EFFECTIVENESS OF TREATMENT OF DEEP AND EXTENSIVE WOUNDS BY SKIN MICROAUTOGRAFTING

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Key Words: number of skin microautografts; new skin; neoepidermis; contraction of wound.

By the existing method of autodermoplasty it is possible to increase the area of a graft (by perforation) by 2-9 times, thus exceeding the area of the wound [3, 4]. In extensive burns an acute deficiency of donated skin resources arises, restricting the use of this method [2]. The method of skin microautografting (SMAG), which is more economical with its use of donated autologous skin, can be used as an adjunct to the basic method. However, the efficacy of this method of treatment of skin trauma has not been fully studied. The aim of this investigation was to study the ability of SMAG in varied concentration to form a neoepidermis.

EXPERIMENTAL METHOD

Male rats weighing 300-400 g were divided into four groups. Under hexobarbital anesthesia, the hair was shaved in the dorsal region of all the rats on the future operation field, after which the skin was treated with 70° alcohol and a full-thickness skin flap measuring 5.4 cm^2 was excised. To prevent the wound from contracting, a polyethylene ring equal in area to the excised skin was used. The ring was fixed to the wound edges with silk thread. A strip whose area relative to that of the wound was 1:10 (group 1), 1:20 (group 2), and 1:40 (group 3) was cut off the skin flap by means of a microtome blade and 108, 54, and 27 pieces of skin measuring 0.5 × 1 mm were prepared from it. After exposure of these pieces for 5-10 min in physiological saline containing 500 U each of penicillin and streptomycin, they were applied randomly and evenly to the surface of the recently excised wound. As a temporary biological covering of the SMAG, a full-thickness fresh perforated skin allograft, treated in the same way as the SMAG, was used. About 10-15 days after transplantation the allograft was rejected, and a neoepidermis covering the whole wound surface was observed beneath it. The ring was then removed and after 2-4 days the area of the neoepidermis was measured every week by applying a piece of cellophane film. The outlines of the new skin were then transferred to squared paper and its area calculated. The area of the open wound was similarly measured in the rats of group 4 (8 animals), which served as the control of the rate of natural healing. The rate of contraction of the wound was calculated from these data by the equation [3]:

$$\Delta S = -[(S - Sn)/-ST] \times 100\%,$$

where S is the area of the recently excised wound, Sn the area of the wound at the time of measurement, and T the number of days between the first and next measurement. Rats of groups 1-3 were killed with an overdose of hexobarbital 6 weeks, and rats of group 4, 4 weeks after the operation. The new skin and skin of intact rats for comparison were fixed in 10% formalin solution or Carnoy's fluid and embedded in paraffin wax. Histological sections 4-5 μm thick, cut perpendicularly to the skin surface in different parts of the wound were stained with hematoxylin and eosin and for glycogen, and examined under the microscope. To estimate the state of the skin quantitatively, an ocular grid with 289 crossing points, with a step of 0.5 mm between them, was used. By the same method, the bulk density (V) of the intercellular substance and vessels in the dermis was measured under a magnification of 280× (with the grid placed immediately beneath the epidermis), by the equation:

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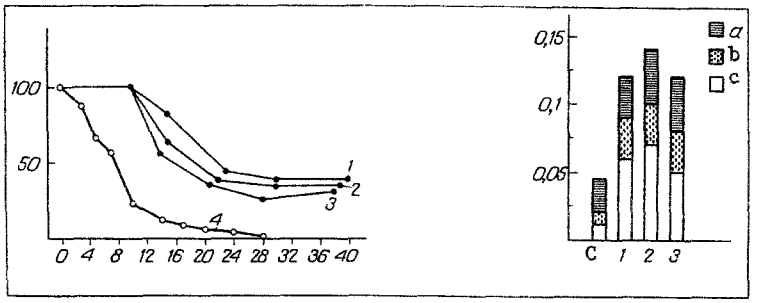

Fig. 1 Fig. 2

Fig. 1. Changes in area of new skin formed after transplantation of MAG to wound in ratio of 1:10 (1), 1:20 (2), and 1:40 (3). Abscissa, days of measuring area of wound: ordinate, area of wound (in per cent). Area of original wound, namely 5.4 cm^2, taken as 100%. 4) Area of open wound during healing.

Fig. 2. Histogram of change in thickness of neoepidermis. Abscissa, no. of group (C — control skin); ordinate, thickness of epidermis (in mm); a) stratum corneum, b) stratum granulare, c) stratum spinosum + s. basale.

$$V = (N/289) \times 100\%,$$

where N is the number of points corresponding to the given structures. The thickness of the epidermis (without the stratum corneum) and of the stratum granulare and stratum corneum was measured with an ocular micrometer. The number of dividing cells among 10^4 keratinocytes was counted in the stratum basale. The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

It was assumed that the larger the number of skin microautografts (MAG) transplanted to the wound, the faster epithelization would take place. It was found, however, that irrespective of the number of MAG transplanted, epithelization of the wound took place at the same rate, and a neoepidermis developed in all variants of the experiment by the 10th-15th days. This phenomenon is evidently attributable to the ability of the MAG to form marginal epithelization from cells migrating from the explant, by analogy with what happened in vitro. These zones of growth merge to form an epithelial sheet, in which morphogenetic processes subsequently lead to the production of new skin tissue. A quantitative and qualitative analysis of this new skin is presented below.

It will be clear from Fig. 1 that during 1-2 weeks after removal of the ring from the rats of groups 1-3 there was a linear increase in area of the new skin, which then ceased. This course of the change in area of the new skin corresponded to contraction of the open wound (Fig. 1), evidence of primary contraction of the neodermis and secondary contraction of neoepidermis. The rate of contraction of the new skin (from the linear region of the curve) was 4.3, 5.2, and 5.8 mm^2/day in groups 1, 2, and 3, respectively, i.e., the lower the concentration of MAG the faster the contraction of the new skin which theoretically (with a decrease in the MAG concentration) ought to approximate to the rate of contraction of the open wound, which was 8 mm^2/day. Thus the reduction in area of the new skin went through two phases: rapid linear contraction and stabilization. Contraction of the open wound also went through two phases: the first was similar to the phase of contraction of the new skin, whereas the second was characterized by slow contraction, ending in complete cicatrization of the wound. A similar reaction was observed previously by other workers studying the course of wound healing [8, 9]. It can be tentatively suggested that the phenomenon of wound contraction reflects a general biological rule, for it also took place in model experiments in vitro, which showed that the degree of contraction of the "dermal equivalent" is proportional to the number of fibroblasts and inversely proportional to the collagen concentration [5, 6]. By analogy with these data it can be postulated that the degree of contraction of the wound also depends on the number of fibroblasts and the collagen concentration in the granulation tissue. In the present study a quantitative analysis of the intercellular substance was carried out, but only after six weeks, before contraction of the new skin had

TABLE 1. Quantitative Parameters of Skin ($M \pm m$)

Ratio of area of MAG to area of wound	Number of rats	Bulk density, per cent		Mitotic activity of cells of stratum basale, promille
		of intercellular substance	of vessels	
1:10	10	57,5±16,0	4,8±1,5	2,8±0,2
1:20	9	54,1±16,3	6,3±2,6	2,7±0,3
1:40	10	61,0±11,7	5,5±1,4	3,2±0,2
Normal skin	6	79,0±1,0	2,5±0,3	1,2±0,1

occurred. Table 1 showed that irrespective of the number of MAG transplanted the bulk density (mass) of the intercellular substance and vessels in the neodermis was equal. The greater deviations from the arithmetic mean than in the control will be noted, an indication of individual differences in the state of the intercellular substance in the neodermis, which may be due to many different factors. Conversely, the parameters of the neoepidermis (Fig. 2) were statistically significantly greater than those of the epidermis of normal skin, evidence of hyperplasia, independent of the MAG concentration. Moreover, hyperplasia developed mainly, not on account of an increase in thickness of the stratum corneum, which was close to normal, but of the other layers of the neoepidermis. For instance, whereas under normal conditions the ratio of the stratum corneum to the stratum granulare was 1:0.36, in the experimental group it was 1:1, i.e., the relative size of the stratum corneum was less than in the control. This fact can be explained by reduction of the proliferated activity of cells of the stratum basale or, which is more probable, increased desquamation of horny scales from the surface of the neoepidermis, due to direct contact of the neoepidermis, deprived of its hair cover, with the surrounding medium. In turn, desquamation of the horny substance ought to lead, by a feedback mechanism, to stimulation of proliferation and shortening of the cycle of terminal differentiation of the keratinocytes, i.e., to an increase in the rate of self-renewal of epidermal cells. Hyperplasia of the neoepidermis, at least until the 6th week of its development, is evidently due to this same mechanism. This is confirmed by the process of desquamation of scales from the surface of the neoepidermis and the increased mitotic activity of cells of the stratum basale (Table 1).

The histological structure of the neoepidermis was indistinguishable from normal. No atypical ingrowth of epithelium into the underlying connective tissue was observed, indicating a sufficiently high level of differentiation of the granulation tissue cells. The dermal component consisted of areas of mature collagen bundles, evidently constituting the dermis of MAG. Leukocytic infiltration persisted in some places in the neodermis. Throughout the thickness of the neodermis epithelioid cysts measuring from 0.2 to 80 mm^2 were frequently found, and were filled mainly with horny material, and occasionally with remnants of hairs. These formations were microorgans, for the cyst walls consisted of stratified keratinizing epithelium, similar to epidermis in situ. The presence of horny material in the cysts indicates the functional activity of these microorgans. Such cysts can migrate beneath the neoepidermis and, as a result of pressure exerted from within on the basement membrane, they frequently formed "pressure sores," accompanied by rupture of the epithelium and escape of the contents of the cysts. Another microorgan is the sebaceous gland, which also has cavities and forms fistulous tracks through the neoepidermis.

The formation of these microorgans evidently reflects a general property of epithelial tissue, described as long ago as in 1940 [1].

The investigation thus showed that the area of covering of skin defects in rats can be increased by 40 times by the SMAG method. An increase of the same sort of order in the efficacy of wound covering, namely by 15 times in rabbits [10] and by 30 times in pigs [7], has been demonstrated by the use of this method. It must be emphasized that all parameters of the new skin were independent of the number of MAG.

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MECHANISMS OF THE THYMOLYTIC EFFECT OF METHANDROSTENOLONE

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Anabolic steroids, testosterone derivatives widely used in clinical practice, give rise to a number of side effects, including depression of activity of the T system of immunity. Some authors [1, 6, 7] have observed that anabolic steroids cause a decrease in weight of the thymus. However, the mechanism of the thymolytic action of the androgenic-anabolic steroids, by contrast with that of the glucocorticoids [3, 4], remains virtually unstudied.

The aim of this investigation was to study the effect of administration of methanodrostenolone for 7 days on protein biosynthesis in the rat thymus and the character of its interaction with the thymus cytosol and thymocytes.

EXPERIMENTAL METHOD

Experiments were carried out on 80 noninbred male rats weighing 160-200 g. Bilateral orchidectomy was performed 5 days before the experiment began. Methandrostenolone (MA, USSR product; equivalent Metandienone) was injected intraperitoneally in a dose of 10 mg/kg (the dose exhibiting maximal anabolic activity of MA in animals [2]) in the form of a solution in aqueous alcohol, in a volume of 0.2 ml. The intensity of protein biosynthesis was investigated by the use of a radioactive indicator [2]. The character of binding of ^{3}H-MA with the soluble fraction of thymus homogenate was determined by the following method: thymus glands were homogenized in TDM buffer (10 mM Tris-HCl, 0.5 mM dithiothreitol, 10 mM sodium molybdate, pH 7.8) and the supernatant was obtained at 10,000*g*. To 0.2 ml of supernatant 20 μl of a solution of ^{3}H-MA was added to final concentrations of between 10^{-8} and $5 \cdot 10^{-7}$ M. Samples were incubated for 2 h on an ice bath, after which 1 ml of a suspension of dextran-coated carbon was added to each sample, which was then shaken and allowed to stand for 10 min on the ice bath. After subsequent centrifugation at 5000*g* for 10 min 0.2 ml of supernatant was taken for radiometry and the quantity of bound ^{3}H-MA was expressed in cpm/mg protein. To determine specific binding of MA, incubation was carried out in the presence of a 500-fold excess of unlabeled MA. The difference between binding of ^{3}H-MA in the absence and presence of an excess of unlabeled MA having been calculated, parameters of MA binding by the thymus cytosol were determined with the aid of standard mathematical software for the EMG 666/B computer (Hungary). Ligand specificity of MA receptors was studied by competitive analysis of binding of ^{3}H-MA and of other unlabeled steroids (testosterone, estradiol, cortisol) by the cytosol.

Chromatography of ^{3}H-MA-receptor complexes was carried out by the method in [8]. To measure binding of ^{3}H-MA by isolated thymocytes, 20 μl of ^{3}H-MA (final concentrations in the incubation medium 10^{-8}-$5 \cdot 10^{-7}$ M) was added to the suspen

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