

Effects of Bischofite Gel on Reparative Processes in Wound Healing

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Abstract

Introduction: The currently available information on the bischofite mineral pharmacodynamics suggests that gels based on it have wound-healing properties. It was shown that bischofite has anti-inflammatory, immunomodulatory, and reparative activity. **Materials and Methods:** Experiments were performed on 36 male rats. Using a blade, a linear wound length of 50 mm was modeled. Animals were divided into four groups: (1) Control; (2) Bischofite (500 mg of gel with bischofite per wound area for 7 days); (3) Actovegin (500 mg of Actovegin gel on the wound area for 7 days); and (4) Contractubex (500 mg of Contractubex gel for 7 days wound area). About the wound healing effect of the drugs was judged by studying the physicochemical properties, determining the concentration of hydroxyproline (HP), and calculating the ratio of collagen 1 and 3 types. **Results:** The greatest strength of the wound defect, significantly greater than in the control ($P < 0.01$), was obtained in the group that received the bischofite gel (13.70 ± 0.76 N). Actovegin also demonstrated a positive reparative effect (12.60 ± 0.63 N, $P < 0.05$), significantly lower ($P < 0.01$) than in the control strength was obtained in the group using Contractubex (9.65 ± 0.59 N). In the calorimetric analysis, it was found that the highest concentration of HP was contained in the tissues of wound defects in animals treated with Contractubex; however, there was no statistically significant difference with the control group, significantly lower in comparison with the control ($P < 0.05$) concentration of selenium, contained tissues of simulated wounds in animals treated with bischofite gel (79.7% of the control). When assessing the ratio of collagens I and III in the tissues of the wound defect when dyeing with picosirius red, it was found that, by the number of mature collagen fibers, the studied groups can be arranged in the following sequence (descending): Bischofite > Actovegin > Control > Contractubex. **Conclusions:** The study showed that the best results were obtained with external use of the gel with bischofite. Actovegin has a less significant but pronounced reparative effect on this model. The least satisfactory results were obtained when applying Contractubex.

Key words: Collagenase, linear wound, rats, reparation

INTRODUCTION

Modern pharmacology has a wide arsenal of compounds with cytoprotective potential.^[1,2] In accordance with the requirements of esthetic and cosmetic medicine, the development of dermatotropic agents with wound-healing activity is actively conducted. One of these tools is a gel based on bischofite, a natural mineral with significant reserves in the territory of the Lower Volga region. The pharmacological activity of this mineral has been studied in detail for several decades^[3-6] and includes an anti-inflammatory, immunomodulatory, and reparative activity.^[7,8]

the 14-day quarantine mode and randomized in accordance with all bioethical norms and rules. Under anesthesia (chloral hydrate 300 mg/kg), after preliminary depilation (80 × 45 mm) and treatment with an antiseptic (70% solution of ethyl alcohol) in the dorsal region, a linear wound 50 ± 1 mm long was modeled by cutting the skin through the paravertebral line with a blade with a terminator depths of 2 mm, after which the edges of the wound were brought together by the imposition of three sutures with sterile threads.^[9]

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MATERIALS AND METHODS

The experiments were carried out on 36 male Wistar rats weighing 193–218 g, who passed

Then, the animals were divided into four equal groups:

- Group I - Control group - imitation of rubbing the drug on the shaved area 10 min after modeling the wound and for the next 6 days (1 time/day).
- Group II - Bischofite - rubbing 500 mg of gel with bischofite on the wound area and adjacent tissues 10 min after modeling the wound and for the next 6 days (1 time/day).
- Group III - Actovegin - rubbing 500 mg of actovegin gel onto the wound area and adjacent tissues 10 min after modeling the wound and for the next 6 days (1 time/day).
- Group IV - Contractubex - rubbing 500 mg of Contractubex gel on the wound area and surrounding tissue 10 min after the wound modeling and for the next 6 days (1 time/day).

On the 8th day, the animals were taken out of the experiment by the method of cranial dislocation under anesthesia, and then, four skin grafts (total surface 25 × 45 mm) were removed from the dorsal surface for further research. About the wound healing effect of the drugs was judged by studying the physicochemical properties, assessing the morphological picture, determining the concentration of hydroxyproline (HP), and calculating the ratio of collagen 1 and 3 types.

The study of the physicochemical properties of the wound defect was carried out using a mechanical early spreading device (Metrotest REM-0.2-1, Russia). The cut skin fragment was fixed in a special installation using threads and metal knitting needles. After launching the device, the force (discreteness = 0.1 N), necessary for tearing tissues along the wound line, was monitored. This value characterizes the ultimate strength of the wound defect. In addition, data on the ultimate strain (tension at the time of rupture) of the skin graft were obtained by analyzing the force-tensile curve. This parameter allows to judge the elasticity of the wound defect.

Calorimetric analysis of the concentration of HP in the tissues of the wound defect

In addition, to assess the degree of reparative reaction, the concentration of HP in tissues was determined. HP is one of the non-proteinogenic (non-coded) amino acids that make up the proteins of plants and animals, mainly collagens.

To determine the concentration of HP in the samples, a calorimetric method was used to determine the reaction products of oxidized HP and Ehrlich reagent.^[6,7] During the reaction, the pyrrolidine ring under oxidative dehydration with chloramine B in a buffer solution with a pH of about 6 becomes pyrrole, which, in turn, can be determined by reaction with 4-(N, N-dimethylamino) benzaldehyde. The resulting compound is intensely colored and is detected calorimetrically.

During the sample preparation, round skin areas without underlying tissues with a diameter of 5 mm and including all its layers were taken from euthanized animals using the Dermal Punch Tool (USA). The samples were taken in such a way as to include the linearly scar, as large as possible, that was formed after wound modeling and treatment. Samples were frozen in liquid nitrogen by immersion for 1–2 s and stored at –72°C in sealed tubes of the Eppendorf type.

On the day of the study, the samples were thawed for 3–5 h at room temperature in the open air. The samples were then weighed and cut so that the weight of one of the fragments was about 20 mg.

Tissue hydrolyzate was prepared as follows: Tissue samples weighing 20 mg were placed in 5 ml glass ampoules and 400 µl of hydrochloric acid was added. The ampoules were sealed and placed for 25 min in an oil bath heated to a temperature of 166°C. After that, the ampoules were opened and in heated to 60°C incubator was evaporated to dryness. The dry residue was diluted with 2 ml of distilled water.

To determine the HP, 1 ml of chloramine B was added to 1 ml of the hydrolyzate, shaken, and kept at room temperature for 20 min. 1 ml of perchloric acid was added and shaken again, and 1 ml of a 20% solution of Ehrlich reagent was added. The tubes were shaken again and placed in a water bath (60°C) for 20 min, then the reaction was terminated by immersing the tubes in an ice bath, and 5 ml of ethyl cellosolve was added. The optical density was determined at a wavelength of 557 nm. For the preparation of standards crystalline HP production Sigma-Aldrich (USA) was used.

Assessment of the ratio of collagen I and III types in a polarizing microscope

For a quantitative assessment of the ratio of mature and immature collagen in the obtained histological preparations, the ratio of collagen fibers of types I and III was studied. For this, sections were stained with picosirius red and were photographed using a polarizing microscope. For each slice, 10 fields of view were photographed at ×400 magnification. The color ratio of the differential coloration was established by automatically analyzing color histograms for each of the micrographs using the Image J program and subsequent statistical processing. A lower ratio indicates a higher proportion of immature type III collagen.^[10]

RESULTS

After awakening and on further days of the study, the animals were active, and the consumption of feed and food was within the normal range. There were no purulent complications, hemorrhages, excoriations, and other undesirable effects.

Determination of the physicochemical properties of the wound defect

When determining the force at the moment of rupture using a mechanical early-opener (Metrotest REM-0.2-1, Russia), it was found that the average force required to rupture the skin graft along the wound defect in the control group was 11.76 ± 0.71 N. The greatest strength of the wound defect, significantly greater than in the control ($P < 0.01$), was obtained in the group treated with bischofite gel (13.70 ± 0.76 N). Actovegin also demonstrated a positive reparative effect (12.60 ± 0.63 N, $P < 0.05$), significantly lower ($P < 0.01$) than in the control strength was obtained in the group using Contractubex (9.65 ± 0.59 N) [Figure 1].

Calorimetric analysis of the concentration of HP in the tissues of the wound defect

In the calorimetric analysis, it was found that the highest concentration of HP was contained in the tissues of wound defects in animals treated with Contractubex; however, there was no statistically significant difference with the control group, significantly lower in comparison with the control ($P < 0.05$) concentration of selenium (SE), contained tissues of simulated wounds in animals treated with bischofite gel (79.7% of the control) [Table 1].

Taking into account the data obtained in determining the physicochemical properties of tissues, the most likely reason for the increase in the concentration of SE in the tissues of animals treated with Contractubex is the presence of processes of secondary alteration and growth of granulation tissue.

On the other hand, a decrease in the concentration of HP in the wound defects of the group treated with bischofite gel indicates a decrease in secondary alteration and an accelerated repair. A similar trend in the group of animals treated with collagenase is more likely due to the failure of reparative processes associated with increased collagen degradation.

Assessment of the ratio of collagen I and III types in a polarizing microscope

When assessing the ratio of collagen I and III in the tissues of the wound defect when dyeing with picosirius red, it was found that, by the number of mature collagen fibers, the studied groups can be arranged in the following sequence (descending): Bischofite > Actovegin > Control > Contractubex [Table 2 and Figure 2].

Statistically significant differences were found in the bischofite and Contractubex groups. In the group that received Contractubex, there is an increased relative content of type III collagen, which indicates a delay in the maturation of collagen and the tendency to form scar tissue.

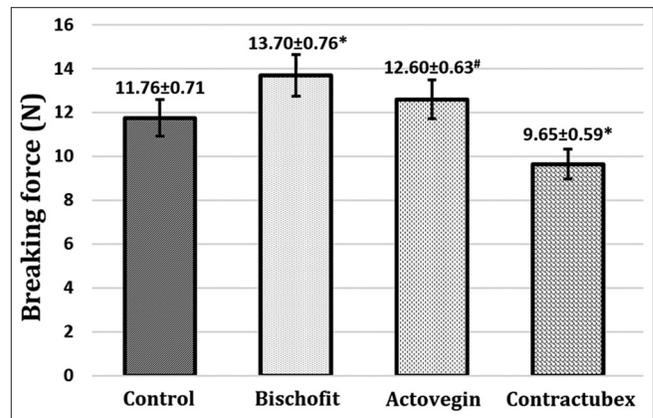


Figure 1: Force at the time of rupture (H) when assessing the strength of a wound defect using a mechanical early-finer.* $P < 0.01$ when compared with the control group; # $P < 0.05$ when compared with the control group

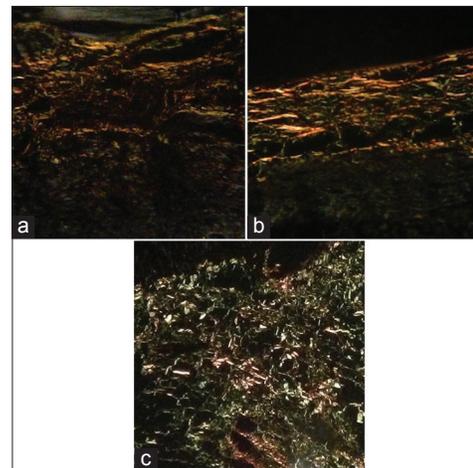


Figure 2: Microscopic picture of the skin in the area of the simulated wound in the control group (a), in the group treated with bischofite (b), and in the group treated with Contractubex (c), Polarization microscopy. Coloring Sirius Red H $\times 400$

CONCLUSIONS

The study showed that the best results were obtained when using the bischofite gel externally. The wound defect in this group was characterized by the greatest strength and lower tendency to form a scar, which can be judged by the low concentration of HP and type III collagen. Actovegin has a less significant but pronounced reparative effect on this model. Actovegin gel showed a positive effect on wound strength, and using laboratory research methods, its preventive effect on the excessive formation of scar tissue was also proved. Less satisfactory results were obtained when applying Contractubex. Without having a significant impact on the physicochemical properties of the wound, Contractubex increased the content of HP and reduced the content of mature collagen. This conclusion is confirmed by the results of histological examination and will be explained in terms of the available information on the pharmacodynamics of the drug.^[11]

Table 1: The concentration of HP in tissue samples of wound defects obtained on the 8th day after the start of the experiment

Group	Control	Bischofit	Actovegin	Contractubex
Concentration of HP, mg/g	16.59±1.08	13.23±1.68	15.89±1.37	17.61±0.67

*The presence of statistical significant differences when compared with the control group on the criterion of Mann–Whitney with $P \leq 0.05$.
HP: Hydroxyproline

Table 2: The ratio of collagen I and III types in tissue samples of simulated wounds received on day 8 after the start of the experiment

Group	Control	Bischofit	Actovegin	Contractubex
The ratio of collagen type I/type III collagen	0.56±0.012	0.73±0.023*	0.67±0.017	0.38±0.02*

* $P \leq 0.05$ when compared with the control group

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