

ORIGINAL ARTICLE

Antimicrobial properties of the new combined dental gel

Antimikrobiální vlastnosti nového kombinovaného dentálního gelu

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Summary

Given the role of the microbial factor in the development of infectious-inflammatory processes in the oral mucosa, the research aim was to study the antimicrobial action of a new combined dental gel containing Rotocan® (10%) and triclosan (0.4%) *in vitro* and in the traumatic stomatitis in albino rats. Rotrin-Denta® exhibited strong antimicrobial activity against etalon strains of gram-positive (*S. aureus* ATCC 6538, *S. pyogenes* DICK 1, *B. subtilis* ATCC 6633) and gram-negative (*E. coli* ATCC 25922) bacteria, which exceeded the action of the reference drug Camident-Zdorovia® and weak effects on pseudomonads (*P. aeruginosa* ATCC 27853) and fungi (*C. albicans* CCV 885-653), which is less to the reference preparation. Rotrin-Denta® reduced microbial insemination and eliminated oral dysbiosis in albino rats with traumatic stomatitis, exceeding the effect of Kamident-Zdorov'ya®. The results open up the prospect of its clinical testing and further implementation in the dentistry practice.

Key words: dental gel • microorganisms susceptibility • dysbiosis • traumatic stomatitis

Souhrn

Vzhledem k úloze mikrobiálního faktoru při vzniku infekčně-zánětlivých procesů na orální sliznici bylo cílem výzkumu studovat antimikrobiální účinek nového kombinovaného dentálního gelu obsahujícího Rotocan® (10 %) a triclosan (0,4 %) v podmínkách *in vitro* a při traumatické stomatitidě u potkanů albinů. Bylo prokázáno, že Rotrin-Denta® vykazoval silnou antimikrobiální aktivitu proti etalonovým kmenům

grampozitivních (*S. aureus* ATCC 6538, *S. pyogenes* DICK 1, *B. subtilis* ATCC 6633) a gramnegativních (*E. coli* ATCC 25922) bakterií, což převyšovalo účinek referenčního přípravku Camident-Zdorovia® a slabší účinky na pseudomonády (*P. aeruginosa* ATCC 27853) a plísně (*C. albicans* CCV 885-653) než referenční přípravek. Rotrin-Denta® snižuje mikrobiální inseminaci a odstraňuje orální dysbiózu u potkanů albinů s traumatickou stomatitidou a převyšuje účinek přípravku Kamident-Zdorov'ya®. Výsledky otevírají perspektivu jeho klinického testování a dalšího uplatnění ve stomatologické praxi.

Klíčová slova: dentální gel • antimikrobiální citlivost • dysbióza • traumatická stomatitida

Introduction

In treating inflammatory and infectious diseases of the oral mucosa, drugs of different pharmacological groups: anesthetics, antiseptics, astringents, and others, are used¹⁾. Both synthetic and natural remedies, particularly herbal preparations, are widely applied to treat this pathology^{2, 3)}. Interest in herbal medicines does not fade due to their polyvalent action on the main links in the pathogenesis of diseases of the oral mucosa as well as the possibility of their combining both with each other and with synthetic drugs⁴⁾. The therapeutic potential of herbal remedies can be increased due to the modern medicinal form, in particular, gel, characterized by hydrophilicity, elasticity, and plasticity; for a long time, retained on the surface of the mucous membrane producing high bioavailability of drugs⁵⁾. Given the above, the original combined dental gel Rotrin-Denta®, developed by Ukrainian scientists, attracts attention. It is plant-based and contains the herbal drug Rotocan® and the synthetic antiseptic triclosan⁶⁾. According to our previous studies, this gel exhibits anti-inflammatory and regenerative properties and is effective in the experimental treatment of traumatic gingivitis in laboratory animals^{7, 8)}.

Due to the role of microbial factors in the development of stomatitis, gingivitis, and other

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infectious-inflammatory processes of the oral mucous membrane, the aim of the work was to study the antimicrobial activity of a new combined dental gel Rotrin-Dent® *in vitro* and its effect on the oral dysbiosis in traumatic stomatitis.

Experimental part

Materials and methods

For the gel base, the gel-forming agent carbomer was used; employing trometamol as the neutralizer; sorbitol as the moisturizer; sodium benzoate as the preservative; and purified water. Active substances were represented by a complex of phytocomponents Rotokan (10%) and triclosan (0.4%). Rotokan (ATC code A01A D11) contains a liquid extract of chamomile flowers, calendula flowers, and yarrow herb (2 : 1 : 1)⁹. Triclosan is a synthetic antimicrobial agent with a broad spectrum of action, which is used in concentrations up to 1% in hygienic products for oral care¹⁰. As the reference preparation was chosen Kamident-Zdorov'ya® gel (ATC code A01A D11) (LLC Pharmaceutical Company Zdorov'ya, Ukraine) similar in design, which contains lidocaine hydrochloride, tincture of chamomile flowers, and thymol. It is intended to treat inflammatory diseases of the oral mucous membrane and gum lesions¹¹.

To determine the *in vitro* antimicrobial properties of Rotrin-Denta® gel and its ingredients in comparison with Kamident-Zdorov'ya® gel, the agar diffusion method (wells method) was used. Etalon strains of gram-positive bacteria (*S. aureus* ATCC 6538, *S. pyogenes* DICK 1, *B. subtilis* ATCC 6633), gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853) and fungi (*C. albicans* CCM 885-653) were used. The susceptibility of microorganisms to the studied agents was evaluated by a zone of growth inhibition greater than 10 mm. If the diameter of the zone exceeded 25 mm, the microorganism was considered highly susceptible; if such a zone was from 16 to 25 mm – moderately susceptible; and if such a zone was from 10 to 16 mm – minimally susceptible. Inhibition zones of bacterial growth were measured after 24 hours of incubation at $\pm 37^\circ\text{C}$. Determination of the susceptibility of the microorganism was repeated 6 times.

In the *in vivo* experiments, the study of Rotrin-Denta gel® was performed under the conditions of traumatic stomatitis. It received permission from the Commission on Bioethics of the Poltava State Medical University, the decision of the Commission on Ethical Issues and Bioethics of the PSMU (order No. 350 dated November 8, 2021). The experiments were done on 70 albino male Wistar rats of 190–230 g body weight. The animals were divided into groups of 10 rats: intact animals; control pathology 5 or 8 days after traumatic injury of the oral mucosa; experimental groups with traumatic stomatitis and application of the tested gel or reference preparation during 3 and 6 days up to 5th or 8th day after the trauma of mucous membrane of the oral cavity. Traumatic stomatitis was simulated by deliberate eye

trepan damage of 3 mm in diameter to buccal mucosa in rats¹². The mentioned procedure was carried out under general ether anesthesia. Treatment was started on the 2nd day after the injury simulation. The duration of a single application of the gel was 2 minutes. Animals with control pathology were treated with saline. Rats were removed from the experiment on day 5 or 8 by total bloodletting from the heart under thiopental anesthesia (20 mg/kg), and the buccal mucosa was taken for the investigation. Homogenate of this tissue was prepared at a ratio of 20 mg to 1 ml of 0.05 M Tris-HCl (pH 7.5).

The activity of urease produced only by the microflora and not characteristic of the macroorganism was studied to assess microbial insemination of the oral cavity. Determination of lysozyme activity was performed by bacteriological method⁷. Based on the above two parameters, the degree of dysbiosis was evaluated as the ratio of the urease activity to the lysozyme content in the mucous membrane of the oral cavity¹³.

The digital material was processed using a one-way analysis of variance ANOVA and Dunnet's test based on the standard computer software package Statistica for Windows 6.0.

Results and discussion

The results of the antimicrobial action of Rotrin-Denta® gel and its ingredients on the reference strains of microorganisms are presented in Table 1. It was shown that Rotrin-Denta® gel demonstrated high antimicrobial activity against etalon strains of gram-positive (*S. aureus*, *S. pyogenes*, *B. subtilis*) and gram-negative (*E. coli*) bacteria with growth inhibition zones greater than 25 mm and weak antifungal activity against *C. albicans* CCM 885-653 with zones of growth inhibition on average 16.0 mm (see Table. 1). As for the ingredients of Rotrin-Denta®, the gel base did not inhibit the development of any test culture of microorganisms and had no antimicrobial activity in this experiment. Two strains of microorganisms, *S. aureus* ATCC 6538 and *S. pyogenes* DICK 1 displayed susceptibility to the gel containing Rotokan without triclosan: their growth inhibition zones ranged between 21.2–24.5 mm. In other microorganisms, growth inhibition zones were less than 10 mm or absent, indicating resistance to this composition.

The reference preparation Kamident-Zdorov'ya® gel delayed the growth of the culture *C. albicans* CCM 885-653 fungi with the formation of a growth inhibition zone greater than 25 mm, which confirmed the high susceptibility of candida fungi to this drug (see Table 1). The susceptibility of the etalon strains of *S. aureus*, *S. pyogenes*, and *P. aeruginosa* to the reference drug was weak or moderate, confirmed by the presence of growth inhibition zones of test cultures from 17.8 to 20.0 mm. Strain *B. subtilis* ATCC 27853 was not susceptible to Kamident-Zdorov'ya gel.

Comparison of the antimicrobial action of Rotrin-Denta® with the reference preparation showed that the susceptibility of the etalon strains of staphylococci was significantly higher to the new combined gel ($p < 0.001$) (see Table 1). Its advantages were also noticeable in the stronger influence on the etalon strains of *E. coli* ($p < 0.001$) and *B. subtilis* ($p < 0.001$). At the same time, Rotrin-Denta® was inferior to Kamident-Zdorov'ya® gel in the susceptibility of *E. coli* ($p < 0.01$) and *C. albicans* CCV 885-653 ($p < 0.01$). All used strains of microorganisms showed greater susceptibility to Rotrin-Denta® gel than to its base with the phytopreparation Rotokan (in all cases, $p < 0.001$).

As you can see, the sample of the new gel with 10% Rotokan and 0.4% triclosan had a pronounced antimicrobial effect. Obviously, its spectrum of action expanded compared to the effect of the composition «gel base + Rotokan» due to the inclusion of triclosan.

This spectrum is wider, and the impact on the etalon strains of *S. aureus*, *S. pyogenes*, *B. subtilis*, and *E. coli* is stronger than that of Kamident-Zdorov'ya® gel. The obtained data suggest that the investigated gel will affect the oral microflora in the body and modify its interaction with the host organism.

Table 2 shows the parameters characterizing the microbial insemination of the oral mucosa by conditionally pathogenic microflora (as urease activity), nonspecific antimicrobial protection of the oral cavity (as lysozyme activity) and a degree of dysbiosis in the cheek tissues of rats with traumatic stomatitis after the treatment with Rotrin-Denta® gel.

Simulation of oral mucosa injury caused an increase in urease activity by 3.3 times ($p < 0.001$) after 5 days and 4.1 times ($p < 0.001$) after 8 days after the trauma against the background of a decrease in lysozyme activity by 1.6 times ($p < 0.02$). These changes were

Table 1. Zones of growth inhibition of test cultures of microorganisms etalon strains with Rotrin-Denta® gel and its ingredients, $M \pm m$ ($n = 6$)

Test cultures of microorganisms	Rotrin-Denta® gel	Gel base	Gel base + Rotokan	Kamident-Zdorov'ya® gel
	Diameter of growth inhibition zone (mm)			
<i>S. aureus</i> ATCC 6538	$53.8 \pm 3.2^{*,\#}$	–	$24.5 \pm 1.5^*$	17.8 ± 1.2
<i>S. pyogenes</i> DICK 1	$51.5 \pm 3.3^{*,\#}$	–	21.2 ± 1.8	20.0 ± 1.7
<i>B. subtilis</i> ATCC 27853	$26.3 \pm 2.3^{*,\#}$	–	–	3.2 ± 0.9
<i>E. coli</i> ATCC 25922	$25.5 \pm 1.8^{*,\#}$	–	$5.5 \pm 1.2^*$	14.0 ± 1.0
<i>P. aeruginosa</i> ATCC 6633	$15.3 \pm 0.8^{*,\#}$	–	$6.6 \pm 2.3^*$	18.3 ± 1.7
<i>C. albicans</i> CCV 885-653	$16.0 \pm 1.0^{*,\#}$	–	$7.8 \pm 2.4^*$	25.5 ± 1.2

«–» no growth inhibition zones of microorganisms

* $p < 0.05$ as compared to Kamident-Zdorov'ya® gel, a reference preparation

$p < 0.05$ as compared to gel base + Rotokan

Table 2. The effect of Rotrin-Denta gel® on the activity of urease, lysozyme, and dysbiosis degree in the buccal mucosa of rats with traumatic stomatitis, $M \pm m$ ($n = 10$)

Conditions of the experiment	Urease activity, mcat/kg	Lysozyme activity, units/g	Dysbiosis degree, units
Intact animals	0.48 ± 0.07	0.364 ± 0.045	1.0 ± 0.02
Control pathology (trauma + saline, 5 days)	$1.60 \pm 0.22^*$	$0.233 \pm 0.020^*$	$5.20 \pm 0.41^*$
Control pathology (trauma + saline, 8 days)	$1.98 \pm 0.13^*$	$0.217 \pm 0.037^*$	$6.88 \pm 0.52^*$
Trauma + Rotrin-Denta® (5 days/3 days of treatment)	$0.71 \pm 0.07^{*,\#}$	$0.258 \pm 0.021^*$	$2.08 \pm 0.24^{*,\#}$
Trauma+Kamident-Zdorov'ya® (5 days/3 days of treatment)	$0.78 \pm 0.10^{*,\#}$	0.269 ± 0.027	$2.20 \pm 0.18^{*,\#}$
Trauma + Rotrin-Denta® (8 days/6 days of treatment)	$0.57 \pm 0.07^{\#}$	$0.312 \pm 0.016^{\#}$	$1.38 \pm 0.11^{*,\#,\oplus}$
Trauma + Kamident-Zdorov'ya® (8 days/3 days of treatment)	$0.72 \pm 0.07^{*,\#}$	$0.298 \pm 0.020^{\#}$	$1.83 \pm 0.14^{*,\#}$

* $p < 0.05$ as compared to intact animals

$p < 0.05$ as compared to the corresponding control pathology

⊕ $p < 0.05$ as compared to the corresponding term of the treatment with Kamident-Zdorov'ya® gel, a reference preparation

accompanied by an increase in the dysbiosis degree 5.2 times ($p < 0.001$) after 5 days and 6.9 times ($p < 0.001$) after 8 days from the trauma of oral mucosa as compared to intact animals. The dysbiosis degree is an integral parameter. Its significant increase, apparently, reflects a violation of the balance between the macroorganism and microflora in rats with traumatic injury of the mucous membrane of the oral cavity.

On day 5 of the experiment, treatment with both Rotrin-Denta® gel and Kamident-Zdorov'ya® gel reduced urease activity in rat buccal mucosa homogenates ($p < 0.002$ and $p < 0.001$, respectively). No significant changes were revealed in the period following the therapy with either Rotrin-Denta® gel or the reference preparation. The dysbiosis degree, despite a probable decrease (2.5 and 2.4 times, respectively) compared with the control pathology, remained high against intact control ($p < 0.001$) (see Table 2).

On day 8 of the experiment, it was found that treating traumatic stomatitis in rats with Rotrin-Denta® gel contributed to normalizing the activity of urease and lysozyme in the buccal mucosa. Still, the dysbiosis degree in animals of this group remained elevated against intact rats ($p < 0.02$), similar to this parameter in the previous term of research (see Table 2).

The use of Kamident-Zdorov'ya® gel also led to a decrease in urease activity ($p < 0.001$) and an increase in lysozyme activity ($p < 0.05$). The activity of urease, as well as the dysbiosis degree, were higher than the corresponding levels in the intact group ($p < 0.02$, $p < 0.001$, respectively).

The obtained results indirectly indicate that the studied gel reduced the microbial insemination of the oral mucosa in the animals with traumatic stomatitis and did so no worse than the reference preparation, provided by the synergistic antimicrobial action of phytocomponents Rotrin-Denta and triclosan. The new gel, as well as Kamident-Zdorov'ya® gel, had a positive effect on the factors of nonspecific resistance of the oral mucosa in traumatic stomatitis, as seen in the example of the normalization of lysozyme activity, which plays a key role in the antimicrobial protection of the oral cavity due to the ability to destroy bacteria and viruses, activate immunoglobulins and phagocytes. It can be explained by the properties of chamomile, calendula, and yarrow extracts in the composition of Rotokan®, based on which Rotrin-Denta® was developed, to affect nonspecific resistance¹⁴. But the greatest attention is drawn to the ability of the new gel to reduce dysbiosis in traumatic stomatitis, i.e., to normalize the balance between oral microflora and macroorganism, which is a necessary prerequisite for successful treatment and prevention of many diseases of the oral cavity⁴.

Therefore, the new combined Rotrin-Denta® gel has antibacterial and antifungal properties and reduces microbial insemination of the mucous membrane of the oral cavity and dysbiosis in experimental traumatic stomatitis, surpassing in some respects the reference preparations. It opens up prospects for clinical testing

and further implementation in the practice of dentistry in patients with infectious and inflammatory diseases of the oral mucosa.

Conclusions

The new dental gel Rotrin-Denta® has a strong antimicrobial activity against etalon strains of gram-positive (*S. aureus* ATCC 6538, *S. pyogenes* DICK 1, *B. subtilis* ATCC 6633) and gram-negative (*E. coli* ATCC 25922) bacteria, which is superior to the reference preparation, and a weak effect on pseudomonads (*P. aeruginosa* ATCC 27853) and fungi (*C. albicans* CCV 885-653), which is inferior to the Kamident-Zdorov'ya®, as a reference preparation. Rotrin-Denta® gel reduces microbial insemination of the oral mucosa of animals with traumatic stomatitis in 3–6 days from the beginning of its local application. It also eliminates dysbiosis in the oral cavity, surpassing the known Kamident-Zdorov'ya® gel.

Conflict of interests: none.

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