

Implementation Potential of the Improved Formula-Biodegradable Wound Dressing

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Abstract: The wound dressing has been developed on the basis of chitosan with the additional components presented by antioxidant, buffering agent and plasticizer. The developed specimen has been tested in 30 white outbred male rats with the mass of 180-220 g with the contrived aseptic dermal wound in the interscapular area. As affected by the wound dressing, acceleration of the wound area's daily reduction, rapid recovery of the epidermal thickness, decrease in intensification of lipid peroxidation processes, normalization of the antioxidant system activity, formation of the connective-tissue structures, characterized by normal histomorphologic features, were observed. Besides, the preventive action of the dressing in respect of the wound infection by the opportunistic flora has been proved.

Key words: Experimental wound • Wound dressing • Chitosan • Glycerin • Asparaginic acid • Ceruloplasmin • Reparative regeneration • Lipid peroxidation processes • Antioxidant system

INTRODUCTION

The wound damage, especially infected, is characterized by a number of local reactions accompanied by the essential metabolic disorders, including the activation of the free radical oxidation processes, which resultants have the damaging effect on the cell membranes; the acidosis development promoting the lesion increase and regeneration process deceleration [1-3]. That requires purposeful correction, in particular, application of the wound dressings with specific properties [4]. The similar curative effect is reached by additional inclusion of the auxiliary components into the structure of the wound dressings.

The natural biodegradable polymer - chitosan - is quite often used as a basis for obtaining of the wound dressings with antibacterial and antioxidant properties [5-10].

Objectives: To develop the biodegradable wound dressing and examine its influence on the contrived aseptic experimental wound. Materials and methods: the wound dressing has been developed on the basis of chitosan; plasticizer (glycerin), antioxidant (ceruloplasmin)

and buffering agent (asparaginic acid) have been also integrated into the formula. The wound dressing has been perforated.

MATERIALS AND METHODS

The wound dressing has been tested in 30 white outbred male rats with the mass of 180-220 g. A full flap dermal wound of 400 sq. mm in size has been simulated with each animal.

The wound dressing of the appropriate size has been once spread on the wound of the experimental group animals, slightly pressing it to the wound on purpose of providing good adhesion to the damaged surface. The repeated application or the change of the dressing in the course of the treatment is not required, it gradually biodegrades in the process of the wound healing. After completion of the epithelization process, if any remains of the wound dressing present on the skin surface, they are carefully washed away.

In the comparison group the wound cleaning has been carried out by single impact by normal saline on the wound surface and removal of necrotic tissues. The changes of the daily wound area of the experimental

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group and the comparison group animals have been examined over time; the bacteriological researches have been conducted; lipid peroxidation indicators (malondialdehyde (MDA) content) in the blood serum and the antioxidant system condition (ceruloplasmin (CP) activity) have been researched.

The dynamic change of neutrophilic leucocytes and fibroblastic cells quantity has been examined for assessment of the reparative processes with the animals of both groups by means of the cytological technique.

RESULTS

Table 1. Daily reduction in the wound area of the experimental animals (as %) in the course of the treatment ($M \pm m$) (Table 1).

While analyzing the daily reduction of the wound area with the animals, the wound dressing application shows the evident dynamics of the daily reduction of the wound area. Complete wound healing has been marked by the 10th day with 7 animals of the experimental group, by the 14th day - with 8 rats more. The wound healing with 11 animals of the comparison group has been marked only on the 14th day. The reparation process with four other rats has ended by the 21st observation day because of the wound infection.

Over the whole experimental period bacteriological researches of the wound surface have been made with all animals. The wound surface inoculation of all rats belonged to the experimental group has remained sterile over the whole period of observation.

E. coli infection (2×10^5 CFU) has been registered with 4 rats of the comparison group on the 5th day. The same microbial flora in the number of 2×10^2 CFU has remained with two animals of the comparison groups by the 21st day; according to the bacteriological research, the wound has been sterile with 2 rats by the 20th observation day.

As concerning the experimental group, CP activity has been considerably ($\delta < 0.001$) increased to 24.23 ± 0.35 c.u. after the treatment, in comparison with the group where the treatment has not been carried out (19.43 ± 0.65 c.u.). The positive effect of the complex preparation may be explained not only by the known antioxidant action of chitosan [11], but also by the fact that the exogenous CP forming a part of the wound dressing, shows the substitutive properties in relation to the endogenous CP, which activity decreases in the conditions of the aseptic wound that leads to activation of the free radical oxidation processes.

Table 1:

Day (24-hour)	Series of Experiments	
	Comparison group	Application of the complex preparation, n=15
the 3 rd day	4.6±1.2	10.8±0.8 p<0.001
the 5 th day	5.4±2.1	33.1±0.5 p<0.001
the 7 th day	6.4±0.5	24.2±1.7 p<0.001

Note: p - confidence level of the indicator distinctions in relation to the comparison group

Under the action of the wound dressing the decrease in intensification of the lipid peroxidation processes, making the damaging influence on the cell membranes and decelerating the wound regeneration process, has been observed. The MDA level has been declined ($\delta < 0.05$) till 2.83 ± 0.11 $\mu\text{mol/l}$ in comparison with the indicators of the untreated animals (3.21 ± 0.12 $\mu\text{mol/l}$).

The 3-days cytogram - since the moment of the contrived aseptic experimental wound simulation - has been taken as an initial indicator. There are polymorphic and nuclear infiltration foci along the wound's perimeter; histiocytes are observed focal; there are granulation tissue areas in the subjacent tissues. The wound bed has full-blooded vessels and a significant amount of myofibroblasts and histiocytes.

On the 5th research day, the cytogram of the comparison group has been mainly presented by neutrophilic leucocytes, which number has been decreased on the average 1.05 times as compared to the 3rd observation day. In the experimental group animals the number of the dominating neutrophils has been decreased 1.07 times. As for the experimental group the appearance of single fibroblastic cells has been marked, as distinguished from the comparison group.

By the 7th day, the further decrease in number of neutrophilic leucocytes has been observed in the comparison group - 1.17 times; in the experimental group - 1.59 times. At the same time, the increase in number of fibroblastic cells, except for the infected wounds, has been marked.

On the 11th day, the impression smear of the comparison group's animals demonstrated the reduction in number of neutrophilic leucocytes - on the average 2.6 times in comparison with the initial indicators, while the number of fibroblasts has been increased - on the average 66.4 times. During the same observation period the wound epithelization has been completed with almost all animals of the experimental group. The obtained results concerning the dynamic change of the average number of neutrophilic leucocytes are given in the table.

The histological research made in the experimental group showed the increase in the epidermis thickness by 42.3% in relation to the comparison group by the 10th day. The number and the structure of the blood vessels located within the epidermic layer, have the features close to normal, particularly, the ordered location, typical for the organized granulation tissue with the formation of the vascular loops. The intensive fibroblast proliferation has been marked within the dynamic blood circulation loci. The dermatitis-epidermal junction is florid. The skin is flexible, never conglomerated with the lower tissue. Recovery of the sebaceous glands' structure, as well as the presence of hair follicles of various maturity degree and hair growth, has been marked. The structural components are restored. The collagen fibers are horizontal. As concerned the comparison group animals, the collagen fibers are located chaotically, rarer - vertically along the vessels, in the skin - the accumulation of acidic (sulfated) glycosaminoglycans. The inflammatory cellular infiltration remains with the majority of the rats.

In the course of healing the wound dressing's gradual biodegradation has ended almost simultaneously with the wound epithelization complete.

CONCLUSION

The results were demonstrating the efficiency of the chitosan-based wound dressing influence on the reparative regeneration in the conditions of the full-flap skin wound.

Deduction: The developed improved formula-wound dressing can be recommended for treatment of experimental wounds as an independent pharmaceutical form, as well as it can subsequently be used as a whole with metal nanoparticles.

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