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THE PHARMACOLOGICAL EFFICACY OF THE EXTEMPORANEOUS GEL DIAVENOL

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Effective treatment of the diabetic foot syndrome - severe complications of type 2 diabetes is the one of the main problems endocrinology. Loss of sensation in the lower extremities due to the later stages diabetic neuropathy over time leads to a foot injury and the emergence of diabetic ulcers. These ulcers are a very long time does not heal and eventually lead to the need for surgery [1]. Currently, effective tools for healing venous wounds do not exist.

As a means of therapy venous ulcers Diavenol gel was developed based on a combination of Chelidonium quinolizidine alkaloid and riboflavin [2]. The gel showed high effectiveness in the treatment of trophic ulcers, including diabetic origin [3]. The drug is produced extemporaneously. As is known, the share of extemporaneous formulations in some countries close to 40% taking into account the packaging of tablets from packages in bulk [4].

The main mechanism action of the drug is based on the synergy quinolizidine celandine alkaloids and riboflavin. These alkaloids are found in most plant material of the genus Papaveraceae, of which the most available Chelidonium majus. Chelidonium majus L., Semin. Papaveracea - celandine (BW), the family of poppies - known medicinal plant, has a diverse pharmacological activity, which is caused by the presence of alkaloids isoquinoline group [5]. In particular, chelidonine has a pronounced analgesic, sedative and antispasmodic action, the strength similar to that of morphine [6]. Grass celandine contains 0,97-1,87%, and the roots - 1,9-4,14% benzophenanthridine alkaloids sanguinarine, chelerythrine, protopine, chelidonine and others. In medicine, used the juice of the aboveground parts of the plant as an external agent in the treatment of warts, Kandil, papillomas, initial forms of lupus erythematosus. Composition bisulfate quaternary benzophenanthridine alkaloids of celandine is similar to that of makleya [7]. Sanguirythrine as liniment successfully applied and allowed as a means of treatment infectious and allergic dermatitis [8]. Sanguirythrine

shows no toxic properties both after oral and the topical application of [9].

Riboflavin, or vitamin B2, it has reparative properties. Back in the 40s XX-th century the most important role of riboflavin in the tissue regeneration has been set [10]. Further investigation showed that the regeneration of the amphibians tail stops using riboflavin function inhibitors [11]. The data on the direct relationship between the rate of stem cell division and riboflavin autofluorescence in cells, from which the authors conclude about the prospects of the use of high doses of riboflavin to enhance tissue regeneration [12]. Riboflavin is an essential component of culture media for culturing stem cells and embryonic cells [13]. Without these vitamin stem cells not only they fall, but not in highly differentiated tissue.

Thus, given the fact that the main active components of Diavenol well studied, are available on the market, non-toxic and approved for use, the pharmaceutical composition on the basis of celandine alkaloids and riboflavin is unsubstantiated.

The aim of this study was to evaluate the activity of wound healing gel Diavenol two compositions - D1 (for medical use) and D2 (cosmetology) - on the model stencil wounds in rats, as well as an assessment of their impact on the amount of their own stem cells (CD34). Both the gel ratio active substances identical and differ only in the presence of auxiliary substances and fragrances in cosmetic version D2.

Materials and methods

Defining Gel Diavenol influence on the healing of wounds stencil

The study of the wound healing properties of the gel Diavenol (two compositions) carried out in 38 male rats Wistar. The animals were divided into 4 groups: 1 - wounds, treatment by gel composition Diavenol D1 (n = 10); 2 - wound treatment by gel composition Diavenol D2 (n = 10); 3 - wounds, treatment by drug comparisons - dexpanthenol (panthenol-Ratiopharm, ointment 5%) (n = 10); 4 - Control wounds (n = 8).

Stencil wound back reproduced in animals under anesthesia with diethyl ether. The original size of the wound was $4 \pm 1,0$ cm². Application of drugs began with the first day after the alteration. Gel samples applied 2 times a day so that they cover the entire wound surface, and approximately 0.5 cm depilated skin around the wound. At 3, 7, 10, 14 and 18 hours was carried out planimetric study from the beginning of the experiment. The resulting area of the wound surface indicators were statistically processed using Student's t test.

Stimulation of the growth of pluripotent hematopoietic cells CD34 +

The drugs studied in the experiment on the model of cytostatic hemo-immunosuppression. Experiments were carried out in 90 male rats Wistar 160-200 g weight. The animals were housed with free access

to food and water. The two experimental and control groups consisted at 30 animals each. In both groups, hemo-immunosuppression invoked fivefold, at 24 hours intervals, the intraperitoneal administration of cyclophosphamide at 10 mg / kg, rubomycin 2 mg / kg, and prednisolone 2 mg / kg in 3 ml saline. On the next day after the end of drug administrations hemo-immunosuppressiv animal from experimental groups on depilated areas of the scalp (withers) gels were applied in an amount of 0.5 g / animal. The animals of the control group in the same terms applied to the skin similar to placebo - carbopol gel without active ingredients. Applications gels were performed daily, 2 times a day. Blood samples were collected from the tail vein at 1, 10, 30, 60 and 90 days of the experiment. Proliferative activity of CD34 + stem cells was studied using a test-system Countkit CD34 [14] for cytometry with the use of flow cytometry FACS Calibur. Digital material is processed using nonparametric statistics to the definition of «U» test by Wilcoxon - Mann - Whitney.

Results and discussion

Gel Diavenol two compositions had a significant effect on the dynamics of wound healing in rats. On day 3 (48 hours of playback stencil wounds) observed maximum intensity of inflammation in control group with pathology. Swelling and redness covered and the surrounding tissue. The area of the wound surface in this period even slightly exceeded the initial parameters of the wounds (Table 1). The general condition of the animals was heavy. They were sedentary, ruffled hair coat, decreased food intake. Diavenol gel in this period did not cause changes in the area of healing, however, it had lower expression of the local inflammatory response and slightly better general condition of the animals.

Later in the experimental groups was observed rapid positive dynamics of the clinical condition of the rats. On day 7, in the experimental groups redness and

swelling was small, centered wounds formed granulation tissue. It marked the beginning of reparative processes - edge overgrowth defect that affected the wound area. In this period there was a significant reduction in wound surface area in relation to the performance 3 days. At the same time, this indicator has not changed in the control group with pathology.

On day 10 of the experiment in both groups experienced marked boundary epithelization of wounds, a significant decrease in the area of the wound surface in comparison with the original data and the untreated control, complete cleansing of the wound surface, the presence of granulation tissue, the lack of inflammation.

In subsequent periods, there was a rapid healing of the wound defect in rats treated with gels Diavenol. On day 14 the wounds were dry their area was 0.8 and 0.7 respectively in the groups 1 and 2, i.e. most of the wound surface was epithelized. At the same time the control pathology, although a number of animals positive trend observed in 4 of 8 rats still remained inflammation, wound surface area and had a significant size.

By 18 days, all animals of experimental groups fully healed wound defects, and in the control group, 3 animals was complete epithelialization of wounds, including a 1 animal with pus.

Dexpanthenol ointment also contributed to the reduction of terms of wound healing. However, its effect was somewhat less pronounced than that of the gel Diavenol, the difference in the area of the wound surface in a significant number of cases. Table 2 shows the results of a study influence the samples compositions on the amount pluripotent CD34 + cells in the rats blood. Normal levels of CD34 cells in the blood ranges from 3 to 6 x 10⁶ cells / liter.

Table 1. Influence of Diavenol gel at stencil on a surface area of wounds in rats

Samples	n	The area of the wound surface, cm ² (M±m)				
		3 day	7 day	10 day	14 day	18 day
Diavenol D1	10	4,87±0,41	3,33±0,30*	**2,23±0,21*	0,82±0,23*	-
Diavenol D2	10	5,01±0,37	3,59±0,24*	2,72±0,24*	**0,72±0,20*	-
Dexpanthenol	10	4,84±0,29	4,04±0,28	3,20±0,29	1,23±0,21*	-
Wounds control	8	5,07±0,30	4,81±0,37	3,90±0,40	2,21±0,35	0,51±0,21

* - reliability of differences in relation to disease control indices ($P \leq 0,05$);

** - reliability of difference between the indices of the studied samples gels and the reference drug ($P \leq 0,05$);

At the beginning, therapy during cytostatics was observed decreased level of CD34-cells to 3-4 times, compared to baseline. Thus, animals in both groups were able to recover their normal values. However, bone marrow regeneration process with restoration of peripheral blood flowed differently. Topical using D1

and D2 contributed to the acceleration of regeneration processes is about 2 times. This was evidenced excess of blood parameters more than 2 times in experimental group animals to the 30th day, while the values in animals from control group came to normal levels only on the 60th day.

Table 2. Number of blood pluripotent cells + CD34 in rats under influence compositions D1 and D2

Samples	n	CD34+ · 10 ⁶ cells/L				
		1 day	10 day	30 day	60 day	90 day
D1	30	1,2±0,4	4,4±0,6	12,3±0,8	15,2±0,9	15,6±1,0
D2	30	1,0±0,3	4,3±0,7	12,0±0,7	14,3±0,8	15,0±1,1
Carbopol**	30	1,7±0,3	1,0±0,2	1,9±0,2	4,2±0,3	4,0±0,4

* - $P \leq 0,05$; ** - The average level in the control group rats without immunodeficiency 4,2±0,3

The maximum effect of Diavenol using observed on the 60th day and the restoration of the physiological level of pluripotent cells have been observed on the 10th day after the start of the gel application. Increasing the number of pluripotent cells coincided in time with the stimulation of reparative processes (stencil wound model in rats), which apparently indicates preferential stimulation of stem cell division in the periphery, rather than an enhancement of bone marrow function.

As gels were applied to intact skin, one can speak about high resorptive properties both gel compositions and the overall effect on the entire body.

Conclusions

1. Gel Diavenol at two compositions (for medical and cosmetic applications) has pronounced reparative properties, significantly speeding up the epithelialization and wound healing in rats stencil. In this kind of action it is slightly superior to dexpanthenol.
2. Diavenol gel has the ability to be absorbed through the skin and have a beneficial effect on the immune system of whole animal's body through stimulation of pluripotent CD34 –cells growth.
3. The joint action on animal's tissues of riboflavin and chelerythrine / Sanguirythrine in form Diavenol gel results in selective stimulation growth pluripotent cells and stimulation of excretion of wound fibroblast growth factors contribute to a 6-fold acceleration of immune reconstitution after animals induced immunodeficiency.

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Introduction. Diavenol - new extemporaneous gel for the treatment of venous ulcers. The gel contains a synergistic combination *C. majus* quinolizidine alkaloids and riboflavin. Activate tissue regeneration shown previously for riboflavin, and cheleretrin / sanguirythrine. The combination of the alkaloids and riboflavin pharmacologically fully justified. The research aim was to check the mechanism action of the Diavenol gel - through stimulation of the pluripotent cells growth

and determination the effectiveness of the gel for wound healing at experimental model in rats. **Materials and methods.** In a study 128 rats were used, of which 38 - in the pharmacological experiment with a stencil wound healing, and 90 in the experiment to study the influence of gel on CD34 cells amount in the blood in rats with chemically induced immunodeficiency. High immunodeficiency caused a five-fold administration of cyclophosphamide, prednisone and rubomycin. CD34 expression level was determined using flow cytometry FACS Calibur. **Results and discussion.** Topical skin application the Diavenol gel samples in rats helped to accelerate the regeneration process is about 2 times, as evidenced by the excess of blood parameters in experimental group animals to the 30th day, more than 2 times, while the indicators in the control group animals came to normal values only on the 60th day. Increasing the number of pluripotent cells as well as acceleration of wound regeneration indicates preferential stimulation of stem cell growth in the periphery, rather than an enhancement of bone marrow function. The maximum effect of the Diavenol observed on the 60th day and the restoration of the pluripotent cells physiological level have been observed on the 10th day after the start of Diavenol application. Due to the fact, that the gel was applied to intact skin, we can say that Diavenol had resorptive properties. Treatment of wounds in rats Diavenol gel resulted in a more rapid removal of inflammation and significant acceleration of stencil wound healing. In terms of the granulation appearance and wounds epithelialization the Diavenol gel has some advantage in relation to the dexpanthenol ointment.

Keywords: diavenol, CD34 + cells, regeneration, wound healing, riboflavin, Chelidonium majus, chelidonine, chelerythrine, sangviritrin.

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