Bulletin of Experimental Biology and Medicine, Vol. 120, № 10, October, 1995

The Quaternary Ammonium Salt of Conidine Oligomer 25 Hastens Healing of Skin Wounds in Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 120, № 10, pp. 407-409, October, 1995
Original article submitted December 12, 1994

Collagen sponges and films containing a synthetic polycation, the quaternary ammonium salt of conidine oligomer 25, stimulate the repair processes when applied to a wound, accelerating the final epithelialization of skin wounds. The effect of the drug manifests itself mainly during the proliferation and cicatrization stages by speeding up replication and transcription in the fibroblasts and producing intercellular matrix components.

Key Words: *skin; repair; polycation; sponges; films; autoradiography; histochemistry*

The quaternary ammonium salt of the monodispersed oligomer conidine including 25 monomer components (QAS) can selectively and rapidly interact with heparin and restore blood clotting when the extracorporeal circulation is disconnected by abolishing the anticoagulant effect [7]. The oligomer is effective in that it combines high antiheparin activity (weight neutralizing ratio 0.75:1) and low toxicity (116 mg/kg).

Medical use of this agent holds good promise. It has been shown to appreciably hasten repair of the liver in animals with acute toxic hepatitis induced by CCl_4 by enhancing replication and transcription in hepatocytes. Such a mechanism of stimulating regeneration can also be envisaged upon exposure of skin cells to the cation polymer after a skin injury.

This paper describes the success achieved with treating skin wounds by means of dressings with QAS.

MATERIALS AND METHODS

Male albino rats weighing 180 to 200 g were used in the study. A 300 mm^2 area of skin was removed with the subcutaneous fat in the interscapular area under ether narcosis. Standardization of the wound size was achieved by inserting a Teflon ring in the wound after a previously described method [4]. Collagen films or sponges, as well as composite coatings containing, in addition to collagen, 6-methyluracil or QAS, were applied once to the wound surface inside the rings [2]. The rings were then covered with perforated cellophane to prevent contamination, drying of the wound, and loss of the collagen coating. The teflon rings were removed on day 5 after the operation; wound size was measured planimetrically on days 7, 10, 12, 14, and 16, and the time of definitive healing of the skin defect was recorded. The relationship between the rate of wound healing and the QAS concentration in the dressing was assessed. The wound-healing effect of a dressing with the agent of interest was compared with that of the commercial collagen sponge Methuracol containing 5% 6-methyluracil, manufactured in Russia.

The animals were divided into 15 groups, 10 rats in each. In control group 1 healing proceeded under a collagen film, in group 2 under a collagen sponge, and in group 3 under a Methuracol sponge. In the experimental groups collagen sponges or films with QAS in concentrations ranging from 1 to 50% were applied to the wounds. The effects of the preparations on wound healing were monitored planimetrically and by histological and histochemical methods (staining with hematoxylin-eosin, pic-

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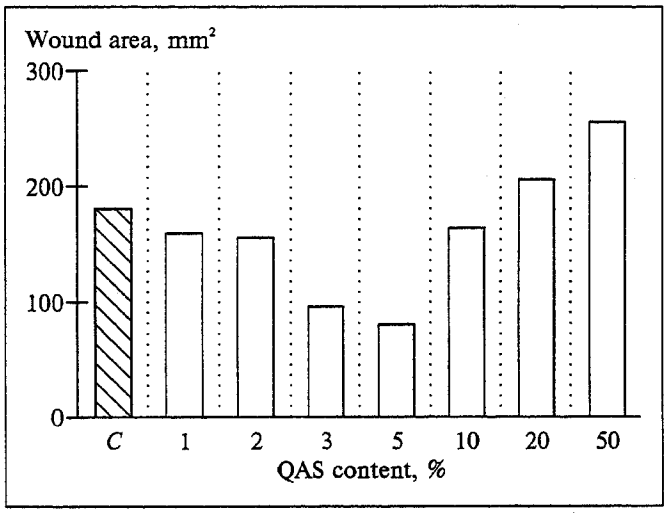

Fig. 1. Relationship between wound healing and QAS content in collagen film (day 12 of experiment).

rofuchsin after Van Gieson, toluidine blue for glycosaminoglycans, and Brachet's test for RNA).

Sponges with 5% ^{14}C-QAS were used to elucidate the rate of QAS transfer from the dressing to organs and tissues. The animals were sacrificed 3 h and 3 days after the sponges were applied to the wounds. Label incorporation was measured by autoradiography in the blood, liver, spleen, and granulation tissue of the wound.

The numerical data were statistically processed, and the reliability of the results was assessed using Student's t test [1].

RESULTS

Study of the time course of wound healing in animals treated with collagen films with different contents of QAS showed that by day 7 postoperation the wounds of animals to which dressings with 2-5% QAS had been applied were smaller than the wounds in animals of the control and other experimental groups. By day 12 the difference in wound size in animals from different groups had increased still further. Figure 1 shows the dose-effect relationship of wound healing acceleration in percent of the control on day 12 of the experiment. The data indicate that by this time application of a coating containing 3 or 5% QAS diminished wound size more than twofold in comparison with control group 1.

Study of the therapeutic effect of collagen films and sponges as the agent carriers showed that their effects at low QAS concentrations were almost the same. The toxicity of high concentrations of the polymer was less expressed in sponges than in films, and hence, sponges less often than films caused hemorrhages in the wounds and death of animals.

Comparison of the size of wounds treated with films and sponges with 5% QAS (Fig. 2) showed that stimulation of repair under the effect of collagen coating with QAS is maximal on day 14 for films and on day 16 for sponges; it remains quite high for a long time, thus shortening the period of final epithelialization. Table 1 shows that wound healing aided by sponges with 5% QAS is observed 19.2±1.5 days postoperation, with a 36.2% index of healing acceleration in comparison with collagen film and a 20% index in comparison with control group 2 (Methuracol). This suggests a higher therapeutic effect of dressings with QAS in comparison with Methuracol sponges, although these sponges are widely used in surgery for the treatment of ulcers, burns, wounds, and other skin injuries.

In order to find out how QAS affects the cell composition and processes taking place in the wound, histochemical studies were carried out at various times of wound healing. The fate of the synthetic polycation coming in contact with the wound surface as a component of the dressing was assessed from the results of autoradiographic analysis of the granulation tissue, liver, and spleen of animals treated with sponges containing ^{14}C-QAS. The agent was shown to be partially retained on the plasma membrane and inside granulation tissue fibroblasts and macrophages, but the bulk of it was delivered to tissues with the blood.

By day 3 QAS was detected in diminishing concentrations in visceral vascular walls, hepatocytes, and splenocytes. On days 3-7 postoperation QAS enhanced the permeability of already existing and newly formed vessels at the site of the skin defect by increasing the content of polymorphonuclear leukocytes and macrophages. Maturation of granulation tissue during this

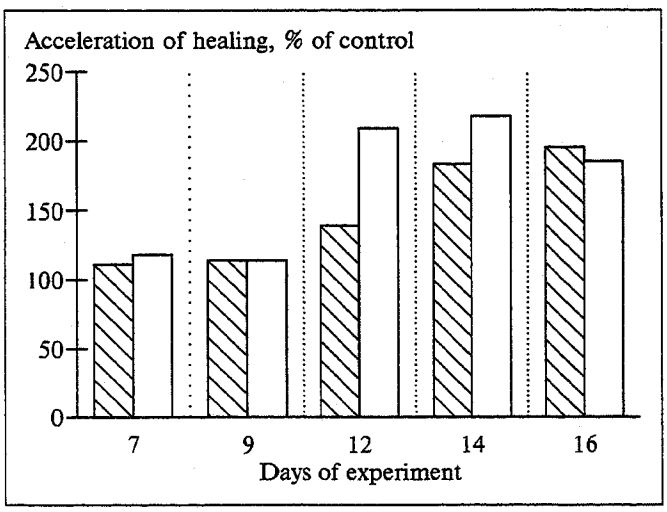

Fig. 2. Acceleration of wound healing under the action of QAS as a component of collagen sponges and films. Dark bars: collagen sponges with 5% QAS; light bars: collagen films with 5% QAS.

TABLE 1. Time Course of Wound Healing under the Action of Collagen–QAS Compositions ($M \pm m$)

Test agent	Number of animals	Wound size, mm²			Time of final healing	Index of healing acceleration, %
		7 days	10 days	14 days		
Control group 1 (collagen sponge)	20	284.3±43.6	238.6±24.0	89.0±22.8	30.1±1.24	
Control group 2 (Methuracol sponge)	20	244.5±18.7	132.6±14.3	71.3±12.1	24.0±1.0	
Collagen sponge with 2.5% QAS	20	247.1±25.0	221.3±18.2	66.9±12.8	21.0±1.3	30.2* 12.5**
Collagen sponge with 5% QAS	20	257.1±25.8	208.6±32.1	48.7±1.8	19.2±1.5	36.2* 20**
Collagen sponge with 10% QAS	20	285.7±35.6	256.4±19.0	147.9±47.4	32.5±2.7	7.9* 35.4**

Note. Healing acceleration index: one asterisk shows values *vs.* control group 1, two asterisks *vs.* control group 2.

period did not differ from that in control group 1 (collagen films), but granulations were more widespread, this intensifying the repair at later periods: the content of fibroblasts increased, as did the biosynthetic activity of cells. This was confirmed by a more expressed pyroninophilia of the cytoplasm and fibroblast nucleoli, an increased content of acid glycosaminoglycans and collagen in the intercellular substance, and an earlier epithelialization of the wound defect.

Experiments with ^{14}C-QAS demonstrated that the agent passes from the wound defect into the liver, where it is retained, exerting a positive effect on the function of this organ [6]. Comparison of the time course of wound healing as a result of treatment with 6-methyluracil and QAS indicates marked differences in the mechanism of action of these drugs. According to a previous report [5], the biological efficacy of 6-methyluracil is based on its participation in the regulation of the antioxidant defense systems of the organism. That is why it is most active at the initial stages of healing, reducing the duration of the phase of inflammation [3].

For wounds under dressings with QAS the healing process is hastened at later stages during proliferation and cicatrization due to boosted replication and transcription in the fibroblasts, stimulated production of intercellular matrix components, and enhanced regeneration of the epithelium.

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