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Research Article

The effectiveness of Soderm® – forte gel and a new injectable dosage form of Rexod® in the complex treatment of experimental periodontitis in rats

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Abstract

Introduction: Insufficient effectiveness of traditional drug therapy in a treatment of patients with chronic generalized periodontitis, as well as high social significance of this problem, determines the need to search for new drugs and their compositions aimed at solving it.

Aim of the study: To increase the efficacy of complex treatment of periodontitis with the administration of Soderm®-Forte gel and a new injectable form of Rexod®.

Materials and methods: Experiments were performed in 50 male Wistar rats. Experimental periodontitis (EP) was simulated by ligation of the necks of lower incisors. We studied the animals with intact periodontium, untreated EP, and when traditional drug therapy (TDT), as well as the combinations of TDT with Soderm®-Forte gel and additionally with the new injectable dosage form (NIF) of Rexod® were administered. The general condition, behavior, nutrition and body weight of the animals were evaluated. The Schiller-Pisarev test and the Muhlemann-Cowell bleeding index were used, and the amount of crevicular fluid (CF) was measured. The contamination of the marginal gum with microorganisms was determined.

Results and discussion: The TDT in EP has a moderate therapeutic effect, which does not lead to a sufficiently high pharmacotherapeutic effect, whereas the combinations of TDT with Soderm®-Forte and, to a greater extent, TDT with Soderm®-Forte and NIF of Rexod® have high therapeutic efficacy, which is statistically confirmed by a sharp decrease in the amount of CF, the Schiller-Pisarev test and the Muhlemann-Cowell bleeding index, as well as absolute suppression of pathogenic microorganisms.

Conclusion: The combinations of TDT with Soderm®-Forte gel and NIF of Rexod® in EP in rats can significantly increase the effectiveness of the treatment. The data obtained indicate the expediency of the administration of Soderm®-Forte gel, as well as its combination with NIF of Rexod® in dental practice in the complex therapy of patients with periodontitis.

Keywords

crevicular fluid, new injectable dosage form (NIF) of Rexod®, antimicrobial effect, Soderm®-Forte, experimental periodontitis.

Introduction

According to Miklyaev et al. (2018) and Gursoy et al. (2018), the morbidity of patients suffering from pathological changes in the periodontal complex is 95–100%. In this aspect, chronic generalized periodontitis (CGP) prevails, which causes not only a disorder of the periodontal function of individual teeth but also leads to degradation of the entire dental system (Miklyaev et al. 2018; Yanushevich and Dmitrieva 2018).

Despite the presence of a significant number of different pharmacotherapeutic regimens and methods of complex treatment of CGP, this nosological pathology continues to be relevant (Tsepov et al. 2019).

In the development of CGP, a crucial role is shown to be played by changes in homeostasis of a body initiated by various factors, among which periodontal pathogenic bacterial flora plays a significant role, causing the formation of an inflammatory process in the periodontium, manifested by cellular and tissue infiltration, emigration of leukocytes to the focus of inflammation, which can produce mediators of secondary alteration and generate reactive oxygen species (ROS), causing oxidative stress (Kovalevskiy and Kovalevskiy 2018; Leontev et al. 2020).

A list of drugs that affect the pathogenic microflora, taking part in the development and progression of CGP is very extensive and includes antiseptics and antibiotics. However, in clinical practice, they do not always lead to the expected effect – sustained remission and especially complete recovery, which is largely due to the development of resistance of microorganisms to antibacterial drugs, in particular to antibiotics, which do not always reach a sufficient concentration in the crevicular fluid and microbial film, which, as is known, is a protective barrier that prevents the action of antimicrobial drugs on bacterial cells.

Silver ions, in particular silver nanoparticles (unlike the former, these have a greater activity and safety for humans), have a wide spectrum of bacteriostatic and bactericidal effects, including antibiotic-resistant gram-positive and gram-negative strains. Silver ions have a short-term blocking effect on the channels, through which vital substances enter the bacteria, while silver nanoparticles destroy the bacterial cell membranes, causing their instant death, and continue to show the antibacterial effect. It is noteworthy that various naturally occurring biologically active substances can be transferred to the microorganism on the surface of nanoparticles. The effect of silver nanoparticles is directly related to their size and shape: the smaller the particle size (optimal 1–10 nm) and the higher their concentration, the more significant the antimicrobial effect; triangular particles are more effective compared to the rod-like and spherical ones (Ahmad et al. 2020; Da Silva et al. 2020; Talapko et al. 2020; Yunusov et al. 2020; Enas et al. 2021).

Silver-based drugs have antifungal activity; they suppress *Candida albicans* and *Candida* spp. – *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and others, which are characterized by a variety of clinical manifestations (lesions of mucous membranes, skin and internal organs) and high resistance to many antimycotic agents (Novikova and Russkikh 2018; Kowalczyk et al. 2021). It is believed that the mechanism of antifungal action of nanosilver, like antibacterial one, may be due to the destruction of the cell membrane of fungi (Zarowska et al. 2019).

Antiviral properties of nanosilver are described (it kills respiratory syncytial virus, hepatitis B virus, herpes simplex virus, human immunodeficiency virus, coronavirus, etc.), which are associated with both the suppression of the initial phase of virus penetration into cells and its virulent effect after leaving the cell (Kryukov et al. 2019; Das et al. 2020; Dung et al. 2020). Currently, studies focused on the effect of nanosilver on the SARS-CoV-2 virus, which induces COVID-19, are being conducted. In particular, there are data on the antiviral effect of silver nanoparticles close in size to the virus, due to damage to its nucleic acid, which leads to the virus failure to self-replicate (Kowalczyketal. 2021).

Nanosilver has been shown to have a wide range of anti-inflammatory properties that have found application in medical practice (Singh et al. 2018; Shin et al. 2018; Fehaid et al. 2020; Kubyshkin et al. 2020). As for the mechanism of the anti-inflammatory action of nanosilver, it has not been sufficiently studied at the molecular level, although the fact of this action has been convincingly demonstrated in various *in vivo* and *in vitro* studies.

The high pharmacotherapeutic activity of nanosilver and its combinations with other antimicrobial agents, in particular with sulfathiazole, have been widely used in the complex treatment of suppurative wounds (Rodin et al. 2018; Savchenko et al. 2018). Many papers also provide data on the successful use of nanosilver in the treatment of periodontitis (Kadam et al. 2020; Leontev et al. 2020; Fernandez et al. 2021).

It should be noted that the activation of free radical oxidation and its pathogenic effect on the periodontal tissues leads to the development of their morphofunctional disorders. In this regard, the administration of pharmacological drugs with combined antimicrobial, anti- and pro-oxidant properties in periodontology appears to be pathogenically substantiated. Among the agents that neutralize harmful

effects of free radicals, in particular ROS and lipid peroxidation (LPO), an important place is given to recombinant human superoxide dismutase (SOD) – Rexod, especially its new injectable form – NIF (Galenko-Yaroshevsky et al. 2020).

Taking into account the pathogenetic mechanism of CGP, which determines a vector of the search for highly effective pharmacotherapeutic agents with the appropriate direction of action, and to improve the effectiveness of the complex treatment of CGP, we have chosen Soderm®-Forte, which is a mixed-type micellar gel containing nanoclustered zerovalent metallic silver in the form of Ag_n^{K+} type cluster monomers and monomer micelles, the structure of which consists of a metal core and a surface double electric layer [the micelles of the emulsion are formed by a mixture of nonionic surfactants (polyethylene glycols), oil and an aqueous phase including SOD], and NIF of Rexod®.

Aim of the study was to increase the effectiveness of the complex treatment of periodontitis with the administration of Soderm®-Forte gel and a new injectable dosage form of Rexod®.

Materials and methods

Animals

The studies were performed following the requirements of the Law of the Russian Federation (RF) "On the Protection of Animals from Cruelty" of 06/24/1998, the Rules of Laboratory Practice in Conducting Preclinical Studies in the Russian Federation (GOST 3 51000.3-96 and GOST R 53434-2009), World Medical Association's Declaration of Helsinki on Animal Use in Biomedical Research (Report of the AVMA Panel on Euthanasia JAVMA, 2001), Directive of the European Society (86/609 EC), international recommendations of the European Convention for the Protection of Vertebrate Animals Used in Experimental Studies (1997), as well as the Rules of Laboratory Practice adopted in the Russian Federation (Order of the Ministry of Health No.708 of 29.08.2010). All the experiments were approved by the local independent ethical committee of Rostov State Medical University (Minutes No.17/18 of 25.10.2018).

Fifty anesthetized male Wistar rats weighing 290–330 g were used in the experiments.

Studied substances

Soderm®-Forte gel and NIF of Rexod® were the studied substances. The veterinary drug Zoletil 100 (Virbac Sante Animale, France) was used as an anesthetic at the dose of 15–20 mg/kg intraperitoneally.

Experimental periodontitis (EP) was simulated by ligation with EUROLON 4/0 suture material (MZKRS Suture Materials LLC, Russia) of the necks of lower incisors, followed by their immersion in the tooth-gingival groove (Leontev et al. 2020). The ligature was fixed with a light-curing composite material Versaflo (Centrics Inc., USA) to the cervical region of the teeth.

Experimental design

All the rats were divided into 5 groups of 10 individuals each: Group 1 – animals with intact periodontium; Group 2 – untreated EP formed within 30 days; Group 3 – EP treated with a traditional drug therapy (TDT), including irrigation of the oral cavity with a solution of chlorhexidine (0.05%), the application of a dento–gingival dressing Septo-Pack (Septodont), which is a combination of amyl-acetate (0.503 g), butyl phthalate (12.96 g), butyl polymethacrylate (1.16 g), zinc oxide (27.5 g), zinc sulfate (8.8 g), and fillers (up to 100 g); Group 4 – EP treated with the combination of TDT with Soderm®-Forte gel, which was injected with a syringe and cannula into parodontal recess and applied to the gingival mucosa; Goup 5 – EP treated with TDT in combination with Soderm®-Forte gel and NIF of Rexod® [both drugs were developed by The State Research Institute of Highly Pure Biopreparations, St. Petersburg, Russia in the form of an aqueous solution (2 ml) in an ampoule with a dosage of 3.2 million units (registration certificate of the Ministry of Health of Russia – LP-004754)], while Rexod was administered intraperitoneally at a dose of 8000 units/kg. The animals with EP (Groups 3–5) were treated for 12 days. The entire observation period of the animals was 74 days and included an assessment of their general condition, behavior, nutrition, and body weight. Attention was paid to the color, presence and/or absence of swelling and bleeding of the gums, while the Schiller-Pisarev test was performed and the sulcus bleeding index (SBI) was determined according to Muhlemann-Cowell, and the amount of crevicular fluids (CF) was measured (in the initial state – control-1; on the 31st day of the EP simulation – control-2; on the 43rd day of EP simulation, or on the 12th day of the EP treatment; on the 74th day of EP simulation, or on the 31st day after EP treatment). CF measurements were performed using the intracrevicular method described by Leontev et al. (2020).

To compare the levels of microbial contamination of the marginal gum mucosa of rats with intact periodontium, untreated EP and EP treated with TDT, TDT + Soderm®-Forte, TDT + Soderm®-Forte+ NIF of Rexod®, the material was sampled as follows: a swab was taken from the gingival surface with a gelatin swab stick, after which the swab stick was immersed into a tube with 0.9 ml of saline solution, which was used for dilutions in a ratio of 1:10, 1:100 and 1:1000. The resulting dilutions were introduced into solid medium – egg yolk high salt agar culture medium and blood agar; anaerobic, Levin and Sabouraud's mediums with subsequent isolation and identification to the genus of staphylococci (*Staphylococcus*), alpha-streptococci (α-*Streptococcus*), beta-streptococci (*β-Streptococcus*), gamma-streptococci (γ-*Streptococcus*), anaerobes, Enterobacteriaceae, bacilli (*Bacillus*), and candida

(*Candida albicans*). The obtained results of microbiological studies were expressed in colony-forming units – CFU (in the decimal logarithm of the number of CFU).

Statistical processing

Statistical processing of the obtained research results was carried out using nonparametric methods in Microsoft Excel software and StatSoft/Statistica 8.0 software package. The significance of differences between the indicators of the control and main groups for different observation periods was determined using the Mann-Whitney criterion, after checking for normality of distribution using the Shapiro-Wilk criterion. P-value was determined using Student's t-test. The difference between the average values was considered reliable at $p<0.05$ (Gerasimov 2007).

Results and discussion

It was found that periodontium in the intact rats at all the follow-up periods (on days 31, 43, and 74) was within the normal range compared with the initial state (control-1): the mucous membrane of the marginal gum had a pale pink color and retained moderate humidity. The interdental papillae retained their anatomical shape and completely filled the interdental spaces. CF conformed to the physiological norm (0.023–0.024 mg). The Muhlemann-Cowell bleeding index was zero. The Schiller-Pisarev test was negative (Tables 1 and 2). The behavior of the animals

Table 1. The Effect of TDT, TDT + Soderm®-Forte and TDT + Soderm®-Forte + NIF of Rexod® on the Amount of CF in EP in Rats (M±m, n = 10)

Group of animals	Amount of crevicular fluid, mg			
	Initial (control-1)	From the beginning of EP simulation		
		On the 31st day (control-2)	On the 43rd day (or 12th day of EP treatment)	On the 74th day (or 31st day after EP treatment)
Intact periodontium [1]	0.024±0.001	0.023±0.002 -0.001/0.0	0.023±0.001 -0.001/0.0	0.024±0.002 0.0/+0.001
Untreated EP [2]	0.023±0.001	0.083±0.003* +0.060/0.0 p_{1-2}<0.001	0.086±0.002* +0.063/+0.003 p_{1-2}<0.001	0.093±0.003*° +0.070/+0.010 p_{1-2}<0.001
EP + TDT [3]	0.024±0.002	0.082±0.004* +0.058/0.0 p_{1-3}<0.001 p_{2-3}>0.05	0.078±0.002* +0.054/-0.040 p_{1-3}<0.001 p_{2-3}<0.02	0.052±0.001*°° +0.028/-0.030 p_{1-3}<0.001 p_{2-3}<0.001
EP + TDT+ Soderm®-Forte [4]	0.023±0.001	0.084±0.002* +0.061/0.0 p_{1-4}<0.001 p_{2-4}>0.05 p_{3-4}>0.05	0.067±0.001*°° +0.044/-0.017 p_{1-4}<0.001 p_{2-4}<0.001 p_{3-4}<0.001	0.028±0.002°° +0.005/-0.056 p_{1-4}>0.05 p_{2-4}<0.001 p_{3-4}<0.001
EP + TDT+ Soderm®-Forte + NIF of Rexod® [5]	0.023±0.002	0.083±0.003* +0.060/0.0 p_{1-5}<0.001 p_{2-5}>0.05 p_{3-5}>0.05 p_{4-5}>0.05	0.056±0.002*°° +0.033/-0.027 p_{1-5}<0.001 p_{2-5}<0.001 p_{3-5}<0.001 p_{4-5}<0.002	0.026±0.001°° +0.003/-0.057 p_{1-5}>0.05 p_{2-5}<0.001 p_{3-5}<0.001 p_{4-5}>0.05

Note: TDT – traditional drug therapy, EP – experimental periodontitis, NIF – new injectable dosage form. The order numbers of animal groups are shown in square brackets. The differences are statistically significant: *p<0.001 – in comparison with the control-1; p<0.05 and p<0.001 in comparison with the control-2.

Table 2. The Effect of TDT, TDT + Soderm®-Forte and TDT + Soderm®-Forte + NIF of Rexod® on Indicators of the Inflammatory Process and Bleeding of the Marginal Gingival Mucosa of Rats (M±m, n = 10)

Group of animals	Index evaluation indicators (in points) at follow-up periods			
	Initial (control-1)	From the beginning of EP simulation		
		On the 31st day (control-2)	On the 43rd day (or 12th day of EP treatment)	On the 74th day (or 31st day after EP treatment)
Schiller-Pisarev test				
Intact periodontium [1]	0.12±0.05	0.15±0.04 +0.03/0.0	0.13 ± 0.04 +0.01/-0.02	0.14 ± 0.05 +0.02/-0.01
Untreated EP [2]	0.15±0.03	2.30±0.15* +2.15/0.0 p_{1-2}<0.001	2.60 ± 0.12* +2.45/+0.30 p_{1-2}<0.001	2.80 ± 0.14*° +2.65/+0.50 p_{1-2}<0.001
EP+TDT [3]	0.13±0.04	2.50±0.12* +2.37/0.0 p_{1-3}<0.001 p_{2-3}>0.05	1.80 ± 0.13*°° +1.67/-0.70 p_{1-3}<0.001 p_{2-3}<0.001	0.90 ± 0.12*°° +0.77/-1.60 p_{1-3}<0.001 p_{2-3}<0.001
EP + TDT+ Soderm®-Forte [4]	0.14±0.04	2.40 ± 0.15* +2.26/0.0 p_{1-4}<0.001 p_{2-4}>0.05 p_{3-4}>0.05	0.90 ± 0.13* +0.76/-1.50 p_{1-4}<0.001 p_{2-4}<0.001 p_{3-4}<0.001	0.16 ± 0.05°° +0.02/-2.24 p_{1-4}>0.05 p_{2-4}<0.001 p_{3-4}<0.001
EP + TDT+ Soderm®-Forte + NIF of Rexod® [5]	0.15±0.05	2.60 ± 0.14* +2.45/0.0 p_{1-5}<0.001 p_{2-5}>0.05 p_{3-5}>0.05 p_{4-5}>0.05	0.60 ± 0.10*° +0.45/-2.00 p_{1-5}<0.001 p_{2-5}<0.001 p_{3-5}<0.001 p_{4-5}<0.001	0.13 ± 0.04° -0.02/-2.47 p_{1-5}>0.05 p_{2-5}<0.001 p_{3-5}<0.001 p_{4-5}>0.05
Muhlemann-Cowell SBI				
Intact periodontium [1]	0	0	0	0
Untreated EP [2]	0	1.75±0.09* +1.75/0.0 p_{1-2}<0.001	1.82±0.14* +1.82/+0.07 p_{1-2}<0.001	1.84±0.12* +1.84/+0.09 p_{1-2}<0.001
EP+TDT [3]	0	1.70±0.16* +1.70/0.0 p_{1-3}<0.001 p_{2-3}>0.05	1.38±0.04* +1.38/-0.32 p_{1-3}<0.001 p_{2-3}<0.01	0.22±0.03*°° +0.22/-1.48 p_{1-3}<0.001 p_{2-3}<0.001
EP + TDT+ Soderm®-Forte [4]	0	1.78 ±0.12* +1.78/0.0 p_{1-4}<0.001 p_{2-4}>0.05 p_{3-4}>0.05	0.16±0.02*°° +0.16/-1.62 p_{1-4}<0.001 p_{2-4}<0.001 p_{3-4}<0.001	0°° 0.0/-1.78 p_{2-4}<0.001 p_{3-4}<0.001
EP + TDT+ Soderm®-Forte + NIF of Rexod® [5]	0	1.74±0.15* +1.74/0.0 p_{1-5}<0.001 p_{2-5}>0.05 p_{3-5}>0.05 p_{4-5}>0.05	0.12±0.04*°° +0.12/1.62 p_{1-5}<0.001 p_{2-5}<0.001 p_{3-5}<0.001 p_{4-5}>0.05	0°° 0.0/-1.74 p_{2-5}<0.001 p_{3-5}<0.001

Note: TDT – traditional drug therapy, EP – experimental periodontitis, NIF – new injectable form. The order numbers of animal groups are shown in square brackets. 0 – no bleeding. The differences are statistically significant: *p<0.001 – in comparison with the control-1; p<0.05 and p<0.001 in comparison with the control-2.

was calm; they ate food well; the hair retained its gloss, the body weight was within the range of 394.6–487.8 g (Table 3), i.e., compared with the initial weight (391.2 g), it increased by 75.4, 116.2 and 186.6 g, respectively.

On the 31st day from the beginning of EP simulation (control-2) in Groups 2–5 of rats, the following indicators changed in comparison with the initial condition (control-1): the periodontium became edematous, hyperemic and cyanotic, the interdental gingiva was a spindle-shaped, the interdental papillae in the area of the lower incisors were enlarged, the dental attachments lost their integrity, while pathological cavities filled with serous-purulent

Table 3. The Effect of TDT, TDT + Soderm®-Forte and TDT + Soderm®-Forte + NIF of Rexod® on the Body Weight of Rats With EP (M±m, n = 10)

Group of animals	Body weight of the animals, g			
	Initial (control-1)	From the beginning of EP simulation		
		On the 31st day (control-2)	On the 43rd day (or 12th day of EP treatment)	On the 74th day (or 31st day after EP treatment)
Intact periodontium [1]	319.2±4.3	394.6±3.9**	435.4±3.0**°	487.8±3.7**°
		+75.4/0	+116.2/+40.8	+168.6/+93.2
Untreated EP [2]	312.6±3.8	247.7±2.9**	224.9±3.1**°	205.2±2.5**°
		-64.9/0	-87.7/-22.8	-107.4/-42.5
		p_{1-2}<0.001	p_{1-2}<0.001	p_{1-2}<0.001
EP + TDT [3]	318.3±4.3	252.8±3.4**	278.0±3.0**°	324.5±2.8°
		-65.5/0	-40.3/+25.2	+6.2/+71.7
		p_{1-3}<0.001	p_{1-3}<0.001	p_{1-3}<0.001
		p_{2-3}>0.05	p_{2-3}<0.001	p_{2-3}<0.001
EP + TDT+ Soderm®-Forte [4]	314.4±3.4	265.2±3.7**	317.5±2.4°	398.2±3.3**°
		-49.2/0	+3.1/+52.3	+83.8/+133.0
		p_{1-4}<0.001	p_{1-4}<0.001	p_{1-4}<0.001
		p_{2-4}<0.002	p_{2-4}<0.001	p_{2-4}<0.001
		p_{3-4}<0.05	p_{3-4}<0.001	p_{3-4}<0.001
EP + TDT+ Soderm®-Forte + NIF of Rexod® [5]	316.0±3.3	234.5±3.6**	328.4±2.2*°	416.8±2.7**°
		-81.5/0	+12.4/+93.9	+100.8/+182.3
		p_{1-5}<0.001	p_{1-5}<0.001	p_{1-5}<0.001
		p_{2-5}<0.02	p_{2-5}<0.001	p_{2-5}<0.001
		p_{3-5}<0.002	p_{3-5}<0.001	p_{3-5}<0.001
		p_{4-5}<0.001	p_{4-5}<0.001	p_{4-5}<0.001

Note: TDT – traditional drug therapy, EP – experimental periodontitis, NIF – new injectable form. The order numbers of animal groups are in square brackets. The differences are statistically significant: *p<0.01 and **p<0.001 – in comparison with the control-1; °p<0.001 – in comparison with the control-2.

discharge were found, the depth of which reached 2 mm, the amount of CF increased by 3.7 times (Table 1), the appearance of dental deposits was noted, gingival abscesses were detected in 40% of rats, I–II degree of pathological mobility of the lower incisors was determined, offensive mouth was noted, the Muhlemann-Cowell SBI index was within the range of 1.5–3.0, the Schiller-Pisarev test values were positive (Table 2), and the body weight of the animals decreased by 49.2–81.5 g. In the subsequent observation periods (on days 43 and 74) in the 2nd group of rats with EP, all the noted signs had a tendency to progress, and, by the 74th day of observation, statistically significantly differed from those in the control-2, in particular, in terms of the amount of CF (Tables 1 and 2).Aggressive behavior of the animals was observed, their hair lost its lustre, body weight decreased compared to the initial values (control-1) by 87.7 and 107.4 g, and compared to the control-2 (EP) – by 22.8 g and 42.5 g, respectively (Table 3).

On the 43rd and 74th days of observation in the animals of the 3rd group, TDT for 12 days caused a decrease in the severity of the inflammatory process in periodontium: hyperemia, cyanosis, edema of the marginal gum, the depth of parodontal recesses, the amount of serous discharge became less in intensity. Forty percent of the animals recovered the hair gloss, the dental deposits on the lower incisors decreased; dental attachment was restored in 60% of the rats; gingival recesses had a depth of 1–1.5 mm; gingival abscesses were absent; no incisor mobility was detected, and there was no offensive mouth. The amount of CF on the 43rd day of the observation period did not significantly change when compared to that on the 31st day (control-2), although there was a tendency to its decrease, and on the 74th day, the amount of CF statistically significantly decreased by 1.6 times (Table 1). The Muhlemann-Cowell SBI score ranged within 1.5–2.5. The values of the Schiller-Pisarev test were moderately positive (Table 2). The body weight of the animals in comparison with that in the control-1 decreased by 40.3 g and increased by 60.2 g, and in comparison with the control-2 increased by 25.2 and 71.7 g, respectively (Table 3).

The inclusion of Soderm®-Forte in the TDT of EP in the 4th group of rats had a more pronounced therapeutic effect than the only TDT. So, on the 43rd day of observation, hyperemia, edema of the marginal gum and the amount of serous discharge from parodontal recesses decreased sharply, and in 2 animals (out of 10 individuals), they were hardly observed. The amount of CF statistically significantly decreased by 1.3 times compared with that on the 31st day of observation (control-2). Thirty days after such treatment of animals with EP, i.e. on the 74th day of the observation, there were practically no signs of periodontitis. The amount of CF did not differ statistically from the control values (Table 1). The Muhllemann-Cowell was 1.0–1.5. The Shiller-Pisarev test was weakly positive (Table 2). In 90% of animals, the hair SBI became glossy. The body weight of rats on 43rd and 74th days of observation was increased by 3.1 and 83.8 g respectively compared to the control-1, and by 52.3 and 133.0 g, respectively compared to the control-2 (Table 3).

Additional intraperitoneal administration of NIF of Rexod® in combination with topical application of the TDT with Soderm®-Forte to the animals of Group 5 with EP on the 12th day of treatment, i.e. on the 43rd day of observation, led to a more pronounced therapeutic effect, involving in 6 rats (out of 10 individuals) mild hyperemia and slight edema of the marginal gum, when compared with the animals of Group 4, which were administered with only TDT + Soderm®-Forte with the same observation period. In the remaining 4 animals, the symptoms of the inflammatory process were absent. The amount of CF statistically significantly decreased by 1.2 times. Thirty days after the selected treatment of animals with EP (on the 74th day of observation), the condition of the gum mucosa and CF practically did not differ from those of intact rats (Table 1).The Muhllemann-Cowell SBI was 0.1–1.0. The Schiller-Pisarev test was weakly positive in the rats with residual periodontitis (Table 2). Hair of all the animals became glossy. The body weight of the rats on days 43 and 74 increased by 12.4 g and 100.8 g, respectively, when compared with that in the control-1 and by 93.9 and 182.3 g, respectively, when compared with that in the control-2 (Table 3).

The microbiological studies showed that in rats with intact periodontium (control-1), only staphylococci were detected on the mucous membrane of the marginal gum, amounting to 1.8±0.1 CFU, anaerobes and enterobacteria up to 20 and 60 cells in a swab, respectively, whereas in EP (control-2), most pathogenic microorganisms were detected: staphylococci, gamma-streptococci, anaerobes

Table 4. The Effect of TDT, TDT + Soderm®-Forte and TDT + Soderm®-Forte + NIF of Rexod® on the Microbial Contamination of the Marginal Gingival Mucosa in Rats With EP (M±m, n = 10)

Group of animals	Microbial contamination, CFU							
	Streptococcus			*Staphylococcus*	*Enterobacteriaceae*	Anaerobes	*Bacillus*	*Candida albicans*
	α	β	γ					
Intact periodontium [1]	-	-	-	1.8±0.1	up to 60 cells per swab	up to 20 cells per swab	-	-
Untreated EP [2]	-	5.2±0.2 (40)	4.8±0.1 (100)	6.2±0.2 (100) p_{1-2}<0.001	4.0±0.2 (100)	4.5±0.2 (100)	-	3.4±0.2 (20)
EP + TDT [3]	-	2.8±0.2 (20) p_{2-3}<0.001	2.6±0.1 (30) p_{2-3}<0.001	3.2±0.2 (40) p_{1-3}<0.001	2.2±0.2 (40) p_{2-3}<0.001	2.4±0.1 (40) p_{2-3}<0.001	-	1.2±0.1 (10) p_{2-3}<0.001
EP + TDT+ Soderm®-Forte [4]	-	-	-	-	-	-	-	-
EP + TDT+ Soderm®-Forte + NIF of Rexod® [5]	-	-	-	-	-	-	-	-

Note: TDT – traditional drug therapy, EP – experimental periodontitis, NIF – new injectable form. The order numbers of animal groups are in square brackets, the number of cases of microorganisms detected are in parentheses (%).

and enterobacteria were screened in 100% of cases and made up 6.2±0.2 CFU, 4.8±0.1 CFU, 4.5±0.2 CFU and 4.0±0.2 CFU, respectively. Beta-hemolytic streptococci and candida were detected in 40% and 20% of cases, amounting to 5.2±0.2 CFU and 3.4±0.2 CFU, respectively; alpha-hemolytic streptococci and bacilli were not detected. It is noteworthy that the microbial contamination of inflamed periodontium is sharply increased in comparison with the intact one (Table 4).

When the TDT in the animals of Group 3 with EP was completed, the mucous membrane of the marginal gum in 58.4% was free of the microorganisms. In other cases, staphylococci, anaerobes and enterobacteria were screened in 100% of swabs amounting to 3.2±0.2 CFU, 2.4±0.1 CFU and 2.2±0.2 CFU, in 60%, 25% and 15% of swabs – gamma streptococci, betta streptococcus and candida in the amount of 2.6±0.1 CFU, 2.8±0.2 CFU and 1.2±0.1 CFU, respectively (Table 4).

These data indicate that the TDT significantly suppresses the vital activity of various microorganisms (gram-positive and gram-negative bacteria, as well as pathogenic fungi) which develop on the gingival mucosa during the EP progression; however, there was no complete microbial decontamination of the studied gingival surface. It should be assumed that this leads to the attenuation of the inflammatory process in the periodontium, induced and maintained by microorganisms, which subsequently entails a relapse of periodontitis. It should be noted that the periodontopathogenic microflora remaining in the gum mucosa continues to activate free-radical oxidation, which, presumably, prevents the reduction of morphofunctional disorders in it.

Complex treatment of rats with EP by administration of the TDT in combination with Soderm®-Forte gel (Group 4), as well as with NIF of Rexod® (Group 5), led to a decontaminating effect on the gingival mucous membrane in 100% of cases, i.e. none of the types of microorganisms were detected (Table 4). It is possible that the nanosilver contained in Soderm®-Forte gel suppresses the vital activity of microorganisms in the deep layers of the gingival mucosa, which may partly be due to its ability to penetrate into tissues, for example, into the skin, which is embryonally close to the oral mucosa and has a common structure with it (Karkishchenko 2004). In any case, comparison of the CF values, Schiller-Pisarev tests, Muhlleman-Cowell SBI scores and the animal body weight on the 74th day after the treatment of rats with EP, a clear pharmacotherapeutic effect was observed for the combination of TDT with Soderm®-Forte gel in comparison with using TDT only. There is hardly any inflammatory process, which becomed especially evident on the 43rd day of the treatment of animals with EP with additional intraperitoneal administration of NIF of Rexod®, which provides a pronounced antioxidant as well as anti-inflammatory effects.

Conclusion

The administration of a combination of TDT with Soderm®-Forte gel (topically) and NIF of Rexod® (resorptively) in EP in rats can significantly increase the efficacy of their treatment, which may be due to the pronounced antimicrobial, antifungal, and anti-inflammatory properties of nanosilver, which is part of Soderm®-Forte. The presence of superoxide dismutase, which has high antioxidant and anti-inflammatory activities, is an essential link in the mechanism of action of these compositions.

The results of our experimental study stated above indicate the expediency of using Soderm®-Forte gel, as well as its combination with NIF of Rexod®, in dental practice in the complex therapy of patients with periodontitis.

Conflict of interest

The authors declare no conflict of interests.

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