PROLIFERATION OF EPITHELIUM SURROUNDING A SKIN WOUND IN HAIRLESS MICE TREATED WITH SODIUM CHLOROPHYLLIN

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The epidermis of the skin surrounding a wound plays an active part in regeneration: during the period of epithelization of the wound there is a sharp increase in mitotic activity in the zone immediately adjacent to the wound edge; when epithelization of the defect is complete, proliferative processes in the epithelium of the skin around the wound do not die away but, on the contrary, increase in intensity and spread to areas of skin more distant from the wound edges. Proliferative processes in the epithelium around the wound continue for a long time and are fluctuating in character: the mitotic index (MI) decreases slightly from the 14th to the 21st day, then begins to increase, and by the 60th day it is five times greater than MI for intact epithelium. Sodium chlorophyllin, which accelerates wound healing, stimulates proliferation of the epithelium around the wound.

In the study of the healing of skin wounds investigators have concentrated their attention on processes taking place in the wound itself: epithelization, contraction of the wound edges, scar tissue formation, and regeneration of the accessory structures of the skin. Changes observed in the skin around the wound have received much less attention. Nevertheless, the intact skin surrounding wounds plays an active part in restoration of the defect and largely determines the outcome of healing, especially on the mobile areas, where increased contraction takes place, and also in sutured wounds, after the excision of large skin grafts. The skin around a wound has been shown to undergo intensive growth, making good the defect [1, 4-7]. The increase in mitotic activity of the epithelium observed under these circumstances spread to intact epithelium adjacent to the wound for a distance of 6-10 mm from the wound edge [2, 9].

Totally insufficient attention has been paid to the study of the action of stimulators of proliferative processes in the skin surrounding the wound. Hitherto most investigations have been undertaken on animals with a thick hair cover, and this makes matters more difficult when proliferation is studied in the interfollicular epithelium. The most convenient animals with which to study processes in the skin around a wound are hairless mice. The absence of hair makes it possible to study the behavior of the interfollicular epidermis in greater detail, makes the skin more like human skin, and at the same time, simplifies the histological analysis.

The object of the investigation described below was to study mitotic activity of the epidermis in hairless mice. The investigation was carried out in the skin around a wound: immediately next to the wound edge and a short distance from it. The action of a stimulator on proliferation in these areas also was studied.

EXPERIMENTAL METHOD

Sodium chlorophyllin, manufactured in the USSR, was chosen as the substance accelerating healing of skin wounds. The writer has previously demonstrated the stimulant effect of sodium chlorophyllin experi-

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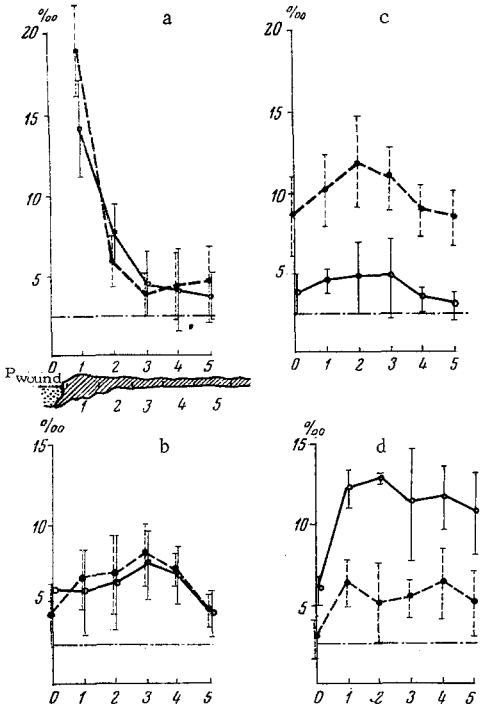

Fig. 1

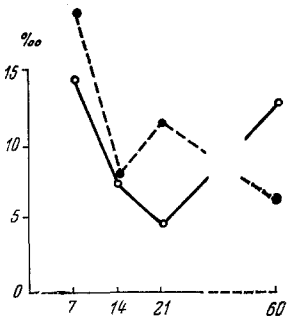

Fig. 2

Fig. 1. Changes in MI at various times of healing of skin wounds in hairless mice: a) 7 days; b) 14 days; c) 21 days; d) 60 days. The wound and adjacent zones are shown schematically beneath the graph. Continuous line, control group; broken line, treatment with stimulator; line of dots and dashes, normal epithelium.

Fig. 2. Changes in maximal values of MI at various times of healing of skin wounds in hairless mice. Continuous line, control group; broken line, treatment with stimulator. Abscissa, days after operation; ordinate, maximal MI (in ‰).

TABLE 1. MI of Epidermis (in ‰) in Control (not treated with stimulator) and Experimental (treated with sodium chlorophyllin) Groups at Different Times after Operation (M ± m)

Day of operation	Group of animals	Scar	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5
7th	Control		14,4±1,3	7,6±0,8	4,4±0,8	4,1±1,2	3,6±1,4
	Experiment		19,2±1,5	5,8±0,6	3,9±0,5	4,1±0,9	4,5±0,8
14th	Control	5,3±0,5	5,6±1,1	6,2±1,2	7,6±0,7	6,7±0,7	4,2±0,6
	Experiment	4,2±0,8	6,6±0,6	6,9±0,9	8,0±0,6	7,1±0,4	4,6±0,5
21st	Control	3,6±0,4	4,4±0,2	4,8±0,9	4,9±1,0	3,4±0,3	3,1±0,4
	Experiment	8,8±0,9	10,2±0,9	12,1±1,1	10,9±0,8	9,1±0,6	8,3±0,7
60th	Control	5,9±0,2	12,3±0,4	13,0±0,2	11,4±1,2	11,7±0,5	10,8±1,0
	Experiment	2,8±0,3	6,0±0,8	5,3±0,9	5,7±0,5	6,4±0,9	5,1±0,7

Note. MI of intact epithelium 2.3 ± 0.2.

mentally [3]. Experiments were carried out on 48 sexually mature male hairless mice weighing 25-30 g. The animals were divided into two groups (control and experimental), with 24 mice in each group. A circular full-thickness skin graft measuring 8 mm in diameter was excised down to the fascia in the interscapular region of the animals of both groups, over the spine [8]. The grafts excised during the operation were used for determining the mitotic index (MI) of the intact epithelium. Drops of an aqueous solution of sodium chlorophyllin in a concentration of 2 mg/ml were applied to the wound and surrounding skin of the experimental mice daily for 60 days.

To avoid diurnal fluctuations the operation and wound treatment were carried out at the same time: 9 a.m. Seasonal variations were also eliminated because the experiment was carried out at the same time

of year (May-June). Each animal was kept in isolation. Material for biopsy was taken on the 7th, 14th, 21st, and 60th days. Six control and six experimental mice were used at each of these times. Only "clean" wounds with no visible evidence of suppuration were considered.

For the histological investigations a piece of skin with the scar and 8 mm of intact skin adjacent to the scar on the cranial end was excised. Sections 7 μ in thickness were cut parallel with the body axis. The MI was calculated in 1000 cells. The area of skin adjacent to the wound was divided into five zones, each of which included 10 fields of vision (450 ×). The number of dividing cells was counted in 10,000 cells of the stratum germinativum of the regenerating epithelium and in 3000-5000 cells of the stratum germinativum of the interfollicular epithelium of the intact skin. Statistical analysis of the result was carried out by the Fisher–Student method.

EXPERIMENTAL RESULTS

MI of the intact epithelium of the skin of the hairless mice was $2.3 \pm 0.2‰$. The results of histological analysis of material from the control group of animals showed that on the 7th day after the operation proliferation in the epithelium was intensified chiefly at the wound edge and to a distance of not more than 2 mm from it. The MI of the epithelium of the marginal zone was seven times higher than MI of the intact epithelium, with a mean value of $14.4‰$. In the more distant zones the increase in MI was not significant (Fig. 1; Table 1).

On the 14th day epithelization of the wound defect was complete in most mice. Young connective tissue filling the wound was organized into scar tissue. Active cell division was observed in the epithelium covering the scar and in all five zones of the intact skin studied. The increase in proliferation was relatively uniform in all zones of the area of skin studied. The mean value of MI in this region was $6.2‰$, 1.5-2 times greater than MI of the intact epithelium. The greatest increase in MI was found not at the wound edge itself, but away from it in zones 3-4. This excess was not statistically significant although there was a definite tendency for it to occur, and this was clearer in animals treated with sodium chlorophyllin.

After 21 days the number of mitoses in the epithelium covering the scar decreased ($P < 0.05$). Some slowing of proliferation also was observed in the skin surrounding the wound. However, MI in the regenerating epithelium was still higher in all zones studied than in normal epithelium.

Proliferation in the epithelium of the scar and in the skin surrounding the wound (in all five zones) was sharply intensified 60 days after the operation. The MI in the regenerating epithelium was increased by $2.3‰$ compared with the previous period. The changes were particularly marked in the epithelium around the wound. The number of mitoses there was twice as high as at the previous period and 5-6 times greater than in the intact epithelium ($P = 0.001$). The mean value of MI for all five zones was $11.8‰$. No decrease in proliferation was observed even in zone 5, i.e., a considerable distance from the wound edge. This is evidence that the increase in proliferative activity extends beyond the limits of the area of skin studied.

Consequently, during the healing of wounds an increase in the intensity of proliferation is observed in the skin surrounding the wound defect; this increase is found at the wound edge in the period of epithelization and in areas of skin more distant from the wound margin after the completion of epithelization. It is important to emphasize that these processes are of long duration and are not yet complete two months after the operation.

The results of histological analysis of material from the experimental group of animals showed that application of sodium chlorophyllin for 7 days leads to stimulation of proliferation in the marginal zone (1) compared with the control. The MI reached its maximum of $19.2 \pm 1.5‰$ ($P = 0.01$; Fig. 1; Table 1).

The changes observed in all five zones on the 14th day represented very little difference from the control.

On the 21st day of treatment with sodium chlorophyllin the most marked proliferative activity was found both in the regenerating wound tissues and around them. MI in the epithelium covering the scar was $8.8 \pm 0.9‰$, and in areas of epithelium around the wound $10.1 \pm 0.8‰$; i.e., it was increased by 4 or 5 times compared with MI of the intact epithelium. The increase in MI was relatively uniform in all five zones.

By the 60th day proliferative processes in the skin surrounding the wound had started to subside. The MI in the scar was reduced to MI of the intact epithelium. The number of dividing cells in the epithelium surrounding the wound was considerably reduced: MI in all five zones studied was approximately the same, $5.7 \pm 0.7‰$.

Sodium chlorophyllin thus not only stimulates epithelization of the wound defect, but also accelerates the course of proliferation in the epithelium surrounding the wound. Both in the experimental series and in the control the increase in MI was fluctuating in character (Fig. 2).

Under the influence of the stimulator (sodium chlorophyllin) wound healing proceeds in the usual manner but at a more rapid pace. No structural differences were found in the scar tissue in the experimental and control groups.

Sodium chlorophyllin has a specific action on regenerative processes in the skin surrounding the wound and accelerates their course. The compound subsequently has no marked effect on MI of the cells of the epidermis.

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