EFFECT OF ALLO-OCIMENE ON THE HEALING OF EXPERIMENTAL SOFT TISSUE WOUNDS

T. V. Pravdich-Neminskaya
and A. P. Kachkov

UDC 616.74-001.4-085.272:
547.979.8-07:616-003.9-07

The effect of allo-ocimene, one of the carotenoid polyenes, which stimulate metabolism in vivo and are active agents promoting normalization of trophic disturbances, on the healing of experimental soft tissue wounds by second intention was studied. Allo-ocimene increased the intensity of healing processes in skin and skin-muscle wounds, in some cases shortened the time required for complete healing, had an antiinflammatory action on the wound and neighboring zones, abolished or reduced edema, hyperemia, laceration, and hemorrhage, and prevented the development of manifestations of acute inflammation. The methods of quantitative analysis of these effects of allo-ocimene used in these experiments provide a method of evaluating its beneficial action on tissue nutrition in the course of wound healing.

KEY WORDS: healing of wounds; carotenoids; trophic action of the nervous system; regeneration.

In connection with progress in the study of nonspecific stimulation as a therapeutic factor and the connected problems of neurotrophic influences in surgery [4-8, 14] the possibility of using substances of biogenic nature, namely carotenoid polyenes, which possess high biological activity and are free from toxic action, for therapeutic purposes is interesting [1, 3].

The carotenoid polyenes, one of which is allo-ocimene, have considerable activity in the presence of trophic disturbances [1, 3]. Allo-ocimene has an analgesic and antiinflammatory action [1, 9, 15], it abolishes the effects of histamine [2] and acetylcholine [3], it reversibly blocks the efferent and receptor function of the sympathetic ganglion [12] and the natural electrical activity of sensory and motor fibers [11, 13], and it can induce central reflex responses through its action on the receptors of the sympathetic ganglion. The compound is nontoxic and is effective in the relief of pain and the correction of trophic disturbances in eye diseases [1, 9, 15].

EXPERIMENTAL METHOD

In chronic experiments on 18 male rabbits weighing 2.5-2.7 kg 330 observations, lasting up to 21-72 days on different individuals, were made on the course of healing of skin and skin–muscle wounds under the influence of 0.025-0.05% solutions of allo-ocimene. Full-thickness pieces of skin measuring 1 × 2 cm^2 were removed for this purpose from symmetrical regions of the animal's back, and in some experiments linear incisions of equal length also were made in the fascia covering the muscle and the first layer of muscle. In some experiments the conditions of wound healing were deliberately aggravated by increasing the initial area of the wound and by making further linear incisions in the muscles compared with the control wound. After removal of these pieces of skin, 0.025-0.05% colloidal solutions of allo-ocimene in water and ethanol, in which the ethanol concentration was 2.5%, made up when required were applied to one of the wounds (experimental), whereas the solvent alone, namely a 2.5% aqueous solution of ethanol, was applied to the other (control) wound. The cotton swabs soaked with the appropriate solutions were changed at intervals of 15-30-45 min during the daily period of observation lasting 6-8-10 h. For quantitative assessment of changes in the state of the wounds in the course of healing, planimetry of the whole wound, of its open part, and of the zone of edema of the edges was carried out on the experimental and control wounds. In addition, the area of the healed part of the wound or the relative area of the healed part in per cent (I) and the decrease in area of the total and open parts of the wound, in

Department of Experimental and Clinical Physiology, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR M. I. Kuzin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 1, pp. 51-54, January, 1978. Original article submitted June 10, 1977.

0007-4888/78/8501-0057$07.50

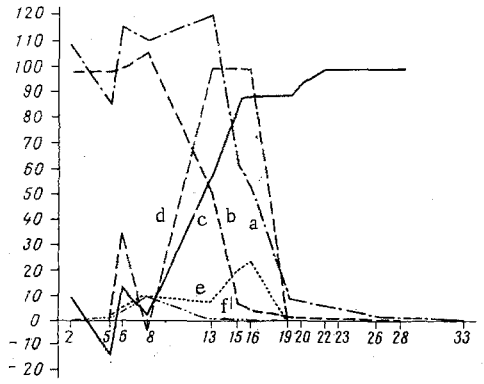

Fig. 1

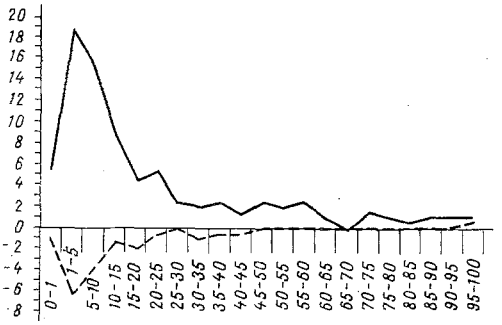

Fig. 2

Fig. 1. Changes in areas of experimental St_{ex} (b) and control St_c (a) wounds, their edematous zones SR_{ex} (f) and SR_c (e), and values of relative decrease in area of experimental wound N_{St} (c) and edematous zone N_R (d) respectively relative to control (in %) during course of wound healing. Experimental wound treated with allo-ocimene, control wound with its solvent (2.5% ethanol solution). Abscissa, time (in days); ordinate, areas of experimental and control wounds and of their edematous zones, and values of relative decrease in area of experimental wound and of edematous zone respectively relative to control (in %).

Fig. 2. Histogram of distribution of differences (Δ) in areas of control and experimental wounds (in %) by number of cases in all experiments. Abscissa, intervals of differences ($\Delta = S_c - S_{ex}$) in areas of control and experimental wounds (in %); ordinate: top – number of cases (frequency) of changes $+\Delta$, where $S_c > S_{ex}$ (in %); bottom – number of cases (frequency) of changes $-\Delta$, where $S_c < S_{ex}$ (in %).

per cent, relative to the corresponding areas of the control wound, i.e., their relative decrease in per cent on a given day of observation (N_{St}), were calculated. This last index was determined by the equation in [10, 16]:

$$N_{st} = \frac{St_c - St_{ex}}{St_c} \cdot 100.$$

This equation also was used when the areas of edema of the edges of the experimental and control wounds were measured at a particular moment. Quantitative analyses of the indices in this way enabled the effect of allo-ocimene on wound healing to be analyzed under dynamic conditions. No antibiotics or sulfonamides were used.

Certain clinical features of the state of the wounds and neighboring zones also were taken into account: hyperemia, maceration, hemorrhage, the presence of pus, necrotic tissue, granulation tissue, and areas of epithelization; the time of first appearance of epithelization of the wound and the corresponding time of the beginning of scar formation also were noted.

EXPERIMENTAL RESULTS

In 12 cases the experimental wounds healed 1-12 days before the control wounds (average 5 days), about 15% of the total time of healing of the control wound. Under the influence of allo-ocimene the healing process was intensified. This was reflected in a decrease in area both of the whole wound (S_t) and of its component parts: the area of the open part of the wound (S_z) and the area of edema of the wound edges (S_R), and also in an increase in the index (N_{St}) of relative decrease in area of the experimental wound, in per cent, compared with the control at the same moment in the overwhelming majority of cases (Fig. 1). Comparison of the positive and negative parts of the histogram (Fig. 2) shows that healing took place more effectively in the experimental than in the control wound. It is important to note that this property of allo-ocimene was still observed even when more severe conditions had previously been deliberately assigned for healing of the experimental wound than of the control, so that the inflammatory reaction of the experimental wound was more severe than in the control and was accompanied by considerable edema of the wound edges, hyperemia, and so on. These results could indicate that under the more severe conditions allo-ocimene was able to stimulate healing of the experimental wound more actively than when the conditions were the same as in the control.

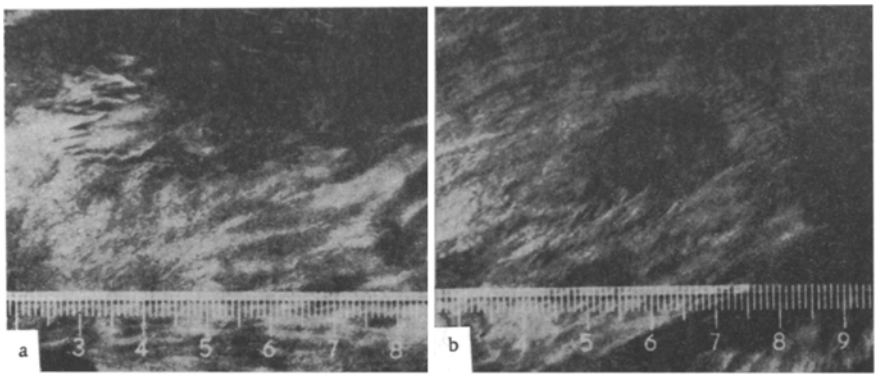

Fig. 3. Difference in intensity of healing by second intention of experimental (a) and control (b) skin–muscle wounds on rabbit's back (15th day after operation). a) Experimental wound: still unhealed area can be seen (in center), surrounded by wide border of epithelization, around which growth of new hairs can be seen; b) control wound, not yet healed, consisting entirely of a region of hyperemia and suppuration, much greater in area than the experimental wound, and with no zone of epithelization.

Observations of the clinical features of wound healing showed that allo-ocimene reduced or prevented the appearance and development of inflammatory changes in the wound and neighboring zones. For instance, whereas only weak signs of inflammation accompanied by slight vasodilatation of the microvessels in the region of the wound were observed on the 1st day in the experimental wound, the intensity of the microcirculatory disturbances and their duration were much greater in the control wound. A characteristic feature of the more marked inflammation in the control wound was the appearance of a focus of inflammation and its spread over a considerable area into zones bordering on the wound, and in some cases changing into a stage of acute suppurative inflammation. The normal regular pattern of reduction in the area of the epithelized control wound during the period of scar formation (asymmetrical shape) also was observed.

Abolition or weakening of edema of the wound edges by allo-ocimene also could be demonstrated by the same methods of comparative quantitative analysis. For instance, the parameters reflecting the decrease in area of edema of the edges of the experimental wound relative to the control were mainly positive and frequently high in value (Fig. 1).

Allo-ocimene thus increases the intensity of healing of skin and skin–muscle wounds, i.e., increases the intensity of regenerative processes in these wounds compared with the control (Fig. 3); in some cases it also shortens the time required for complete epithelization of wounds compared with the control in the same animal. The methods of quantitative analysis of these effects of allo-ocimene used in this investigation enable the beneficial effect of the compound on tissue nutrition in the course of wound healing to be estimated.

LITERATURE CITED

1. S. D. Balakhovskii et al., Dokl. Akad. Nauk SSSR, 92, 377 (1953).
2. S. D. Balakhovskii, D. E. Ryvkina, and N. N. Drozdova, Dokl. Akad. Nauk SSSR, 88, 527 (1953).
3. S. D. Balakhovskii, in: Vitamins [in Russian], Vol. 2, Kiev (1956), pp. 70-85.
4. D. F. Blagovidov and T. V. Pravdich-Neminskaya, in: Collection of Scientific Papers in Commemoration of the 70th Birthday of Academician of the Academy of Medical Sciences of the USSR A. A. Vishnevskii, edited by M. I. Kuzin [in Russian], Moscow (1976), pp. 81-96.
5. A. V. Vishnevskii and A. A. Vishnevskii, Procaine Block and Oil-Balsam Antiseptics as a Special Type of Pathogenetic Therapy [in Russian], Moscow (1952).
6. A. A. Vishnevskii, Selected Works on Surgery and Allied Topics [in Russian], Vol. 1, Moscow (1970).
7. A. A. Vishnevskii and T. V. Pravdich-Neminskaya, Vest. Akad. Med. Nauk SSSR, No. 8, 67 (1974).
8. M. I. Kuzin (Editor), The Prolonged Crush Syndrome [in Russian], Moscow (1970).
9. Therapeutic Substances. Brief Annotations [in Russian], Minsk (1961).
10. V. S. Peschanskii, A. B. Shneider, and V. D. Bondarenko, Proceedings of the 29th All-Union Congress of Surgeons [in Russian], Kiev (1975), pp. 105-107.
11. T. V. Pravdich-Neminskaya, Vest. Akad. Med. Nauk SSSR, No. 1, 87 (1962).
12. T. V. Pravdich-Neminskaya, Byull. Éksp. Biol. Med., No. 8, 6 (1967).

13. T. V. Pravdich-Neminskaya, Éksp. Khir., No. 6, 70 (1968).
14. S. P. Protopopov, in: Anesthesia in Surgery [in Russian], Moscow (1954), pp. 91-108.
15. A. V. Roslavtsev et al. (Editors), Handbook of Ophthalmology [in Russian], Moscow (1967).
16. A. M. Khil'kin, A. B. Shekhter, L. P. Istranov, et al., Collagen and Its Use in Medicine [in Russian], Moscow (1976).