STIMULATION OF CONTRACTION AND EPITHELIZATION OF SKIN WOUNDS BY SOLUBLE COLLAGEN

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Much has been published on the study of changes in the composition and properties of collagen in wounds of superficial tissues [9]. There are also indications of the promising use of collagen-containing coverings (sponges, films, etc.) in the treatment of wounds of the skin and cornea [1, 2]. However, the action of soluble collagen on wound healing has so far received little study. In particular, it is no clear how local applications of different forms of soluble collagen affect stages of healing of skin wounds such as contraction, epithelization, and restoration of the connective-tissue matrix.

The aim of this investigation was to find an answer to this question which may be useful both for the development of new, and for rendering more effective the existing collagen-containing agents for wound treatment.

METHODS

Male Wistar rats weighing 200-250 g were kept on an ordinary diet, one to a cage. The animals were anesthetized by intraperitoneal injection of pentobarbital in a dose of 40 mg/kg body weight. Before wounding of the interscapular region the hair was removed by plucking from an area of about 10 cm^2. Collagen solution (2 mg/ml, pH 6.5-6.8) was obtained by dissolving bands of collagen from rats' tails in 0.01% acetic acid [5]. Ointment containing collagen (0.4 mg/ml) was prepared by introducing collagen solution (4 mg/ml) into an ointment base, consisting of emulsion of oil—water type, containing up to 50-52% of fatty components (stearine, lanoline). (The authors are grateful to T. I. Chizhov for preparing the ointment.) Deepithelization of four areas of skin 5 mm in diameter, located at the corners of a square with side of 20 mm, on the dorsum of each rat was carried out simultaneously by creating a negative pressure [8]. The negative pressure situation was maintained automatically (250 mm Hg, 60 min) by means of an "Epitom" multichannel apparatus (Biotekh-Elektron, Moscow). The two wounds on the right of the midline of the dorsal region served as controls, the two on the left as experimental. Full-thickness circular wounds 6 mm in diameter also were inflicted in the interscapular region at the corners of a square with a side of 20 mm. The two wounds located on one diagonal of the square in this case served as the control, the other two as the experiment. Full-thickness linear wounds 40 mm long were inflicted as linear incisions along the midline of the dorsum, to which three transverse sutures were applied 12 mm apart. The test preparations were applied once daily to the wounds, in a volume of 50-70 μliters. When collagen solution was used, physiological saline was applied to the control wounds; when ointment containing collagen was used, the ointment base was applied. The rate of epithelization was recorded by two methods: a decrease in area and increase in resistance of the deepithelized regions. The essentials of the first method, fully described previously [7], included separation of the epidermis from the dermis of a previously excised skin flap containing the deepithelized region, followed by measurement of the area of the epithelial "window" under the microscope by means of an ocular micrometer. By repeating the procedure described above at each of the previously chosen times of observation,

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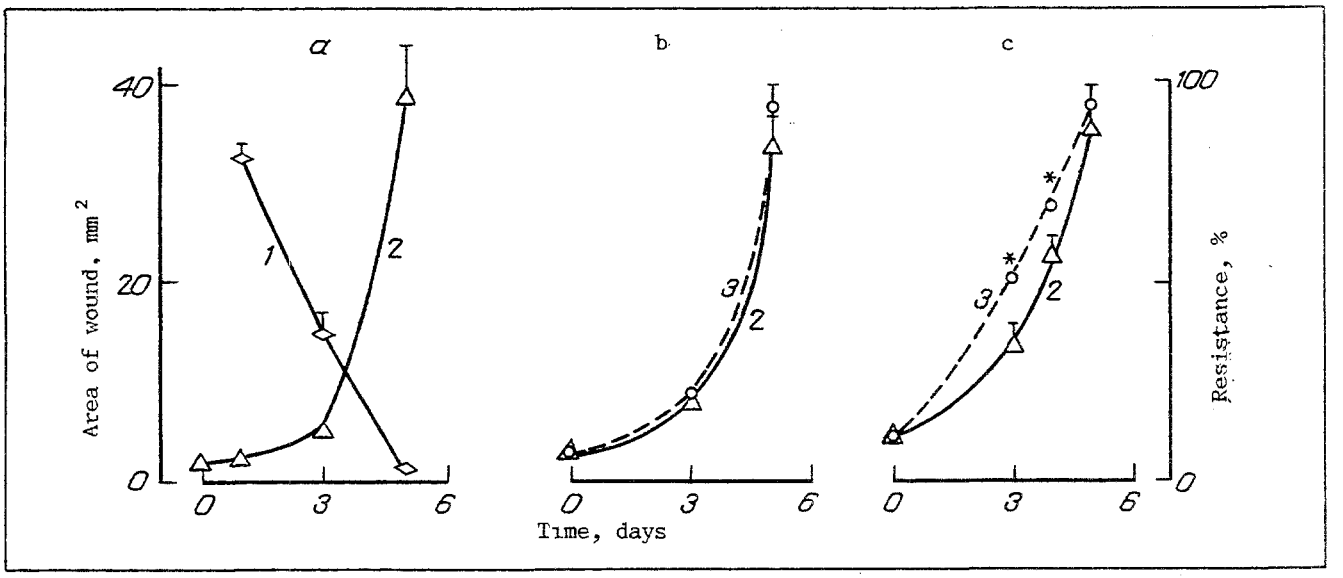

Fig. 1. Mean area of epithelial "window" (1) and its resistance (2 — experiment; 3 — control) as a function of time in the absence of exposure (a), under the influence of collagen solution (b), and of collagen-containing ointment (c); $p^* < 0.05$. Resistance of intact skin taken as 100%.

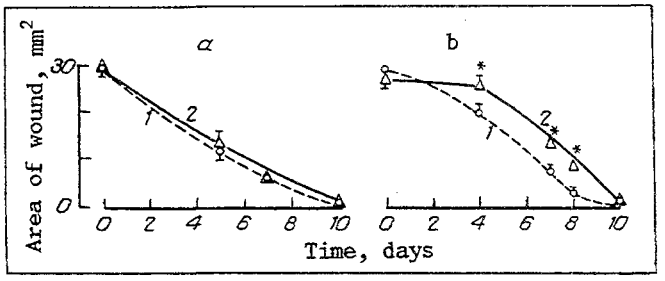

Fig. 2. Average area of full-thickness wound as a function of time under the influence of collagen solution (a) and of collagen-containing ointment (b) (1 — experiment; 2 — control).

dependence of the change in area of the "window," on average for all the animals used in the experiment, on time could be determined. The resistance of the region of the wound surface of the skin to an alternating current (1 kHz) was measured by means of Ag—AgCl electrodes of membrane type on an "Epilar" apparatus (Biotekh-Elektron, Moscow). The rate of contraction was estimated from the decrease in area of the circular wounds, measured by applying a graticule to them [3]. Restoration of the connective-tissue matrix was monitored by measuring the strength of the healing suture of the linear wounds of the rats by the method described in detail in [4]. For this purpose rectangular skin flaps measuring 10×40 mm, containing the suture, were ruptured by means of a "Dermatest" strain-gauge apparatus (Biotekh-Elektron, Moscow) at the rate of 20 mm/min. The results were subjected to statistical analysis by nonparametric tests and by Student's *t* test. Each experimental measurement was accompanied by a control. The number of animals in each group was not less than five.

RESULTS

It will be clear from data illustrating the trend of changes in the area and resistance of the epithelial "window" in the control (Fig. 1a) that these two parameters correlate closely with each other ($R = -2.1S + 63.9$, coefficient of correlation $r = -0.86$). These results also confirm the known conclusion that the epidermis makes the main contribution to skin resistance [6].

On application of collagen solution to the wound, no evidence of speeding up of epithelization was observed (Fig. 1b), whereas after application of the collagen-containing ointment, stimulation of epithelization was manifested perfectly clearly (Fig. 1c). A similar result also was observed during healing of full-thickness wounds: this process was stimulated only by the collagen-containing ointment and only from the 4th through the 9th days (Fig. 2a, b).

Measurements of the strength of the healing suture, made 35 days after wounding, showed that application of the collagen-containing ointment increased this parameter to $450 + 50$ g/mm^2, whereas application of the ointment base increased it to only $430 + 30$ g/mm^2 (five measurements, $p > 0.05$), i.e., no statistically significant differences could be found in this case.

We know that the resistance of the skin is determined mainly by the integrity of the epithelial cover, and that if this is removed the resistance falls sharply [6]. Our data show that the resistance of the skin is restored parallel with the course of reepithelization.

Reduction of the area of the epithelial "window" is the most obvious indicator of reepithelization. However, the use of the corresponding method rules out any possibility of repeated observations. Conversely, recording the resistance of the wound offers the possibility of repeated measurements on the same animal and continuous monitoring of the regeneration of the skin defect, as the results of our observations demonstrate.

Monitoring of reepithelization conducted by this approach showed that collagen in the ointment, but not in solution, stimulates this process. Since the ointment base was found not to have stimulating activity, it remains to suggest that manifestation of this effect requires prolonged entrance of collagen into the wound, as provided by the ointment base.

A similar result was obtained during observation on healing of full-thickness circular wounds: collagen in ointment form but not in solution accelerates the reduction of area of a skin defect.

The most informative parameter of recovery of the connective-tissue matrix is the strength of a healing linear suture [4, 10]. We were unable to find any changes in this parameter compared with he control under the influence of the collagen-containing ointment.

Thus collagen can stimulate wound healing and the degree of its stimulating effect increases if it is used in the long-acting form; not all the stages of regeneration studied are stimulated, but only contracture and epithelization.

The results of the investigations show that the use of collagen-containing ointments evidently is most effective in erosive skin lesions, and in the initial period of healing of full-thickness wounds.

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