under intra-abdominal anesthesia of thiopental sodium in a dose of 20 mg/kg of rat weight by introduction to the gastric cavity at supramedian laparotomy of glass electrode (EL-40) with the help of a pH-meter ("pH-340", Plant of Measuring Instruments, Gomel, Belarus). The level of basal acidity during the simulated hyperacid gastritis was 1.80–2.00. In addition, the gastric mucous membrane structure has been studied by histology to confirm the presence of hyperacid gastritis in the experimental animals.

The rats of the III and IV groups after simulation of hyperacid gastritis and II group at the first day of experiment in the first series of tests under the thiopental anesthesia (20 mg/kg) were simulated with periodontitis by the "ligature" model by applying a ligature around the

neck of the central lower incisor with fixation near the gingiva edge by the dental cement. The essence of the model consists in formation of the retention point for the dental plaque, which initiates inflammation and periodontal tissue destruction.[26] The induction of periodontitis in rats by this method was carried out by Y. Chumakova (2014), E. Zhulev (2015), A. Vishnevska (2018), D. Smaiilkulov (2019) and others.[27-39] The conditions of tobacco smoking were created for the IV group rats.

For tobacco smoking simulation, a plastic impermeable chamber with volume of 28 L with three different compartments was used, in which under pressure by motor, tobacco smoke was delivered from 15 cigarettes ("Prima red" PJSC "Imperial Tobacco Production Ukraine" Kiev, Ukraine, with 1.0 mg nicotine and 10 mg tar level) through opening inward during 30 minutes, daily, during 15 days.[40-43] Simultaneously, 7 animals were in the chamber. During fumigation, behavioral reactions of rats were observed: 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, and came around in 15 minutes.

The animals were removed from the experiment within a few stages. Euthanasia of rats of the I–III groups and IV (the 1st subgroup) of the first series was carried out immediately after the last procedure of the tobacco smoke inhalation (on the 15th day) under the thiopental anesthesia (20 mg/kg) by the total bloodletting from the heart. All the animals, treated after simulated periodontitis with hyperacid gastritis and smoking (the 2nd and 3rd subgroups of IV group), were decapitated on the 8th and 14th day after the treatment onset.

The gingiva biopsy sampling for the biochemical experiments was conducted. In the gingival homogenate supernatant the level of lipid peroxidation – malonedialdehyde (MDA) end product was conducted by thiobarbituric method, the state of antioxidant defense (AOD) was estimated by catalase activity, the level of inflammation — by elastase activity, the nonspecific defense index — by lysozyme activity.[44-47] The antioxidant-prooxidant index (API) was calculated according to catalase activity and MDA level correlation.

During the conduct of clinical investigation the general principles of animal experiments were used approved by the National Congress on Bioethics (Kiev, Ukraine, 2001) and coordinated with provisions of European Convention conerning vertebrate protection, used for the experimental and other scientific purposes (Strasburg, France, 1985).

The statistical information of obtained data was conducted by the "Statistica 6.0" program with the Student t-test. The changes were considered reliable at $p \leq 0.05$.

Results

The experimental animals, before simulated pathological states, had healthy gingival mucosa, without visible pathological changes, gingival bleeding was not revealed during probing. After ligature-induced periodontitis already on the third day all the rats had clinical symptoms of the periodontal tissues inflammation, namely, plaque was detected at the cervical part of the teeth. Hyperemia, edema, gingival bleeding occurred in the incisors area. Gingival inflammation was determined in 5 days in the molars area too, so the inflammatory process generalization in the periodontal tissues took place. There was bacterial plaque accumulation and the microscopic ulcers appeared on the gingival groove epithelium, which contributes to the penetration of periopathogenic bacteria into the connective tissue. The loss of periodontal ligament and bone resorption occurred within 7 days. The animals with simulated hyperacid gastritis became weak, ate little, had signs of the oral inflammation — hyperemia and edema. After the simulated periodontitis on the 2nd day the rats had a distinct picture of gingival inflammation manifested as edema and marginal edge hyperemia, gingivitis. After 5 days, the gums looked cyanotic, swollen toward the alveolar bone basis. The periodontal pockets with a depth of 1.5 mm appeared at the area of teeth. At the 8–10th day of simulated periodontitis and hyperacid gastritis occurred alveolar process swelling at the upper and lower parts, severe hyperemia of the dental neck, gingival pockets depth up to 3 mm, marginal part is easily detached by flat plastic instrument, gums bleeding. At the 14th day, the distribution of edema on the alveolar process with increasing size remained unchanged. The edema of the anterior part of the alveolar process was white-red, and the lateral parts were blue. The enamel in the cervical region had a dark color. Gingival pocket was 6 mm, easily bleeding during probing. The similar manifestations of the pathological process of the periodontal tissues in rats with simulated gastroduodenitis were underlined by N.I. Sidlairuk, O.V. Avdeev (2016).[48]

Histology of the gastric mucosa of rats with hyperacid gastritis revealed significant structural changes throughout the stomach thickness. The superficial epithelium had multiple small necrotic patches and desquamation. The pronounced desquamation of the superficial epithelium with edema and hyperemia with the formation of surface erosions was observed. The surface erosions localized mainly at the top of folds and were multiple. There were dystrophic and inflammatory changes at the edges and at a distance from defects. These changes were combined with hemomicrocirculatory disorders — hyperemia, stasis, margination in the lumen of dilated vessels. The erosion's bottom was covered by mucus with epithelial cells and leukocytes. Circulatory disturbances were detected — hypertrophy, edema. The similar morpholog-

Table 1. The biochemical parameters changes in the gingival tissues of rats with simulated periodontitis and hyperacid gastritis (M ± m)

Groups of animals	MDA level, mmol/kg	Elastase, mckat/kg	Catalase, mckat/kg	Lysozyme, U/kg	API
I - healthy (control), n=10	8.42±0.34	34.0±2.00	7.18±0.33	276±24	8.52±0.32
II - simulated periodontitis, n=10	14.70±0.62	40.0±3.00	6.74±0.41	188±14	4.58±0.52
P*	<0.05	>0.05	>0.05	<0.05	<0.05
III - hyperacid gastritis and simulated periodontitis, n=10	17.80±1.20	43.0±3.00	5.86±0.48	176±22	3.29±0.84
P*	<0.05	<0.05	<0.05	<0.05	<0.05
P_1 #	<0.05	>0.05	>0.05	>0.05	>0.05
IV - hyperacid gastritis and simulated periodontitis+ tobacco smoke intoxication, n=30	19.30±1.10	46.0±4.00	4.80±0.38	154±26	2.48±0.57
P*	<0.05	<0.05	<0.05	<0.05	<0.05
P_1 #	<0.05	>0.05	<0.05	>0.05	<0.05
P_2 ǂ	>0.05	>0.05	>0.05	>0.05	>0.05

Notes: *: probability in relation to the control group; #: probability in relation to parameters before the treatment; ǂ: probability of distinctions between the basic group and comparison group.

ical changes in the gastric mucosa with experimental gastroduodenitis were observed in the literature sources of Karczewska E., Konturek J., Konturek P. (2002), Misula N., Avdeyev O. (2014).[49-50]

Metabolic alterations of the periodontal tissues were marked in rats of II and III groups as compared with healthy animals. The analysis indicated that the rats with periodontitis with hyperacid gastritis and the tobacco smoke intoxication had the most pronounced changes of the inflammation biochemical markers in the gingival tissues (group IV).

The simulated periodontitis resulted in growth of MDA level in the gingival tissues, which proved the lipid peroxidation intensification with the lowered antioxidant defense activity (the catalase activity decreased) in periodontal the tissues. Still more considerable similar changes took place in the gingival samples of animals with simulated periodontitis and hyperacid gastritis. The most pronounced metabolic disorders of the oral tissues were detected in the IV group (with simulated periodontitis with hyperacid gastritis and tobacco smoking), when combination of two damaging factors took place, especially in the lipid peroxidation-AOD system. The catalase activity decreased as a result of its utilization during the active participation in the lipid peroxidation products deactivation processes. The most low indices of the catalase activity in the gingiva (4.80 ± 0.38 mkat/kg) and the most high level of MDA (19.30 ± 1.10 mmol/kg) are marked in the IV group, which 2.3 times exceeds the given index in healthy animals ($p<0.05$) and 1.3 times — in the rats of II group with periodontitis (Table 1).

In the gingival tissues of the hyperacid gastritis rats with periodontitis, which were under tobacco smoke influence, the elastase activity became 1.35 times as much as in healthy animals ($p<0.05$), without the tobacco smoke intoxication this parameter growing was cut by 25% ($p<0.05$). The concomitant hyperacid gastritis substantially affected the level of the oral metabolic disorders in animals with the induced inflammation of the periodontal tissues, intensifying the oxidative stress phenomena, inhibiting AOD system function, which caused the biological membranes damage, structural-functional changes of OM with the inflammation elements. The elastase activity in the gingival tissues increased in rats with periodontitis and hyperacid gastritis 1.26 times as compared with healthy animals, exceeding this parameter in rats without the concomitant pathology. Simultaneously there was decline of the oral tissues local resistance in the rats of III and IV groups, which was proved by the fact that the lysozyme activity in the gingival homogenates was by 36.3% and 44.3% less as compared with healthy animals. It is known that lysozyme renders a local anti-inflammatory and immune modulating action: inhibits the neutrophils chemotaxis and the toxic oxygen radical production.[51-53] The decline of lysozyme activity can be the cause of local inflammatory process.

Apigel application in the local therapy of simulated periodontitis with hyperacid gastritis and the tobacco smoke intoxication promoted lowering of damage factors influence on the oral cavity of animals and improved the tissue condition. The oral examination revealed a considerably less damage to oral mucosa, namely, the gingival edema and reddening. After a local application of Apigel, the periodontal tissues condition improved already in 5 days after the beginning of the therapy, but with a comparison product application — only in 10 days. The results of conducted biochemi-

Table 2. Correction of metabolic disorder of the rats' periodontal tissues with local treatment of simulated periodontitis and hyperacid gastritis – IV group rats (M ± m)

Subgroups of animals	MDA level, mcmol/kg	Elastase, mckat/kg	Catalase, mckat/kg	Lysozyme, U/kg	API
The 1st – untreated subgroup, n=10	19.7±1.30	46.0±4.0	4.96±0.39	154±22	2.52±0.60
P*	<0.05	<0.05	<0.05	<0.05	<0.05
The 2nd - Apigel subgroup, before treatment, n=10	18.8±0,9	46.0±4.0	4.58±0.4	148±26	2.43±0.52
P*	<0.05	<0.05	<0.05	<0.05	<0.05
The 2nd - Apigel subgroup, after treatment, n=10	9.87±0.48	37.0±2.0	6.88±0.58	218±28	7.07±0.53
P*	<0.05	>0.05	>0.05	>0.05	>0.05
$P_1^{\#}$	<0.05	<0.05	<0.05	>0.05	<0.05
The 3rd - The Propolis subgroup, before treatment, n=10	19.4±1.1	45.0±4.0	4.86±0.36	160±30	2.5±0.6
P*	<0.05	<0.05	<0.05	<0.05	<0.05
The 3rd - The Propolis subgroup, after treatment, n=10	13.31±0.74	40.0±3.0	6.23±0.42	197±22	4.68±0.58
P*	<0.05	>0.05	<0.05	<0.05	<0.05
$P_1^{\#}$	<0.05	>0.05	<0.05	>0.05	<0.05
P_2^{+}	<0.05	>0.05	>0.05	>0.05	<0.05

Notes: *: probability in relation to the control group; #: probability in relation to parameters before the treatment; +: probability of distinctions between the basic group and comparison group.

cal experiments showed that a new preparation considerably lowered the inflammation markers in the gingival tissues. Their level in the substrate, studied in animals treated with Apigel applications on the simulated periodontitis areas, registered the lower values as compared to a comparison group. On the 8th day after the treatment with Apigel the most animals (86%) had indicators normalization of the antioxidant-prooxidant system, the inflammation markers in the gingival tissues.

The applications with a comparison product gave a positive effect only in 38% rats on the 8th day after the beginning of application, but the rest of animals (62%) had metabolic disorders, which were removed mainly by the end of experiment. The complex investigation revealed that Apigel renders a more pronounced curative effect than a comparison product, which was proved by the improvement of biochemical values of the periodontal tissues of rats (Table 2).

Discussion

Today there are many methods and regimens for the treatment of periodontal diseases, which includes various medications and treatment modes, but it is not clear which of the available ones is a sufficiently effective treatment. Periodontitis is a polyethiological disease, the basis of which is the complex of pathological disorders that occur in the oral cavity under the influence of exogenous and endogenous factors. Presently Propolis is widely used in inflammatory dental diseases, as it stimulates reparative processes, activates protective mechanisms and has antimicrobial properties. So, the study of herbal medicines usage undoubtedly has practical and scientific interest, since it promotes the introduction of new methods of treatment and prevention in this category of patients.

Summing up results of experiments, it is possible to establish that Apigel usage as applications in rats with periodontitis and hyperacid gastritis after the tobacco smoke intoxication considerably decreased the inflammation processes in the periodontal tissues, having an effect on normalization of the lipid peroxidation processes, inflammation and activation of the protective systems of the oral cavity.

The obtained data are in agreement with the data of researchers Smaylkulov D., Belov G., Subanova A. (2018), Karczewska E., Konturek J., Konturek P. (2002), Misula N., and Avdeyev O. (2014).[30,49-50]

The results obtained in the experiment indicate to a necessity of studying influence of a developed preparation on the parameters of the nonspecific resistance in the oral cavity at periodontitis with GIT pathology and making indication for its usage in the complex therapy of dental diseases. So, we consider the search and study of efficient new ways of the local therapy of periodontal diseases as a concomitant pathology of hyperacid gastritis under conditions of tobacco smoking for practical dentistry.

The results of experimental studies show that the development of inflammation in the stomach leads to failure of the protective mechanisms of the oral cavity to the action of damaging factors that contribute to the onset or complication of the inflammatory processes in

the periodontium, which must be taken into account in practical dentistry during treatment of patients with concomitant diseases of GIT. In the complex treatment of patients with hyperacid gastritis with inflammatory diseases of the periodontal tissue, we recommend the local therapy with the use of a new Apigel to correct changes in the oral cavity.

Conclusion

During experimental periodontitis with hyperacid gastritis and the tobacco smoke intoxication the changes in the periodontal tissues typical for the inflammatory process develop: the lipid peroxidation activity increases and the antioxidant system activity decreases, inflammation markers increase and the nonspecific defense decreases.

The local therapy of simulated periodontitis with hyperacid gastritis and tobacco smoke intoxication in rats with the use of a new Apigel resulted in correction of the definite metabolic disorders in the gingival homogenates. Apigel removed a harmful influence of damage affects and restored the periodontal tissues better than a comparison product.

Curative effect of Apigel is conditioned by a normalizing influence on the lipid peroxidation processes, inflammation and activating the protective systems of the oral cavity.

The results of experiments give reason to recommend a local application of Apigel for inflammatory processes prevention in the oral tissues with the concomitant hyperacid gastritis and creation of optimal terms for the removal of the structural-functional disorders caused by the endogenous and exogenous factors of risk.

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