Savelieva N. N., Suslova O. V., Schneider S. A., Tkachenko E. K. The effect of anthracene derivatives on the state of the extracellular matrix of the periodontal connective tissue and the oral mucosa of old rats. Journal of Education, Health and Sport. 2019;9(4):63-69. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.2620542
http://ojs.ukw.edu.pl/index.php/johs/article/view/6770

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 18.03.2019. Revised: 25.03.2019. Accepted: 01.04.2019.

The effect of anthracene derivatives on the state of the extracellular matrix of the periodontal connective tissue and the oral mucosa of old rats

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Abstract

In the experiments on 27 old female rats, the effect of the anthracene derivative preparation on the state of the intercellular matrix of the connective tissue of the periodontal and oral mucosa in intact animals, as well as on the periodontitis model, was studied.

Key words: hydroxyproline, collagen, glycosaminoglycans, periodontal disease, oral mucosa, anthracene derivatives, periodontitis model, rats.

Drugs based on Hypericum perforatum (Hypericum perforatum L.) have long been widely used in medical clinical practice. St. John's wort is known for its antiviral, antidepressant, cytotoxic, anti-inflammatory properties. Hypericum grass contains a significant amount of various types of biologically active substances of polyphenolic nature: flavonoids, phenol carboxylic acids, tannins, catechins, etc. A special group is anthracene derivatives, in particular, hypericin and pseudohypericin [1].

These substances are currently recognized anti-inflammatory agents. Thus, it was found that light-activated pseudohypericin is able to inhibit the production of prostaglandin E2 (PGE2), caused by lipopolysaccharides [2].

The structural integrity of the tissue is maintained by multicellular macromolecules - collagens, elastins, glycosaminoglycans (GAG), which are the intercellular matrix (MKM) of connective tissue. The balance between degradation and synthesis of MKM components determines the state of soft and hard periodontal tissues in normal conditions and during its pathology.

With periodontitis, pathological degradation of periodontal tissues occurs. The change of the MKM state of the connective tissue of periodontal with periodontitis is carried out with the help of matrix metalloproteinases (MMPs) or collagenases, which break down almost all the components of MKM. Disruption of the relationship between activated MMPs and their endogenous inhibitors (TIMP) in the direction of MMPs leads to pathological degradation of periodontal tissues. Thus, TIMP levels are lower in periodontal tissues of patients with periodontitis [3]. The use of exogenous TIMP may contribute to the treatment of periodontal disease.

Earlier, in the laboratory, we obtained a preparation of anthracene derivatives (AP) of the aboveground part of Hypericum perforatum [4]. The total content of anthracene derivatives (AP) in the preparation was 0.25 mg / ml of extract.

All of the above predetermined the purpose of the study - to study the effect of anthracene derivatives on the state of the intercellular matrix of the connective tissue of the periodontal and oral mucosa of old rats in normal and reproduced periodontitis models.

Materials and methods

In the experiment were taken 27 rats females 18 months. age Intact group - 5 individuals. In the 2nd group, 6 rats were given per os a preparation of anthracene derivatives (working name AP) at 0.1 ml / 100 g of body weight of rats 5 times a week in the morning. In the 3rd group, in 8 rats, periodontitis was simulated by injecting a solution of collagenase (from Clostridium histolyticum, Merk, Darmstadt, Germany) twice at a dose of 0.2 mg / ml and once at a dose of 1 mg / ml. In the 4th group, 8 rats on the background of the simulation of periodontitis per os injected the drug AP (5 times a week). The duration of the experiment was 55 days. All animal experiments were carried out in accordance with the European Convention for the Protection of Vertebrate Animals.

After the slaughter of rats by total bleeding from the vessels of the heart (sodium thiopental at a dose of 40 mg / kg), the gum, buccal mucosa (JIN) were separated, and the jaws were extracted. The objects of biochemical studies were supernatant of SOSH homogenates, gums (25 mg / ml); liver and bone of the alveolar process (50 mg / ml).

The state of MKM was assessed by the content of hydroxyproline [5], GAG [6]. To determine alkaline phosphatase (alkaline phosphatase), the content of Ca2 +, Mg2 + ions, phosphorus, total protein, commercial reagent kits were used (DAC - SpectroMed, Moldova). The level of lipid peroxidation (LPO) was determined by the content of malonic dialdehyde (MDA) [7].

The results of the experiments were processed statistically.

Research results

The study of the effect of the preparation of anthracene derivatives (AP) was carried out both on intact old animals and in conditions of periodontitis modeling.

The state of collagen was determined in the gums and bones of the alveolar process. The drug, administered to intact animals, increased 2.1 times (p = 0.012) the content of bound hydroxyproline in the gingiva of rats as compared with the intact group (Table 1). The concentration of total hydroxyproline increased in the gum 1.9 times (p = 0.009).

Table 1

The influence of the preparation of anthracene derivatives on the content of hydroxyproline in periodontal tissues of rats (M ± m; p; p1)

Groups of animals	Hydroxyproline content (μmol / g)		
	Free	related	common
Gum			
Intact	0,75±0,22	1,13±0,16	1,88±0,27
AP	1,22±0,60	2,35±0,27 p=0,012	3,58±0,38 p=0,009
Model(M)	1,27±0,082	1,27±0,082	2,54±0,0010
M + AP	1,32±0,60	1,88±0,44	3,20±0,65 p=0,09
alveolar bone			
Intact	0,94±0,30	1,88±1,23	2,82±0,82
AP	4,47±1,78 p=0,07	3,53±1,23	8,00±1,09 p=0,009
Model(M)	1,18±0,27	1,41±0,41	2,59±0,27
M + AP	4,47±1,37 p=0,05 p_1=0,05	4,00±1,78	8,47±0,81 p=0,003 p_1=0,001

In the bone of the alveolar process, the drug increased the level of free hydroxyproline by 4.8 times (p = 0.07). Under the influence of the drug, the indicators of total hydroxyproline in the periodontal bone exceeded those of the intact group by 2.8 times (p = 0.009; Table 1).

The drug AP in the buccal mucosa increased by 67% (p = 0.05), and in the gum it slightly reduced the level of GAG (Table 2). In periodontal solid tissues, the GAG content under the influence of the drug increased by 17% (p = 0.04; Table 2).

Table 2

The effect of anthracene derivatives on the content of glycosaminoglycans in the tissues of the oral cavity of rats (M ± m; p; p1)

Groups of animals	GAG content(mg / g)		
	the mucous membrane of the cheek	gums	the alveolar bone of
Intact	1,22±0,32	2,35±0,34	3,53±0,00
AP	2,04±0,11 p=0,05	1,18±0,00 p=0,02	4,12±0,24 p=0,04
Model(M)	0,18±0,034 p=0,02	0,39±0,23 p=0,05	–
M + AP	1,59±0,10 p_1<0,001	1,18±0,00 p=0,02 p_1=0,02	1,96±0,46 p=0,02

The content of total soluble noncollagen protein in different tissues under the influence of the drug AP changed in different ways. Thus, in the liver and in the mucous membrane of the oral cavity (SAG and gums) the level of protein did not significantly differ from the data of intact groups (Table 3). In the bone of the alveolar process, the protein content increased under the influence of the drug by 20% (p = 0.02): 1.16 ± 0.031 mg / g versus 0.97 ± 0.049 mg / g.

Table 3

The influence of the preparation of anthracene derivatives on the content of soluble protein in the tissues of rats (M ± m; p; p1)

Groups of animals	Protein content (mg / g)			
	liver	the mucous membrane of the cheek	gum	Alveolar bone
Intact	1,38±0,18	0,14±0,015	0,20±0,040	0,97±0,049
AP	1,17±0,24	0,14±0,019	0,23±0,017	1,16±0,031 p=0,02
Model(M)	0,33±0,097 p=0,003	0,12±0,020	0,21±0,049	1,45±0,00
M + AP	1,31±0,16 p_1=0,003	0,15±0,019	0,24±0,054	1,09±0,26

A study of the effect of AP on mineral metabolism in periodontal bone tissue revealed the following. The drug tripled the content of Ca2 + ions (p <0.001) and did not significantly change the phosphate content in comparison with the data of intact groups (Table 4). The activity of alkaline phosphatase did not increase significantly (by 11%; p> 0.05; Table 4)

Table 4

The influence of the preparation of anthracene derivatives on the state of mineral metabolism in the periodontal bone tissue of rats (M ± m; p; p1)

Groups of animals	Activityalkaline phosphatase(nmol / s · g)	Contain	
		Ca (mmol/g)	P (mmol/g)
Intact	0,18±0,015	0,016±0,0012	0,016±0,0010
AP	0,20±0,010	0,048±0,00026 p<0,001	0,015±0,0021
Model(M)	0,24±0,035	0,0086±0,0017 p=0,014	0,011±0,0013 p=0,03
M + AP	0,35±0,053 p=0,04 p_1=0,12	0,049±0,020 p=0,13 p_1=0,08	0,011±0,0010 p=0,03

The drug in these experimental conditions significantly reduced the level of peroxide products in the bone of the alveolar process - the content of MDA decreased by 25% (p = 0.001; Table 5).

Table 5

The influence of the preparation of anthracene derivatives on the content of MDA in the tissues of rats (M ± M; p; p1)

Groups of animals	Содержание МДА (нмоль/г)		
	liver	gum	Alveolar bone
Intact	5,53±0,84	1,48±0,34	2,79±0,12
AP	7,59±1,65	2,16±0,15	2,08±0,15 p=0,001
Model(M)	18,1±2,50 p=0,003	2,07±0,023 p=0,01	1,91±0,38
M + AP	6,36±2,044 p_1=0,006	1,30±0,26 p_1=0,02	2,47±0,46

The effect of AP was studied in old rats also under conditions of periodontitis modeling using subgingival administration of collagenase.

The level of free and associated hydroxiproline gums under the influence of the drug in the periodontitis simulation conditions did not change significantly (Table 1). The total

hydroxyproline content of the gums increased 1.7 times (p = 0.09) as compared with the intact group, which almost corresponded to the data on the use of the drug in intact animals (Table 1). The preparation of AP in periodontal bone when modeling periodontitis increased the content of free hydroxyproline by 3.8 times (p1 = 0.05) compared with the "Model" group and 4.8 times (p = 0.05) compared with the intact group (table 1). The level of total hydroxyproline in the bone of the alveolar process was three times higher (p = 0.003) data of the intact group and 3.3 times (p1 = 0.001) - the data of the "Periodontitis Model" group. The content of bound hydroxyproline was not significantly changed (Table 1).

The preparation of AP in the gums and SES rats in the simulation of periodontitis increased the content of GAG 3 times (p1 = 0.02) and 8.8 times (p1 <0.001), respectively, compared with the "Periodontitis Model" groups (Table 2). At the same time, the GAG content in the bone of the alveolar process was lower than that of intact rats (Table 2).

The content of Mg2 + ions in the gum under the influence of the drug AP increased 2.9 times (p1 <0.001): 0.017 ± 0.0011 mmol / g versus 0.0059 ± 0.0020 mmol / g. In the bone of the alveolar process, the level of Mg2 + ions was not significantly changed compared with the "Periodontitis Model" group.

It is known that Mg2 + ions are necessary for the normal functioning of MKM, and their lack contributes to the slowing down of protein synthesis and deterioration of the mechanical properties of the gel forming the main substance of MKM.

The content of soluble noncollagen protein under conditions of periodontitis modeling under the influence of the drug AP increased in the liver by 4 times (p = 0.003): 1.31 ± 0.16 mg / g versus 0.33 ± 0.097 mg / g and corresponded to the data of the intact group - 1 38 ± 0.18 mg / g. The level of soluble protein did not change significantly in the table, the gingiva and periodontal bone tissue (Table 3).

The preparation of AP in terms of modeling periodontitis improved mineral metabolism in the bones of the periodontal animals (Table 4). Thus, it significantly increased the content of Ca2 + ions by 5.7 times (p1 = 0.08), and also increased by 1.5 times (trend; p = 0.12) alkaline phosphorus activity relative to the group "Model of periodontitis". The content of phosphate in this case was not significantly changed (Table 5).

Under the influence of the drug in the gum, the MDA content decreased 1.6 times (p1 = 0.02); 2.8 times - in the liver of rats (p1 = 0.006), which indicated a decrease in the level of POL processes in these research objects. In the periodontal bone tissue, the content of MDA was not significantly changed (Table 4).

Conclusion

Studies have shown that the preparation of anthracene derivatives from the herb St. John's wort in intact old animals improved the condition of periodontal collagen and gel, which forms the basis of the periodontal intercellular matrix and oral mucosa.

When reproducing the model of periodontitis in old rats, the preparation restored the structural and functional state of periodontal collagen and gang of the gums and the oral mucosa disrupted by modeling. The normalization of the state of the gum extracellular matrix was also confirmed by an increase in the content of Mg2 + ions in it. In the periodontal bone, the preparation of anthracene derivatives (AP) significantly improved mineral metabolism. The preparation of anthracene derivatives showed antioxidant properties, reducing the level of POL processes in the gums of old rats.

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