



# Benzofurocaine: effects on experimental periodontitis, anti-diabetic activity and molecular mechanisms of action

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## Abstract

**Introduction:** A promising compound for the treatment of inflammatory periodontal diseases is **benzofuracaine** (BFC). BFC has pronounced anti-inflammatory, analgesic, reparative, hypoglycemic and other effects. **Objective:** To assess the influence of **benzofuracaine** on experimental periodontitis and to study its antidiabetic activity and molecular mechanisms of action

**Materials and methods:** The work was performed on 232 white nonlinear rats of both sexes and 55 male mice. To assess the effect of BFC on experimental periodontitis, the amount of gingival fluid (AGF) was determined, an in-depth morphofunctional study of periodontal tissues was conducted, as well as the study of the biochemical composition of the gingiva and blood. Antidiabetic properties were studied using the glucose tolerance test (GTT), as well as the assessment of endothelium-dependent vasodilation and transmembrane K<sup>+</sup>-currents on an isolated aortic segment of rats with streptozotocin-induced diabetes mellitus (DM).

**Results and discussion:** In experimental periodontitis, BFC improves the morphological picture and decreases AGF, increases the level of blood microcirculation in the attached gingiva, has a normalizing effect on the prekallikrein-kallikrein system, reduces the synthesis of bradykinin, proinflammatory cytokines and PGE<sub>2</sub>, increases the level of VEGF, reduces the level of endothelin-1, increases the ATP content and NADH-ubiquinone-reductase activity, exhibits antioxidant properties, reduces the content of nitrosothiols and NO metabolites (NO<sub>2</sub>/NO<sub>3</sub>), increases the level of transferrin; when conducting a glucose tolerance test in mice, it reduces the level of glucose in the blood, but in this respect is inferior to glibenclamide, has a hypoglycemic effect in the early stages of diabetes (30 days), tends to restore endothelium-dependent dilatation of smooth muscle cells (SMC) of the aorta and does not affect the functioning of the K<sup>+</sup>-channels of the aortic SMC.

**Conclusion:** BFC is an effective drug for the treatment of periodontitis and can be recommended for further preclinical and clinical studies.

## Keywords

**benzofurocaine**, periodontitis, normoglycemia, experimental diabetes mellitus.

## Introduction

Pathology of periodontal tissues is a major problem in modern dentistry. Periodontitis dominates in the group of inflammatory diseases. Patients of any age can be affected by this disease, and the pathological process itself tends to be chronic (Kaner et al. 2009, Petersen et al. 2016).

The prevalence of chronic generalized periodontitis ranks second in the structure of dental pathology after caries (Petersen and Ogawa 2012).

The etiological factors underlying periodontitis are represented by a wide range of genetic, epigenetic, immunological, and microbiological disorders (Hajishengallis 2014, Hajishengallis 2015, Laine et al. 2012, Larsson et al. 2015, Rettori et al. 2014, Silva et al. 2015).

Periodontitis occurs in almost 100% of cases in patients with diabetes mellitus (DM) GJU (Lalla 2007) and is characterized by a pronounced aggressive course, specific morphological features of the lesion and high resistance to pharmacotherapy. The development of the pathogenetically sound principles for the treatment of inflammatory periodontal diseases against the background of diabetes remains an urgent task of modern dentistry (Ide et al. 2011, Preshaw et al. 2012, Zhou et al. 2015).

For the implementation of complex pharmacotherapy of periodontitis, various pharmacological drugs are used, among which are both nonsteroidal antiinflammatories and hypoglycemic drugs in case of diabetes, but they do not always result in the desired therapeutic effect, and sometimes can cause various side effects (Renvert and Persson 2016, Salvi and Lang 2005, Zandbergen et al. 2016).

Recently, much attention has been paid to the use of multitarget drugs, which are capable of specifically modulating several biological targets with one molecule. (Anighoro et al. 2014, Bansal and Silakari 2014). In this regard, of great interest is the Russian drug **Benzofurocaine** (BFC) – ethyl 4-chloro-6-[(dimethylamino)methyl]-5-hydroxy-2-methyl-1-benzofuran-3-carboxylate. This compound has obvious anti-inflammatory (mainly, antikinin) and analgesic (peripheral and central) effects, exhibiting local anesthetic, antiarrhythmic and antianginal properties, stimulating the processes of proteosynthesis, tissue respiration and oxidative phosphorylation, which has an antioxidant effect, with a positive effect on microcirculation; predetermines a decrease in blood sugar levels; has neither ulcerogenic nor nephrotoxic actions (Bedrosova et al. 2013, Bedrosova et al. 2015, Lebedev et al. 1989, Ornostaj and Stepniuk 1991).

These data served as the basis for studying the effect of BFC on the course of periodontitis against the background of normal and hyperglycemia caused by experimental (streptozotocin-induced) DM.

**Objective:** to assess the influence of **benzofuracaine** on experimental periodontitis and to study its antidiabetic activity and molecular mechanisms of action

## Materials and methods

The study was performed on 232 white nonlinear rats of both sexes, each weighing 190–390 g, and 55 male mice of 20–26 g each. The work complied with the requirements of the Law of the Russian Federation “On the Protection of Animals Against Cruel Treatment” of June 24, 1998, Good laboratory practice rules for preclinical studies in the Russian Federation (GOST 3 51000.3-96 and GOST R 53434-2009), Directives of the European Community (86/609 EC), the international recommendations of the European Convention for the Protection of Vertebrate Animals Used in Experimental Studies (1997) and the Laboratory Rules adopted in the Russian Federation (Order of the Ministry of Healthcare of the Russian Federation № 708 of 29.08.2010).

All the manipulations, species and the number of animals were approved by the local Bioethical Committee (Minutes No. 06-07/02-1).

**Modeling of experimental periodontitis (EP)** was performed according to the method described by A.M. Polson et al. (1983) and G.C. Keles et al. (2005). EP was created by applying tight ligatures on the gums under zolazepam-induced anesthesia (Zoletil by Virbac Sante Animale, France) (Fig. 1). Animals were monitored daily, from Day 43 to Day 61 after the start of the experiment.

**A comparative study of the effects from the standard therapy (ST) and from the combination of ST with BFC on periodontal tissues and the amount of gingival fluid (AGF) in rats with EP** AGF was determined by the intrasulcular method by N. Brill and B. Krasse (1958). A Schiller-Pisarev test was made (Nágel et al. 1970).

ST included oral irrigation with **chlorhexidine digluconate** (0.05%), intraperitoneal (ip) injection of **lincomycin hydrochloride** (0.1 ml/100 g), application of periodontal dressings (**anesthesin** – 1.0 g; **methyluracil** – 0.5 g; **metronidazole** – 0.25 g; **carotolin** – 1.0 ml; water dentine – 1.0 g). The course of ST was 12 days.

BFC was introduced into the composition of periodontal dressings, and also injected to the animals at a dose of 10 mg/kg. The course of administering BFC was 12 days.

**A study of the effect of BFC on the state of capillary blood flow in the gingival mucosa against the background of EP**

was carried out on 50 anesthetized (**urethane** 1.1 g/kg ip) rats. In the study of microcirculation, Laser Doppler flowmetry (LDF) was used (Krupatkin and Sidorov 2013). A light guide probe was fixed in a silicone splint for the lower incisors and placed on the attached gingiva (AG) on the labial side. The measurement time was 1 min. The average blood flow (M) was recorded under conditions of a healthy periodontium before administration of BFC at a dose of 10 mg/kg (ip) and then 5, 10, 20, 40, and 60 min after its administration.



**Figure 1.** Modeling of EP by applying ligatures on the gum adjacent to incisors

EP was modeled according to the method described by A.M.Polson et al. (1983) and G.C.Keles et al. (2005). The animals were randomized into 4 groups.

- 1) *Intact* – with a healthy periodontium (n = 10)
- 2) *Control* – with EP without treatment (12 days, isotonic NaCl solution was injected ip) (n = 10)
- 3) *Group of ST* – with EP and ST (12 days) (n = 10)
- 4) *Group of ST+BFC* – with EP and administering ST+BFC (BFC was used according to the method described above) (n = 10)

The parameters of the basal blood flow (M) were recorded before modeling EP, on Day 31 (against the background of established EP), Day 44 (after 12 days of treatment) and Day 74 (1 month after treatment). At the same time intervals, microcirculation was also studied in animals with a healthy periodontium.

***Effect of BFC on the activity of kallikrein-kinin, immune and endothelial systems with EP*** was studied on 33 rats:

- 1) *Intact* – without EP (n = 11);
- 2) *Control* – on Day 30 after simulating EP, the saline solution (in the equivalent volume) was injected ip once a day (n = 11);
- 3) *Group of BFC* – on Day 30 after simulating EP, BFC was injected at a dose of 10 mg/kg, also once a day. The efficacy of BFC was evaluated 10 days after the administration (n = 11).

In the homogenates of the gingiva, the content of endothelin-1 was determined, using the test system by R&D Systems for Human Endothelin-1 Immunoassay (UK),

and that of the vascular endothelial growth factor (VEGF) by means of a VEGF ELISA Kit (Quantikine human VEGF; R&D Systems, Minneapolis MN, USA).

The activity levels of kallikrein and prekallikrein were evaluated by a spectrophotometric method according to the rate of hydrolysis of N- $\alpha$ -benzoyl-L-arginine ethyl ester (Paskhina et al. 1974).

The level of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) was determined using an ELISA kit (Enzo Life Science, USA), sensitivity 13.4 pg/ml of PGE<sub>2</sub>, measuring range 39.1-2.500 pg/ml.

Plasma bradykinin concentration was determined by the enzyme immunoassay method (Enzyme Immunoassay system Bradykinin kit, Peninsula Lab., Inc. California, USA), and concentration of thromboxane A<sub>2</sub> – by ELISA method using kits by Assay Designs Correlate-EIA™ Thromboxane B<sub>2</sub>.

***A study of the effects of BFC on hypoxic syndrome, oxidative stress and nitric oxide metabolism under EP conditions*** was performed on 30 rats. The methods to simulate EP, to determine intracellular ATP, activity of succinate-ubiquinone-reductase and NADH-ubiquinone-reductase systems, the content of cytochrome C, lactate and pyruvate, the content of O<sub>2</sub> by measuring the inhibition of cytochrome C reduction by superoxide dismutase (SOD); the rate of H<sub>2</sub>O<sub>2</sub> formation using the fluorescent method and malonic dialdehyde (MDA), as well as to record the electron paramagnetic resonance (EPR) spectra on the RE-1307 radio spectrometer (Russia) were used according to Danhier and Gallez (2015), Keles et al. (2005), Polson and Zander (1983).

Determination of NO metabolites (NO<sub>2</sub>/NO<sub>3</sub>) in blood was performed spectrophotometrically by excretion of stable metabolites (ENOX) with prior deproteinization

of serum and reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  using granulated cadmium (Golikov and Nikolaev 2004).

All rats after EP simulation were randomized into 2 groups, control and experimental. To the animals of the control group ( $n = 10$ ) on Day 30 after EP simulation, normal saline was administered (in the same volume as BFC), in the experimental group ( $n = 10$ ), BFC was injected ip at a dose of 10 mg/kg once a day, and its efficacy was studied in periodontal homogenates 10 days after injection.

***A comparative study of the effect of ST and combination of ST with BFC on the morphofunctional state and regeneration of damaged gingival tissues during EP*** was carried out on 45 anesthetized (urethane 1.1 g/kg ip) rats. The animals were randomized into 3 groups:

*Control* – with EP without treatment ( $n = 15$ )

*Group of ST* – with EP with 14-day course of ST ( $n = 15$ )

*Group of ST+BFC* – with EP with 14-day course of ST+BFC ( $n = 15$ ).

Biopsy sampling of tissues from the marginal gingival area in rats with EP was performed before treatment and 28 days after the start of treatment.

The biopsy material was fixed in a 10% solution of neutral formalin and embedded in paraffin. Paraffin sections were stained using the following dyes and methods: hematoxylin and eosin; Mallory's method; alcianic blue (at pH 1 and 3), toluidine blue; the PAS alcian blue method (Lilly 1969) and Hotchkiss-McManus' method, with hematoxylin staining cell nuclei. In addition, the methods of combined staining were used: by aldehyde-fuchsin, chromotrope and aniline blue, alcian blue and neutral red.

In order to increase the objectivity of the results of the study, morphometry was used, which was performed using a stereometric counting grid. At the same time, the relative area occupied by inflammatory cell infiltrates, areas of sclerosis, as well as the area restored via restitution (complete regeneration) were determined. Assessment of vascularization of the regenerate was carried out by determining the percentage of the area of the own gingival plate with blood vessels.

***A comparative study of hypoglycemic activity of BFC and glibenclamide on the model of glucose tolerance test (GTT)*** was performed on 55 mice. To determine hypoglycemic properties in BFC, a model of glucose tolerance test was used, by means of a single subcutaneous injection of 40% aqueous glucose solution (3 g/kg) (Buzlama et al. 2013). Control and experimental animals were deprived of food for 12 hours. Sixty minutes prior to conducting the glucose tolerance test, 1% aqueous solution of BFC was once administered ip to the animals of the experimental group at the doses of 5 and 10 mg/kg. To the animals of the comparison group *Glibenclamide* was intragastrically administered once at a dose of 0.36 mg/kg. The glucose concentration (mmol/L) in blood was measured using a Bionime Rightest GS300 electronic glucose meter (Bionime GmbH, Switzerland) several times: 1) 5

times before drug administration; 2) after administration of drugs before injecting glucose; 3) 30, 60 and 120 minutes after the administration of glucose. In the control, glucose concentrations were measured 4 times - on an empty stomach, before glucose administration, and 30, 60, and 120 minutes after it.

Then, A.A. Pokrovsky's coefficient (C) was calculated (Buzlama et al. 2013) – the ratio of the difference between the maximum and initial levels of glucose in blood to the original level, expressed in %, according to Formula 1.

**Formula 1.** The calculation of A.A. Pokrovsky's coefficient

$$C = (B-A)/A \times 100 \quad (1),$$

where **A** is the initial glucose level, **B** is the maximum glucose level.

***A study of the antidiabetic properties of BFC, its effects on endothelial dysfunction and aortic  $\text{K}^+$ -channelopathy*** was performed on 40 rats. Diabetes was modeled using a single injection of *Streptozotocin* (65 mg/kg ip, once). *Streptozotocin* was dissolved in citrate buffer solution containing 0.9% NaCl and 10 mmol of citrate, pH = 4.6. In the experiment, the animals were used on  $30 \pm 7$  and  $90 \pm 7$  days after the induction of diabetes.

The glucose levels of the control ( $n = 8$ ) and diabetic rats ( $n = 32$ ) were measured before injection of *streptozotocin* every 30 days for 3 months of diabetes development and on the day of the experiment using a Bionime Rightest GS300 electronic glucose meter (Bionime GmbH, Switzerland). With administering BFC to diabetic rats ( $n = 16$ ), the glucose level was measured daily in all groups of animals. BFC was injected im at a dose of 15 mg/kg in the early (30 days,  $n = 8$ ) and late (90 days,  $n = 8$ ) periods of development of diabetes. BFC (200  $\mu\text{l}$ ) was injected twice a day for 5 days.

*Registration of vascular tone* was conducted on the prepared segments of the thoracic aorta. After pre-anesthesia (*ketamine* 45 mg/kg, *xylazine* 10 mg/kg), the animals were euthanized by decapitation. The thorax was opened, the aorta was isolated and transferred to Krebs solution at a temperature of 18–23° C. For animals with diabetes, the glucose concentration in the solution was increased to 25 mmol/l. The thoracic aorta was cut into circular segments, 1–2 mm wide.

Registration of contractile activity of vascular preparations was performed in isometric mode using voltage sensors (Danish Myo Technology, Aarhus, Denmark) and the LabScribe 2 software (World Precision Instrument Inc., USA).

To determine endothelium-dependent vasodilation, acetylcholine (AC) was used at a concentration of  $10^{-10}$ – $10^{-4}$  M for preparations previously contracted by means of phenylephrine (PE) at a concentration of  $10^{-6}$  M.

The values of the average effective concentration of AC are shown as a negative logarithm of AC concentration necessary for the development of a half-maximal response (pD<sub>2</sub>). This parameter was calculated using Formula 2.

**Formula 2.** Calculation of AC average effective concentration.

$$T = 100 / (1 + 10^{\text{Log}(x_0 - x) \times p}) \quad (2),$$

where **T** is the normalized level of tone (% of the initial level of contraction), **x** is the concentration of the test substance (expressed as negative logarithm),  $x_0$  is the middle point of the dose-response curve (i.e.  $T = 50\%$ ) and **p** is the curve slope factor. The pD2 totals were obtained by averaging pD2 calculated separately for each vascular preparation.

Transmembrane  $K^+$ -currents were studied using the method of fixing the membrane potential (patch-clamp) in the modification “whole-cell perforated patch”. SMC of vessels were isolated from the prepared thoracic aorta of rats. Ion currents were recorded and cells were stimulated using an Axopatch 200B amplifier (Axon Instruments Inc., USA), a Digidata 1200B analog-to-digital converter (ADC) (Axon Instruments Inc., USA) and pClamp Software (V.6.02, Axon Instruments Inc., USA).

**Statistical processing of research results** was carried out using the tables of the mean error values and the confidence interval of the arithmetic mean value of the variation series using Student’s t-test for paired and unpaired measurements (Dakhale et al. 2012, Glantz 2005), packages of licensed applications – Statistica 10.0, Origin 8.1 (OriginLab Corporation, USA), EXCEL 5.0 (Microsoft, USA) and Microsoft Office Professional Plus 2013; the differences were taken as significant at  $p \leq 0.05$ .

**Anesthesia and reference drugs:**

- Zoletil – 6% solution, 60 mg/kg ip (Virbac Sante Animale, France)
- Anesthezin – 1.0 g as part of the solution for gingival dressings (Pharmstandard-Ufa, Russia)
- Xylazine (Xyla) – 2% solution, 10 mg/kg ip (Interchemie, Netherlands)
- Ketamine – 5% solution, 45 mg/kg ip (Moscow Endocrine Factory, Russia)
- Methyluracil – 0.5 g as part of the solution for gingival dressings (Veropharm, Russia)

- Metronidazole – 0.25 g as part of the solution for gingival dressings (Galderma Laboratory, France)
- Carotoline – 1.0 ml as part of the solution for gingival dressings (Biostimulator LLC, Ukraine)
- Aquatic dentin – 1.0 g as part of the solution for gingival dressings (STOMADENT LLC, Russia)
- Chlorhexidine digluconate – 0.05% solution for irrigation (ROSBIO LLC, Russia)
- Lincomycin hydrochloride – 0.1 ml/100 g ip (Omela LLC, Ukraine)
- Glibenclamide (Maninil®) – 0.37 mg/kg ig (Berlin-Chemie AG/Menarini Group, Germany)
- Urethane (Diprivan®) – 1% solution, 1.1 g/kg ip (Astra-Zeneca UK Ltd, UK)

## Results and discussion

### A comparative study of the effect of ST and the combination of ST with BFC on the state of periodontal tissues and AGF in rats with EP

In rats with EP, the combination of ST+BFC showed the most pronounced therapeutic effect both in the immediate and distant periods of observation, which significantly exceeded only ST. The Schiller’s test was negative in all animals. AGF on Day 44 of the experiment decreased 2.6 times as compared with Day 31 and was close to the initial ones (Table 1).

After one month (on Day 74 of observation), pharmacotherapy (BFC+ST) showed that periodontal tissues in rats with EP did not differ much from their condition in the group of intact animals. In the group of rats where only ST was used, the symptoms of the pathological process (edema, hyperemia of the marginal edge, discharge from the gingival pockets) persisted in most animals.

### Effect of BFC on the state of capillary blood flow in the gingival mucosa in healthy periodontium and in rats with EP

*The effect of BFC on the microcirculation in a healthy periodontium.* At the intervals of 5, 10, 20 and 40 min-

**Table 1.** Effect of ST and Combination of ST with BFC on AGF in Rats with EP (M±m)

Type of impact, number of rats	AGF, mg		
	Before simulation	Day 31 of Experiment	Day 44 of Experiment
1. Intact periodontium (n = 10)	0.024±0.002	0.024±0.002	0.024±0.001
2. Periodontitis without Treatment (n = 10)	0.024±0.002	0.082±0.004	0.091±0.006*
3. Periodontitis+ST (n = 10)	0.024±0.002	$p_{1,2} < 0.001$	$p_{1,2} < 0.01$
		0.084±0.002*	0.053±0.001*
4. Periodontitis+ST+ BFC (n = 10)	0.024±0.006	$p_{1,3} < 0.001$	$p_{1,3} < 0.001$
		$p_{2,3} > 0.5$	$p_{2,3} < 0.001$
		0.089±0.003*	0.034±0.005
		$p_{1,4} < 0.001$	$p_{1,4} > 0.05$
		$p_{2,4} > 0.1$	$p_{2,4} < 0.001$
		$p_{3,4} > 0.1$	$p_{3,4} < 0.02$

**Note:** \*  $p < 0.001$  - in relation to the data in intact control;  $p < 0.001$  – in relation to the data before treatment, i.e. on day 31 of the experiment.

utes after the injection of BFC, the capillary perfusion decreased significantly. So, M at the above time intervals was  $19.9 \pm 0.2$ ,  $17.6 \pm 0.2$ ,  $19.1 \pm 0.3$  and  $20.4 \pm 0.3$  P.U., which is statistically significantly ( $p < 0.001$  in all cases except M at the 40<sup>th</sup> minute, for which  $p < 0.02$ ) lower – 7.0, 17.8, 10.7 and 7.0% lower than the initial value of  $21.4 \pm 0.2$  P.U. The most pronounced decrease in M was noted at the 10<sup>th</sup> minute of the study. By the 60<sup>th</sup> minute of the experiment, M was almost comparable with those at the outcome.

*The effect of BFC on microcirculation in AG in conditions of EP.* It was revealed that in animals with a healthy periodontium (intact) microcirculation parameters in the AG area were almost stable, as evidenced by the absence of statistically significant differences between the initial data and data on Days 31, 44 and 74 of the study: M was within the range of 21.8-22.4 (Table 2).

In the control group of animals on Days 31, 44, and 74, M was significantly ( $p < 0.001$  in all cases) – 37.1,

39.7, and 43.2 – lower than the outcome (22.9 P.U.) (Table 2).

The use of ST for 12 days in case of EP caused significant increase in M in animals M. So, on Day 44 and Day 74 of the study, M statistically significantly ( $p < 0.001$  in all cases) increased by 26.6 and 20.9. At the same time, M on Day 74 of the study, compared with that in Day 44, significantly ( $p < 0.05$ ) decreased by 4.5%. However, the positive changes in the microcirculation that occurred did not reach its baseline at any time during the study (Table 2).

When using the combination of ST+BFC in the conditions of EP, more significant changes in M were observed than was the case when only ST was used. So, on Day 44 and Day 74 of the study, the average level of microcirculation was statistically significant ( $p < 0.001$ ) increased by 40.8 and 53.5% (Table 2).

When comparing the parameters of microcirculation in AG of 4 groups of animals with one another, it was found out that the outcomes of M were almost comparable (Table 2).

**Table 2.** Effect of ST and Combination of ST+BFC on the State of Microcirculation (M, P.U.) in the AG Area of the Lower Incisors of Rats (M $\pm$ m, n = 10)

Initial data	Day of experiment		
	31; with EP	44; with EP after 12-day treatment	74; with EP after 1-month treatment
<b>Intact</b>			
22.4 $\pm$ 0.3	22.0 $\pm$ 0.3 $p_{1-2} > 0.05$ [-1.8]	21.8 $\pm$ 0.2 $p_{1-3} > 0.05$ [-2.7] $p_{2-3} > 0.05$ [-0.9]	22.2 $\pm$ 0.2 $p_{1-4} > 0.05$ [-0.9] $p_{2-4} > 0.05$ [+0.9] $p_{3-4} > 0.05$ [+1.8]
<b>Control</b>			
22.9 $\pm$ 0.3	14.4 $\pm$ 0.2 <sup>+++</sup> $p_{1-2} < 0.001$ [-37.1]	13.8 $\pm$ 0.3 <sup>+++</sup> $p_{1-3} < 0.001$ [-39.7] $p_{2-3} > 0.05$ [-2.1]	13.0 $\pm$ 0.3 <sup>+++</sup> $p_{1-4} < 0.001$ [-43.2] $p_{2-4} > 0.002$ [-9.7] $p_{3-4} > 0.05$ [-5.8]
<b>Group ST</b>			
21.8 $\pm$ 0.2	13.9 $\pm$ 0.3 <sup>+++</sup> $p_{1-2} < 0.001$ [-36.2]	17.6 $\pm$ 0.2 <sup>+++xxx</sup> $p_{1-3} < 0.001$ [-19.3] $p_{2-3} < 0.001$ [+26.6]	16.8 $\pm$ 0.3 <sup>+++xxx</sup> $p_{1-4} < 0.001$ [-22.9] $p_{2-4} > 0.001$ [+20.9] $p_{3-4} < 0.05$ [-4.5]
<b>Group ST+BFC</b>			
22.3 $\pm$ 0.3	14.2 $\pm$ 0.2 <sup>+++</sup> $p_{1-2} < 0.001$ [-36.3]	20.0 $\pm$ 0.3 <sup>+++xxx***</sup> $p_{1-3} < 0.001$ [-10.3] $p_{2-3} < 0.001$ [+40.8]	21.8 $\pm$ 0.3 <sup>xxxx***</sup> $p_{1-4} > 0.05$ [-2.2] $p_{2-4} < 0.001$ [+53.5] $p_{3-4} < 0.001$ [+9.0]

**Note:** The differences are statistically significant relative to: + - healthy periodontium, x- periodontitis without treatment, \* - periodontitis with use of ST; three signs -  $p < 0.001$ .

### Effect of BFC on the activity of kallikrein-kinin, immune and endothelial systems in EP in rats

The course of EP is accompanied by activation of the prekallikrein and a 45% increase in kallikrein. Course administration of BFC leads to inhibition of the synthesis of kallikrein by 23%, which is not observed in the control group of animals. At the same time, the content of prekallikrein increases by 22% relative to the group with EP and by 15.5% relative to the norm, which is a factor confirming a decrease in the formation of inflammation mediator bradykinin. Moreover, its level is reduced by 10.4%, but exceeds the control by 25% (Table 3).

In the course of the study, it was established that the content of pro-inflammatory interleukins increases in periodontal tissues, the content of IL-1 $\beta$  increases by 9%, TNF- $\alpha$  – by 21%, inflammatory mediator PGE<sub>2</sub> – by 40%, which is a consequence of activation of cyclooxygenase-2 (COX-2).

A decrease in the content of TNF- $\alpha$  to the norm and PGE<sub>2</sub> by 18% relative to the model of EP shows the activation by BFC of reparative processes in periodontal tissues and the reduction of the pain effect. At the same time, BFC does not affect the activity of COX-1, since the level of thromboxane A<sub>2</sub>, which is 31% higher with EP, does not change significantly. A close relationship between the PGE<sub>2</sub> level and bradykinin was also established ( $r = 0.8$ ,  $p < 0.001$ ).

The course application of BFC normalizes the level of the vasoconstrictor component of endothelin-1 (decreases by 50%) and activates VEGF by 64% relative to the norm. Such an increase in VEGF expression, along with a decrease in the level of IL-1 $\beta$  and TNF- $\alpha$  (by 21%) is considered as markers of lymphogenesis.

### Effect of BFC on hypoxic syndrome, oxidative stress and nitric oxide metabolism in rats with EP

Under the influence of BFC, the content of ATP, which was 26% lower in EP, increased by 15%, though there were no changes in the 20%-reduced content of cytochrome C, the activity of succinate dehydrogenase (SDH) and succinate-ubiquinone-reductase, and a 76% increase in

NADH-ubiquinone-reductase. The content of pyruvate more than doubled relative to the level observed in EP, and was two times higher than its content in normal tissue. The content of lactate under the influence of BFC increased by only 29%. Consequently, BFC dramatically activates aerobic glycolysis with the constant lactate/pyruvate ratio (Table 4).

Under the influence of BFC, a decrease in O<sub>2</sub> generation rate by 15%, in H<sub>2</sub>O<sub>2</sub> by 22%, in nitrosothiols by 13% and positive changes in the EPR signal against a 14% of NO (NO<sub>2</sub>/NO<sub>3</sub>) metabolites, as well as the absence of significant changes in MDA level indicates the ability of BFC to shift the imbalance in the pro-/antioxidant system towards the latter and the preparation having a pronounced antioxidant activity. An elevated level of NO metabolites has an adaptation value, since it can be an indicator of a decreased intensity of endothelium dysfunction, especially against the background of the normalization of half-width and EPR signal intensity, as well as a decrease in endothelin-1 (Table 4).

### A comparative study of the effect of ST and combination of ST with BFC on the morphofunctional state and regeneration of damaged gingival tissues in rats with EP

During the morphological study of biopsy specimens of the mucous membrane of the gingiva of animals with EP in the acute period of the disease, there were changes both in the epithelial covering of the gingiva and in its connective tissue basis (Fig. 2). In the gingival *lamina propria*, there was edema, circulatory disorders, fibrinoid changes in the walls of blood vessels and connective tissue structures, as well as massive inflammatory cellular infiltrate, in which mast cells were detected. The presence of neutrophilic leukocytes in the infiltrate characterized the intensity of the inflammatory process. It should be noted that inflammatory cellular infiltrates did not occupy the entire territory of the *lamina propria*. There were always areas free of infiltrates not only in the papillary, but also in the reticular layers.

The epithelial covering of the gingiva (Figs. 3 and 4) suffered from acanthosis, often along with the thickening and lengthening of acanthotic processes that go deep into

**Table 3.** Effect of BFC on the Activity of Kallikrein-kinin, Immune and Endothelial systems in rats with EP (M $\pm$ m, n = 11)

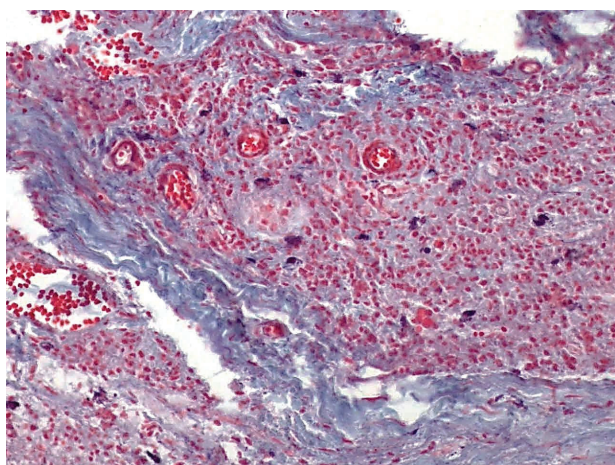
Marker	Intact	Control (Periodontitis)	Periodontitis + BFC
Tromboxan A <sub>2</sub> , ng/ml	427.0 $\pm$ 36.0	561.0 $\pm$ 82.0	531.0 $\pm$ 47.0
PGE <sub>2</sub> , ng/ml	18.4 $\pm$ 0.6	25.8 $\pm$ 2.0**	21.2 $\pm$ 1.1*
Prekallikrein, $\mu$ mol/ml per hour	71.0 $\pm$ 3.0	67.0 $\pm$ 4.0	82.0 $\pm$ 4.0 <sup>o</sup>
Kallikrein, mU/l	62.0 $\pm$ 4.0	90.0 $\pm$ 4.0***	69.0 $\pm$ 3.0 <sup>oo</sup>
Bradykinin, ng/ml	5.5 $\pm$ 0.2	7.7 $\pm$ 0.2***	6.9 $\pm$ 0.3 <sup>o</sup>
IL-1 $\beta$ , pg/ml	4.8 $\pm$ 0.3	5.25 $\pm$ 0.35	4.6 $\pm$ 0.3
TNF- $\alpha$ , pg/ml	10.8 $\pm$ 0.4	13.1 $\pm$ 0.6**	10.7 $\pm$ 0.5 <sup>o</sup>
Endothelin-1, fg/ml of protein	1.30 $\pm$ 0.08	2.58 $\pm$ 0.27***	1.25 $\pm$ 0.09 <sup>oo</sup>
EGF, fg/ml of protein	81.0 $\pm$ 11.0	104.0 $\pm$ 15.0	133.0 $\pm$ 9.0**

**Note:** reliability of differences in average values: when compared with the group indicator, the norm (control 1) – \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ; when compared with the indicator group control 2 (periodontitis) – <sup>o</sup> $p < 0.05$ , <sup>oo</sup> $p < 0.01$ , <sup>ooo</sup> $p < 0.001$ .

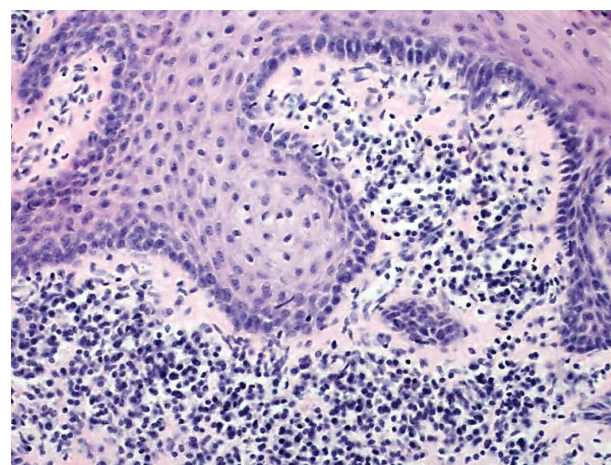
**Table 4.** Effect of BFC on the Indicators of Energy Supply, Oxidative Stress and NO Metabolism in rats with EP (M±m, n = 10)

Marker	Intact	Control (Periodontitis)	Periodontitis +BFC
Level of NO metabolites in plasma (nitrites/nitrates), $\mu\text{mol/L}$	12.3 ± 0.6	17.2 ± 0.6***	15.2 ± 0.5**°
Nitrosothiol content, $\mu\text{mol/g}$ of protein	16.6 ± 0.5	23.1±0.8***	20.2±0.8**°
O <sub>2</sub> <sup>-</sup> generation rate	1.98 ± 0.21	7.58±0.41***	6.44±0.27**°
Hydrogen peroxide, H <sub>2</sub> O <sub>2</sub>	1.52 ± 0.12	5.55±0.18***	4.63±0.25***°
Signal intensity	23.8 ± 0.9	15.6±0.6***	20.2±0.8***°
Half width of signal	6.9 ± 0.3	4.2±0.3***	5.3±0.3*°
FeS, g = 1.54	37.0 ± 2.0	32±1*	39±2°
FeS-NO, g = 2.03	5.3 ± 0.2	6.6±0.4**	6.3±0.4*
Transferrin	32.4 ± 1.9	22.3±1.3***	26.5±2.6
Oxidized Ceruloplasmin	21.1 ± 0.3	23.8±0.4***	21.2±0.9°
MDA, $\mu\text{mol/g}$ wet tissue	40.0 ± 2.0	44±3	44±2
ATP, $\mu\text{mol/g}$ wet tissue	1.83 ± 0.15	1.36±0.07	1.56±0.09
Cytochrome C, nmol/g wet tissue	8.2 ± 0.3	6.6±0.2***	6.7±0.2***
Lactate, mmol/g wet tissue	1.12 ± 0.12	1.19±0.07	1.45±0.16
Pyruvate, mmol/g wet tissue	0.075 ± 0.008	0.065±0.004***	0.15±0.07***°
Lactate/Pyruvate	19.9 ± 3.3	19.5±1.9	18.1±2.1
NADH-ubiquinone reductase, $\mu\text{mol}/(\text{mg of protein} \times \text{min})$	23.4 ± 0.8	17.4±0.5***	30.7±9.2***°
Succinate-ubiquinone reductase, $\mu\text{mol}/(\text{mg protein} \times \text{min})$	1.27 ± 0.06	0.88±0.04***	0.91±0.06***
SDH, nmol/mg protein	58.0 ± 2.0	55±2	59±4
Endothelin-1, fg/mg protein	1.30 ± 0.08	2.58±0.27***	1.25±0.09°

**Note:** the significance of differences in average values: when compared with the indicator of the intact group – \* p<0.05, \*\* p<0.01, \*\*\* p<0.001; when compared with the indicator control group (periodontitis) – ° p<0.05, °° p<0.01, °°° p<0.001.



**Figure 2.** Edema, plethora, foci of hemorrhages, fibrinoid changes in the walls of blood vessels, thick polymorphic cellular infiltration in the reticular zone of the gingiva. Mast cells in the composition of the infiltrate (Stained with aldehyde-fuchsin-chromotrope-aniline blue, ×200)



**Figure 3.** Acanthosis. Inflammatory cellular infiltrate (Stained with hematoxylin and eosin, ×400)

the lamina propria of the gingival mucosa and came in contact with inflammatory cellular infiltrate (Fig. 5).

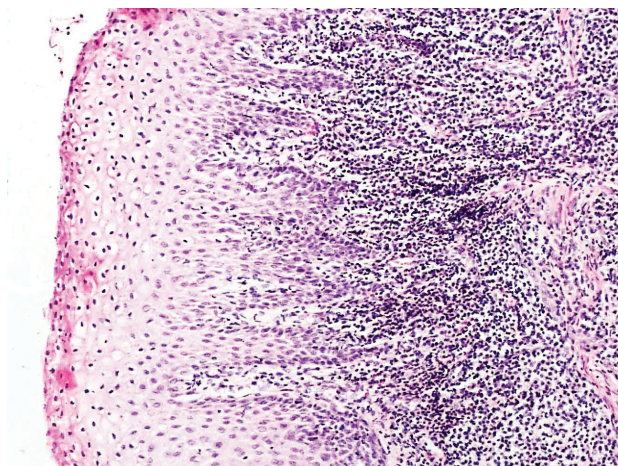
The thickness of the epithelial layer was  $376 \pm 18$  microns. A diapedesis of neutrophilic granulocytes was observed in hyperplastic epithelial tissue. As a result of focal accumulations of neutrophils, there appeared foci of suppuration with the melted superficial areas of the epithelial layer, which led to erosion of the mucous membrane (Fig. 6).

Alterative changes in the form of vacuole and even ballooning degeneration were often detected in the epithelial covering (Fig. 6). At the same time, foci with dystrophically altered cells were near the morphologically intact areas

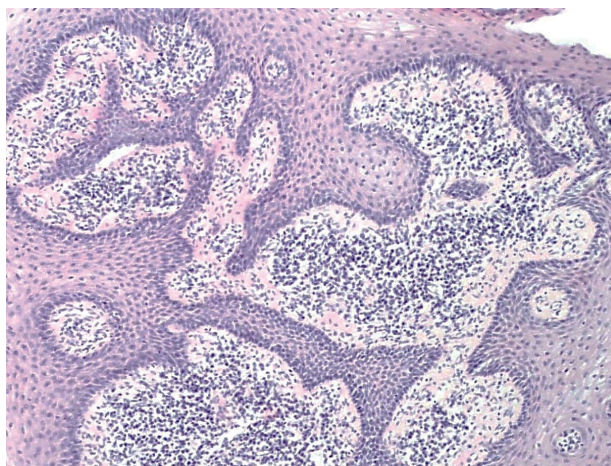
of the mucosa. The same cell focality was characteristic of the areas of excessive keratinization of the superficial areas of the epithelial covering (Figs. 7 and 8). Dyskeratosis of individual cells and their complexes was detected (Fig. 8).

*The results of the 14-day ST in animals with EP (comparison group).* In this group, the effectiveness of the 14-day course of ST (without the use of BFC) was morphologically evaluated in the study of biopsy specimens 28 days after the start of ST. In the lamina propria of the gingival mucosa, all signs of edema were completely stopped; there were neither hemorrhages, nor fibrinoid changes of blood vessels and connective tissue. In the area of

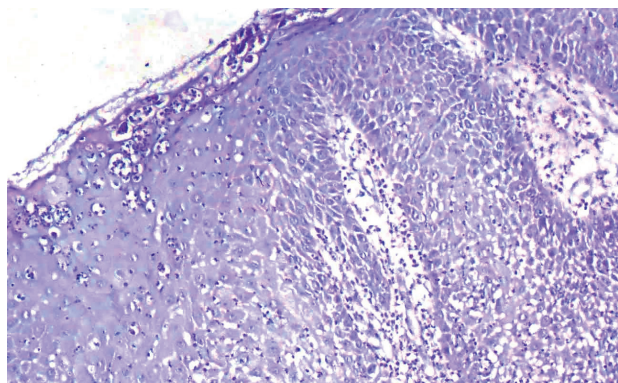




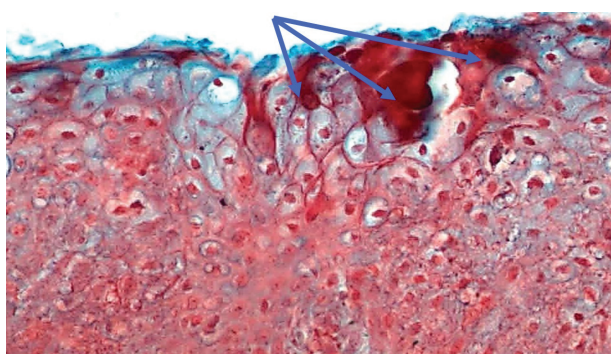
**Figure 4.** Acanthosis. Massive inflammatory cellular infiltrate. Vacuolar dystrophy of gingival epithelial cells (Stained with hematoxylin and eosin,  $\times 100$ ).



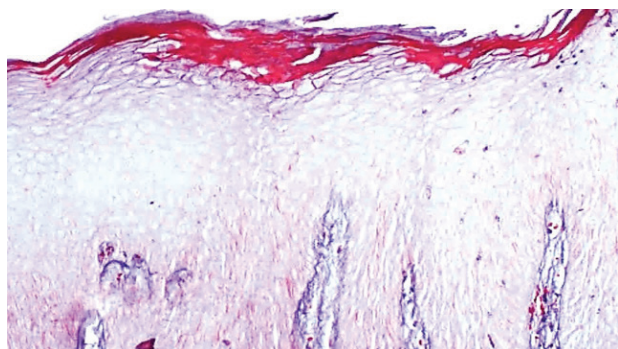
**Figure 5.** Rete acantotic pegs in contact with thick inflammatory cellular infiltrate (Stained with hematoxylin and eosin,  $\times 100$ )



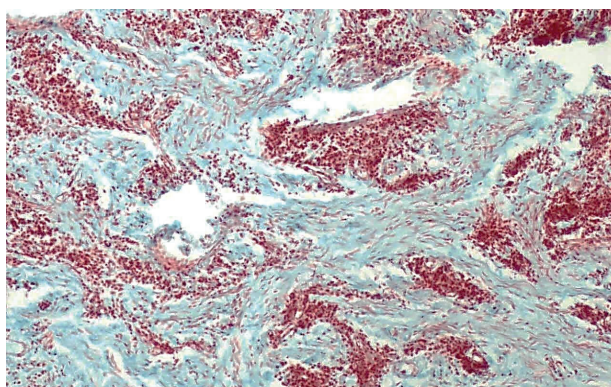
**Figure 6.** Acanthosis. Leucodiapedesis in the thickened epithelial layer. Multiple abscesses formed in the superficial area of the epithelial layer with the eroded mucous membrane (indicated with red arrows) (Stained with hematoxylin,  $\times 100$ )



**Figure 7.** Vacuole and ballooning degeneration of epithelial cells. Areas of dyskeratosis (indicated with blue arrows) (Stained with alcian blue–neutral red,  $\times 400$ )



**Figure 8.** Acanthosis. Excessive keratinization of the superficial areas of the epithelial covering (corneous masses are colored red). Vacuole degeneration of epithelial cells (“wicker basket”) (Stained with aldehyde-fuxin-chromotrope-aniline blue,  $\times 100$ )



**Figure 9.** Compact lymphohistiocytic infiltrates among fields of proliferative fibrous connective tissue (Stained with alcian blue with neutral red,  $\times 100$ )

massive cellular infiltrate, strands of fibrous connective tissue were formed (Fig 9); therefore, the area of sclerotic tissues increased 8.5 times (Table 5). A significant decrease in the thickness of the gingival epithelial cover-

ring was recorded; in it, however, there remained areas of impaired keratinization with accumulated oxyphilic and PAS-positive substances, as well as dystrophic changes in the superficial epithelium cells (Fig. 10).

Thus, under the influence of ST in the lamina propria of the gingival mucous membrane, there was observed positive dynamics in the treatment. However, at the same time incomplete regeneration of recovering tissues clearly prevailed. Sclerotic tissue was detected on the area, which accounted for more than 45% of the entire reticular layer.

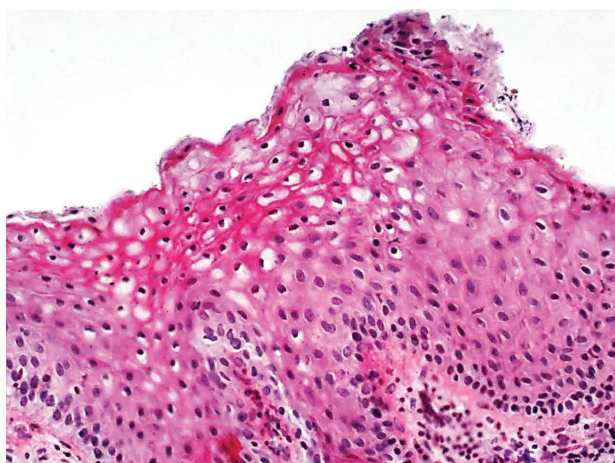
The results of the 14-day ST+BFC in animals with EP (main group). In this group of animals, as a result of adding BFC to the complex of ST treatment measures, a more favorable effect was obtained, which affected the regenerative processes in the gingiva as compared with only the basic ST. Along with reverting morphological signs of inflammation, an ability of BFC to enhance angiogenesis became apparent. At the same time, adding BFC to pharmacotherapy of rats with EP resulted in the vascularization index increasing more than 2.9 times (Table 5). The resulting regenerate contained a network of newly formed blood vessels (Figs. 11–13), which contributed to the activation of high-level metabolic processes in the tissues of the regenerate and eliminated hypoxia.

Due to such changes, the structure of the gingival epithelial covering was normalized, and dystrophic processes in epithelial cells were effectively eliminated. The thickness of the epithelial covering decreased 2.2 times in comparison with this indicator in the group before treat-

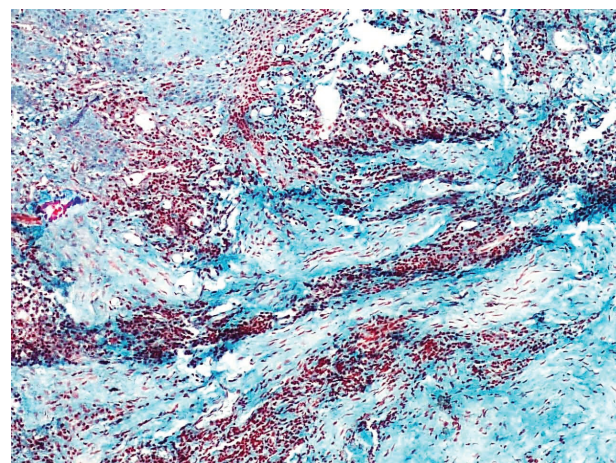
ment, and the character of keratinization also became normal. Along with the areas of incomplete regeneration with proliferative nonspecific fibrous tissue, almost throughout the entire network of the reticular layer, significant areas were constantly detected, restored by the full regeneration type, i.e. loose connective tissue was formed, identical to that which had been there before it was damaged initially (Figs. 14–15).

BFC, additionally included in ST, determined the complete restoration of the structure of the normal gingival reticular layer in the area of  $31.5 \pm 3.2\%$ . The area of sclerotic fields without BFC was  $45.0 \pm 3.3\%$ , whereas with the addition of BFC, this indicator decreased more than 1.6 times (Table 5). These data are objective evidence that there is a possibility in principal to considerably increase by means of BFC the share of full regeneration in the gingival connective tissue basis with the normalization of the morphology of its epithelial covering.

Thus, when carrying out morphological studies, BFC was found to act as a stimulant and corrector for restorative processes in the gingiva. In combination with ST, it stimulates angiogenesis, increasing vascularization of the regenerate 2.9 times, which ensured the delivery of necessary substances to regenerating tissues. The developed vascular network served as a source for supplying regenerating tis-



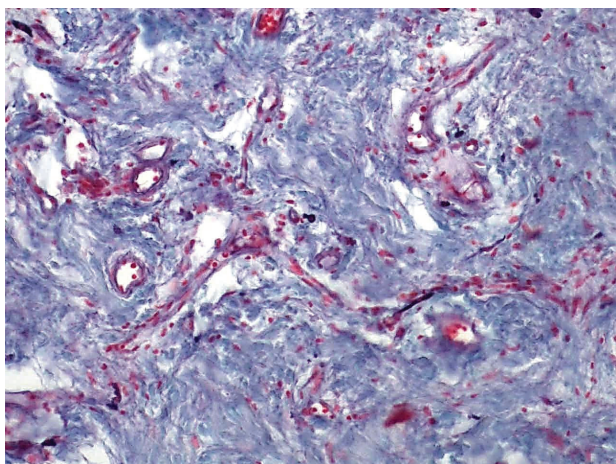
**Figure 10.** Vacuole degeneration of epithelial cells. Increased content of PAS-positive substances in them (stained with hematoxylin and according to Hotchkiss-McManus' method,  $\times 200$ )



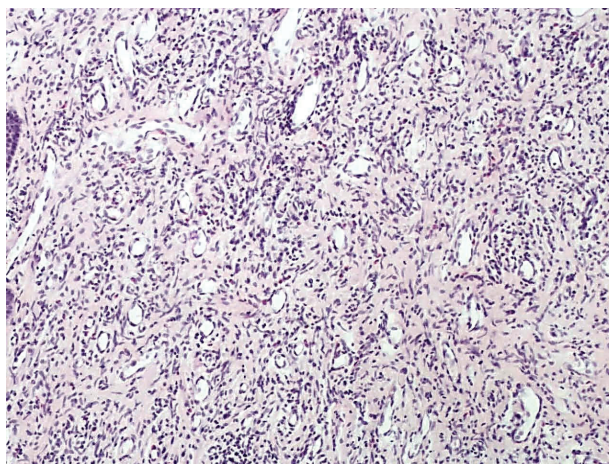
**Figure 11.** Pronounced vascularization of cellular infiltrates separated by strands of fibrous connective tissue. In the lower right corner, there is loose connective tissue in the area of restitution (Stained with alcian blue and neutral red,  $\times 100$ )

**Table 5.** The Results of Morphometric Studies of Gingival Biopsy Specimens in Treatment of EP in rats ( $M \pm m$ ,  $n = 15$ )

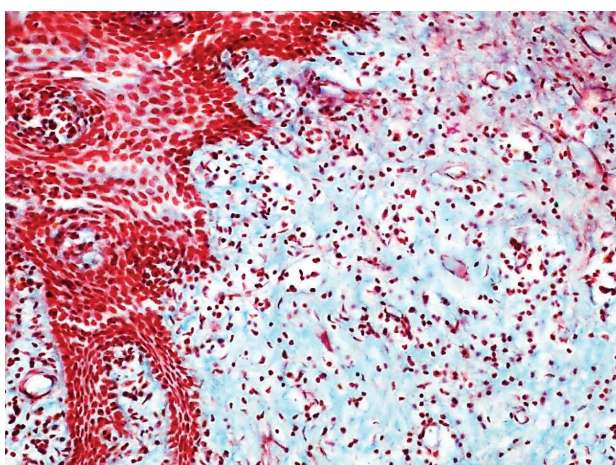
Groups of animals	Average thickness of the epithelial covering of gingiva, $\mu\text{m}$	Area of the vascular bed in the reticular layer of gingiva, %	Total area of cellular infiltrates in the gingival connective tissue base, %	Total area of sclerotic tissue in gingiva, %	Total area of the connective tissue basis of gingiva, restored by restitution type, %
Before Treatment (1)	$376 \pm 18$	$5.1 \pm 0.4$	$87.2 \pm 8.4$	$5.3 \pm 0.8$	–
ST (2)	$254 \pm 22$	$5.2 \pm 0.5$	$44.4 \pm 4.0$	$45.0 \pm 3.3$	$5.1 \pm 1.4$
	$p_{1-2} < 0.001$	$p_{1-2} > 0.5$	$p_{1-2} < 0.001$	$p_{1-2} < 0.001$	
ST+BFC (3)	$171 \pm 21$	$14.9 \pm 1.3$	$26.6 \pm 2.5$	$27.0 \pm 1.4$	$31.5 \pm 3.2$
	$p_{1-3} < 0.001$	$p_{1-2} < 0.001$	$p_{1-3} < 0.001$	$p_{1-3} < 0.001$	$p_{2-3} < 0.001$
	$p_{2-3} < 0.001$	$p_{2-3} < 0.001$	$p_{2-3} < 0.001$	$p_{2-3} < 0.001$	



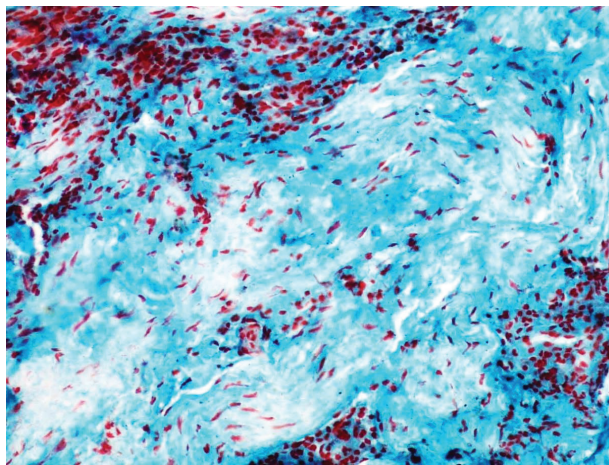
**Figure 12.** The network of newly formed small blood vessels in the composition of the formed regenerate (Stained with aldehyde-fuchsin-chromotrope-aniline blue,  $\times 200$ )



**Figure 13.** Multiple vascular lumens in the area of the reticular layer, restored by the full regeneration type (Stained with hematoxylin and eosin,  $\times 100$ )



**Figure 14.** Vascularized restitution area in the gingival reticular layer. In the loose connective tissue, the main substance clearly dominates over the fibrous structures (Stained with alcian blue-neutral red,  $\times 200$ )



**Figure 15.** Loose connective tissue in the focus of complete regeneration (stained with alcian blue with neutral red,  $\times 200$ )

sues with specialized fibroblasts capable of creating a connective tissue matrix, similar to that before its damage by the inflammatory process (Serov et al. 1998). At the same time, the capacity of tissues for a more perfect, namely, organotypic regeneration of the mucosal *lamina propria* (its share increased 6.2 times) was more pronounced in combination with the reduction of alternative manifestations in the epithelial covering. Under the influence of ST only (without BFC), regeneration in the gingiva was mainly characterized by the replacement of damaged tissues with nonspecific fibrous tissue, which eliminated neither trophic disturbances, nor the hypoxic effects on the superficial epithelium.

Special emphasis should be laid on the fact that the studies based on the analysis of morphological criteria made it possible for the first time to establish that BFC has a pronounced angioprotective effect and also stimulates the processes of full regeneration; at the same time

the ability of the damaged gingival tissues to restore the typical (original) structure of the gingival mucosa lamina propria increases along with the normalization of the structure of its epithelial covering.

#### **The influence of BFC and glibenclamide on the indices of hypoglycemic activity in the GTT**

In the animals of the control group, the fasting blood glucose concentration was  $4.37 \pm 0.32$  mmol/L. With GTT, the highest level of glycemia was observed 30 minutes after the administration of glucose and was  $11.98 \pm 0.71$  mmol/L, which is statistically significantly 2.7 times higher than the initial level. After 60 min, the concentration of glucose in blood decreased to  $9.49 \pm 0.63$  mmol/L, which was significantly 1.3 times lower than the previous measurement of glucose concentration and 2.2

times higher than the initial level. After 120 min, the concentration of glucose in blood was  $5.97 \pm 0.47$  mmol/L, and compared with the initial level, it was significantly 1.4 times higher. A.A. Pokrovsky's coefficient (Buzlam et al. 2013) was found to be 174.1, which indicates glucose intolerance.

When glibenclamide (0.36 mg/kg) was used, 60 minutes after its administration, there was a statistically significant 1.3-fold decrease in fasting blood glucose (to  $4.45 \pm 0.48$  mmol/L) compared with the baseline values ( $5.90 \pm 0.49$  mmol/L). Under GTT conditions, 30 minutes after glucose administration, the glycemia level was significantly 1.4 times lower compared to the absolute value of the corresponding indicator in the control ( $8.63 \pm 0.62$  mmol/L versus  $11.98 \pm 0.71$  mmol/L). After 60 and 120 minutes, affected by glibenclamide, when compared with the control data in absolute values ( $6.25 \pm 0.42$  and  $3.87 \pm 0.19$  mmol/L versus  $9.49 \pm 0.63$  and  $5.97 \pm 0.47$  mmol/L), glycemia was significantly 1.52 and 1.54 times less. A.A. Pokrovskiy's coefficient turned out to equal 46.3, which shows that glibenclamide eliminates glucose tolerance.

BFC at doses of 5 and 10 mg/kg 60 minutes after the injection reduced (by 18.8 and 15.1%, respectively) fasting blood glucose. With GTT 30 minutes after the administration of glucose, the glycemia level became 1.4 and 1.5 times lower than the absolute value of the corresponding indicator in the control ( $8.64 \pm 0.69$  and  $7.92 \pm 0.39$  mmol/L versus  $11.98 \pm 0.71$  mmol/L) and 146.0 and 140.0% lower than the initial value. After 60 minutes, affected by BFC, when compared with the control by absolute values ( $7.50 \pm 0.70$  and  $6.50 \pm 0.34$  mmol/L versus  $9.49 \pm 0.63$  mmol/L), glycemia was significantly 1.3 and 1.5 times (or by 21.0 and 31.5%) less, and after 120 minutes – was hardly different from the control. Pokrovsky's coefficients when using BFC at doses of 5 and 10 mg/kg, respectively,

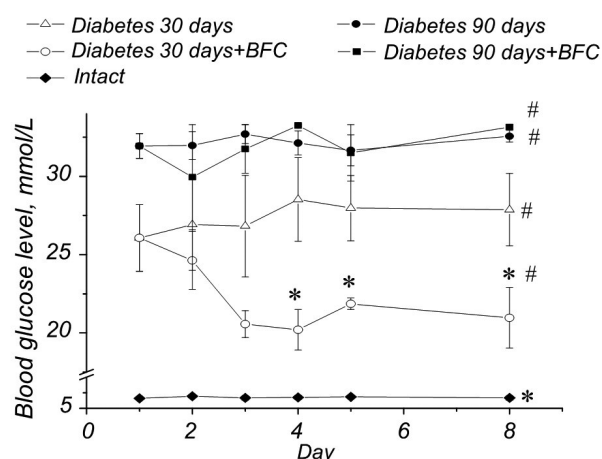
were 43.5 and 52.9, which indicates the decreasing influence of this drug on glucose tolerance.

### The effect of BFC on glucose concentration in plasma of rats with experimental diabetes

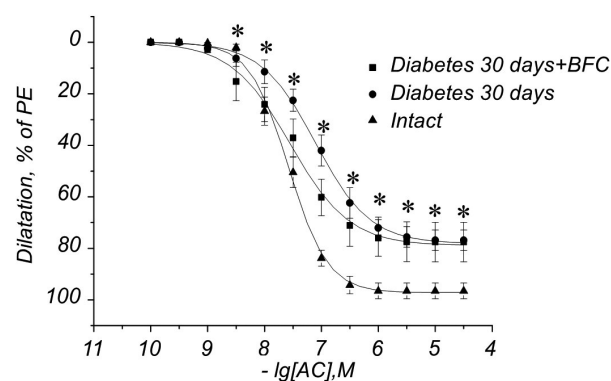
The level of blood glucose after the 30<sup>th</sup> day of development of diabetes was  $27.9 \pm 2.55$  mmol/L ( $n = 16$ ,  $p < 0.05$ ), and after the 90<sup>th</sup> –  $32.6 \pm 0.38$  mmol/L ( $n = 16$ ), which is significantly higher ( $p < 0.05$ ) than that in the intact animals ( $6.7 \pm 0.38$  mM/L,  $n = 8$ ). On Days 2, 4, and 5, BFC contributed to a significant decrease in the level of glucose (Fig. 15). The injection of BFC did not affect the blood glucose level of rats after the 90<sup>th</sup> day of development of diabetes (Fig. 16).

**A study of endothelium-dependent dilatation of isolated aortic preparations** showed that the application of AC at a concentration of  $10^{-10} - 3 \times 10^{-5}$  mmol/L caused a dose-dependent dilatation of isolated aortic preparations with a maximum of  $96.5 \pm 3.05\%$  of the precontraction of the preparations caused by PE at a concentration of  $10^{-6}$  mmol/L ( $n = 8$ ) (Fig. 17). The value of pD<sub>2</sub> was  $7.59 \pm 0.08$  ( $n = 8$ ).

In the aorta preparations of rats with experimental diabetes, there was a significant suppression of endothelium-dependent dilatation on AC with a maximum of  $76.88 \pm 3.98\%$  ( $n = 8$ ,  $p < 0.05$ ) with a duration of diabetes of  $30 \pm 7$  days (Fig. 17) and  $78.05 \pm 5.57\%$  ( $n = 8$ ,  $p < 0.05$ ) with the duration of diabetes  $90 \pm 7$  days (Fig. 17). The value of pD<sub>2</sub> with respect to the control decreased to  $7.13 \pm 0.09$  ( $n = 8$ ,  $p < 0.05$ ) and  $6.7 \pm 0.18$  ( $n = 8$ ,  $p < 0.05$ ). The shift of the dose-response curve, characterized by the pD<sub>2</sub> parameter, is an indicator of the changing sensitivity of vascular preparations to the action of the test substance. In this case, the curve shifts to the left.



**Figure 16.** Blood glucose level in rats (control), diabetic animals on the 30<sup>th</sup> and 90<sup>th</sup> day after administration of streptozotocin, diabetic rats after the course of administration of BFC (diabetes 30 days + BFC; diabetes 90 days + BFC). Note: #  $p < 0.05$  with respect to the control, \*  $p < 0.05$  with respect to diabetic rats.

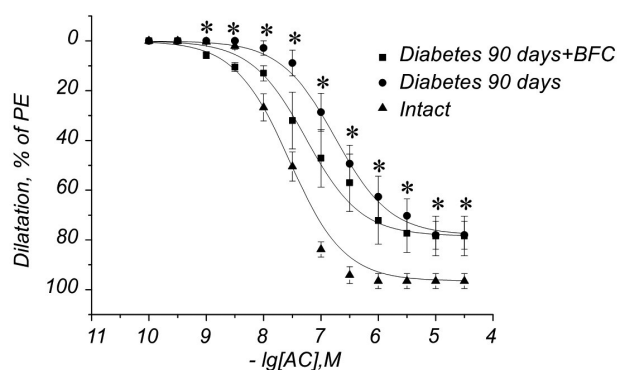


**Figure 17.** Dilator reactions of isolated rat aorta segments, previously contracted by PE ( $10^{-6}$  mmol/L), under the influence of AC ( $10^{-10} - 3 \times 10^{-5}$  mmol/L) in aorta of control animals (control), diabetic animals at early stages of the development of diabetes (diabetes 30 days) and diabetic animals after the course of administration of BFC (diabetes 90 days + BFC). Note: \*  $p < 0.05$  with respect to the control

The course administration of BFC did not cause changes in the maximum amplitude of endothelium-dependent dilatation of aortic preparations of diabetic rats under the influence of AC at the early stages (Fig. 17) and at the later stages of the development of diabetes (Fig. 18). The maximum vasodilation in this case was  $77.53 \pm 7.64$  (n = 8,  $p < 0.05$ ) and  $78.39 \pm 7.95\%$  (n = 8,  $p < 0.05$ ), respectively. At the same time, an increase in the pD2 value to  $7.57 \pm 0.19$  (n = 8,  $p > 0.05$ ) was observed at the early stages of the development of diabetes, which had no significant differences from the indicators of healthy animals, and to  $7.05 \pm 0.21$  (n = 8,  $p < 0.05$ ) at the later stages of the development of diabetes.

The data obtained indicate that the course introduction of BFC to diabetic animals does not affect the maximum amplitude of the endothelium-dependent aortic dilatation suppressed in conditions of DM; however, it increases the sensitivity of the aorta to the action of AC. It should be noted that the positive effect of BFC depends on the duration of the development of diabetes.

**Total outward  $K^+$ -current isolated SMC of aorta** was studied on diabetic animals after the 30<sup>th</sup> day of the injection of streptozotocin. The total outward  $K^+$ -current was recorded in response to the step-by-step depolarization of the isolated SMC membrane from -100 to 70 mV every 3 seconds at a maintained potential of -60 mV. The total outward  $K^+$ -current in the SMC of control animals with a maximum membrane depolarization level of -70 mV was  $51.4 \pm 4.76$  pA/pF (n = 8). The density of the outward current in the aortic SMC of diabetic animals was lower than in the control and was  $18.1 \pm 1.84$  pA/pF (n = 10,  $p < 0.05$ ). The course injection of BFC did not cause changes in the density of the outward  $K^+$ -current, which at the maximum depolarization level of -70 mV did not differ from the indicators of the diabetic animals and was  $17.8 \pm 1.38$  pA/pF (n = 14,  $p > 0.05$ ) (Fig. 19).



**Figure 18.** Dilator reactions of isolated rat aorta segments previously reduced by PE ( $10^{-6}$  mmol/L), under the influence of AC ( $10^{-10}$ – $3 \times 10^{-5}$  mmol/L) in aorta of control animals (control), diabetic animals at later stages of the development of diabetes (diabetes 90 days) and diabetic animals after the course administration of BFC (diabetes of 90 days + BFC). Note: \*  $p < 0.05$  in comparison to the control

## Conclusion

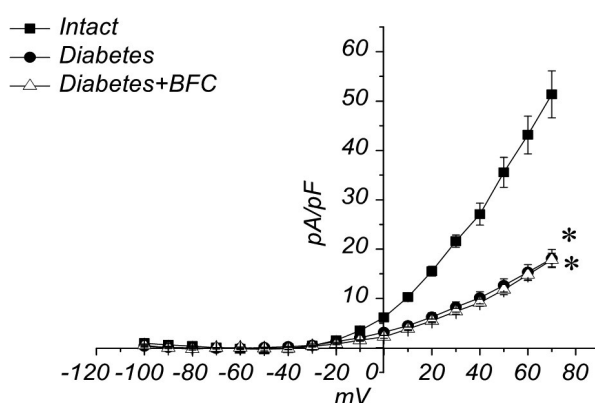
The use of the combination of BFC with ST in rats with EP has a pronounced therapeutic effect both in the short-term (on the 11<sup>th</sup> day) and long-term periods after treatment (after 1 month), significantly exceeding ST, carried out separately.

In case of healthy periodontium, BFC reduces the flow of erythrocytes and its variability in the capillary bed of AG of lower incisors in rats; at the same time while vasomotor activity remains stable compared to the baseline, which may be due to an increase in total peripheral vascular resistance.

The use of the combination of BFC with ST in EP causes a significant increase in microcirculation in AG of rats, both immediately after the 12-day treatment and 1 month after its completion, with microcirculation reaching the level like it was before periodontitis simulation, while when used separately, ST induces an increase in microcirculation in the selected area only after treatment, whereas microcirculation decreases 1 month later.

The improvement of the dental status and a decrease in the periodontal index in rats with EP affected by BFC course administration is accompanied by normalization of the prekallikrein-kallikrein system, and a decrease in the synthesis of bradykinin. The anti-inflammatory effect of BFC may be due to a decrease in the level of pro-inflammatory cytokines and  $PGE_2$ ; there is a close relationship between the levels of the latter and bradykinin. BFC significantly increases the level of VEGF and reduces that of vasoconstrictor endothelin-1, which may indicate stimulation of the growth of lymphatic vessels and the capillary network in periodontal tissues.

Using BFC, which has a pronounced anti-inflammatory effect, along with inhibiting COX-2 and anti-bradykinin activity, in the treatment of periodontitis, will help elimi-



**Figure 19.** Volt-ampere characteristic of the total outward  $K^+$ -current membrane of isolated aortic SMC of control rats (control), diabetic animals after the 30<sup>th</sup> day of development of diabetes (diabetes) and diabetic animals after the course administration of BFC (diabetes + BFC). Note: \*  $p < 0.05$  in comparison to the control

nate neurohumoral imbalance of interleukin-1, thromboxane A<sub>2</sub>, TNF- $\alpha$ , endothelin-1 and prostaglandins.

BFC has a positive effect on the energy supply system of periodontal tissues, increasing the ATP content and activating NADH-ubiquinone reductase, and also stimulating the processes of aerobic glycolysis in rats with EP. The drug reduces the intensity of hypoxic syndrome, which is due to its antioxidant activity – the ability to suppress the generation rate of superoxide anion, hydrogen peroxide, to reduce the content of nitrosothiols and NO metabolites (NO<sub>2</sub>/NO<sub>3</sub>), and to increase the level of transferrin.

BFC in combination with ST in rats with EP stimulates angiogenesis, increases vascularization of the regenerate, which ensures the delivery of necessary substances to the regenerating tissues. At the same time, the developed vascular network serves as a source supplying the regenerating tissues with specialized fibroblasts which create a connective tissue matrix, similar to that before its damage by the inflammatory process. In this context, the ability of the tissue to more perfect organotypic regeneration of the mucosal lamina propria in combination with reduction of alternative manifestations in the epithelial covering is more pronounced. ST, carried out separately (without BFC), causes, as a rule, sclerotic changes, with no normalization of the morphofunctional state of the gingival epithelial cover occurring.

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BFC, when GTT is made in mice, like glibenclamide, is capable of reducing the blood glucose level, but the degrees of hypoglycemic effect of BFC and glibenclamide are different, with glibenclamide having a more pronounced effect. In intact animals, BFC has no significant effect on fasting blood glucose levels; the detected hypoglycemic effect of the drug in GTT should be regarded as normoglycemic.

In streptozotocin diabetes in rats, BFC has a hypoglycemic effect on early diabetes (30 days), while it tends to restore endothelium-dependent dilatation of the aortic SMC; has no significant effect on these indicators during late periods of diabetes (90 days) and has no effect on the functioning of the K<sup>+</sup>-channels of the aortic SMC during both periods of the study. The hypoglycemic effect of BFC may be due to its antioxidant, antihypoxant and anti-inflammatory properties, as well as its ability to increase the activity of NAD and NADPH-dependent  $\beta$ -cells of islets of Langerhans.

## Conflict of interests

The authors state no conflict of interest concerning with the present submitted manuscript.

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