HISTOMORPHOLOGICAL STUDY OF WOUND REGENERATION IN ANIMALS FOLLOWING LONG-TERM EXPOSURE TO LOW-INTENSITY MICROWAVES

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UDC 612.6.03.014.424.5

Linear skin wounds in guinea pigs exposed to low-intensity (5 mW/cm^2) microwaves healed by first intention faster than in unirradiated animals. The postoperative scar was stronger (more than 1.5 times). In the irradiated animals on the third day after the operation regeneration of the epithelium was stimulated and granulation tissue developed intensively in the lower parts. The rate of synthesis of proteins, including collagen, in the wound was observed later. On the 7th-9th day, young connective tissue formed in the place of the granulation tissue, and it was more mature than in the unirradiated animals.

KEY WORDS: *microwave field; healing of skin wounds; tensimetry.*

Conflicting information on wound healing in animals exposed to microwave radiation and also in persons in contact with a low-intensity microwave field is to be found in the literature. Some workers [2, 3] describe acceleration of wound healing in irradiated animals, but others [1] found that exposure to the microwave field delayed regeneration and proliferation.

Healing of skin wounds in animals following prolonged exposure to low-intensity (5 mW/cm^2) microwaves was studied.

EXPERIMENTAL METHOD

Eighty guinea pigs were used, 30 of them in the control group. The technique of irradiation of the animals was described earlier [3]. Linear incised wounds 5 cm long were inflicted down to the dermis under aseptic conditions in the dorsal region of all animals, both experimental (after the end of the course of irradiation) and control. The wounds were immediately closed by interrupted sutures to ensure good apposition of the wound edges. The animals were killed on the 3rd, 5th, 7th, 9th, and 11th days after the operation and a flap of skin containing the wound and postoperative scar was excised; the wound area was investigated histomorphologically. A tensimetric investigation of the scar was carried out simultaneously.

Pieces of tissue were fixed in 10% neutral formalin solution. Celloidin histotopographical sections were stained with hematoxylin–eosin and with picrofuchsin by Van Gieson's method and impregnated with silver by the methods of Gordon and Sweet. Acid mucopolysaccharides were stained with toluidine blue, glycoproteins and glycogen by the PAS reaction, RNA by Brachet's method, DNA by the Feulgen reaction, and elastic fibers with orcein.

Department of Surgery, Central Postgraduate Medical Institute, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 8, pp. 116-118, August, 1975. Original article submitted December 31, 1974.

TABLE 1. Tensimetric Investigation of Postoperative Scar after Wound Healing by First Intention in Unirradiated Animals and Animals Irradiated with Low-Intensity Microwaves (M ± m)

Series of experiments	Number of animals		Time of invest., days after irrad.	Tensimetric readings (in g)		P
	not irradiated	irradiated		not irradiated	irradiated	
I	6	10	3	220±2,3	340±2,7	<0,001
II	6	10	5	360±2,5	520±2,5	<0,001
III	6	10	7	460±2,1	790±2,3	<0,001
IV	6	10	9	680±2,4	1050±2,3	<0,001
V	6	10	11	1100±2,7	1420±2,6	<0,001

EXPERIMENTAL RESULTS

The wounds in both groups of animals healed by first intention. After the operation a moderate degree of traumatic inflammation developed in the region of the canal and floor of the wound (congestion, serofibrinous exudation, infiltration by leukocytes). By the third day after the operation the upper layers of the wound in both experimental and control animals were glued together by exudate containing some fibrin and erythrocytes. The regenerating epidermis in the region of the wound at this time was marked by thickening and acanthotic foci of proliferation. The number of mitoses was increased, RNA was abundant, and the glycogen content was high in the basal cells of the stratified squamous epithelium. These changes were more marked in the irradiated animals. In the lower layers of the dermis, in the muscular layer, and in the subcutaneous fatty areolar tissue, on the other hand, foci of necrosis were observed at the edges and in the floor of the wound, due primarily to disturbance of the circulation as a result of division of blood vessels by the incision and compression of the tissues by the sutures. From the third day, however, granulation tissue rich in thin-walled vessels began to develop in this region and proliferation of cells of the dermis and fatty areolar tissue, principally fibroblasts, increased in intensity. Despite the marked difference revealed by tensimetric investigation of the strength of wound healing (Table 1), no sharp morphological differences in wound healing could be observed in the two groups of animals on the third day, except for increased regeneration of the epithelium and also, evidently, better adhesion of the wound edges in the irradiated animals. On the fifth day the line of incision could not be identified in the upper part of the wound, and on impregnation with silver thin argyrophilic fibrils appeared in this region. Proliferating fibroblasts were rich in RNA and an increased content of acid mucopolysaccharides was found in the ground substance, indicating active synthesis of proteins, including collagen. In the lower parts of the wound progressive maturation of the granulation tissue and formation of ground substance continued, and proliferation of fibroblasts was observed, as was confirmed by appropriate histochemical staining methods.

Wound healing by first intention thus takes place differently at different depths. Whereas in the upper parts, through intensive regeneration of the epithelium, early adhesion of the wound edges, and the development of connective tissue following proliferation of fibroblasts, the process takes place more rapidly, in the lower parts of the wound in control animals slit-like spaces often were seen. Granulation tissue, subsequently (7-9th day) filling their lumen, developed in the walls of these spaces. When necrotic areas appeared in the muscular layer as a result of interference with the nutrient supply, the filling of these spaces was delayed until the 11th day. By the 7th day further maturation of the scar connective tissue in the upper parts was observed; the content of acid mucopolysaccharides and the RNA level in the fibroblasts were lowered, the fibroblasts differentiated into fibrocytes, and young fuchsinophilic collagen fibers were formed to replace the argyrophilic fibrils. In the lower parts maturation of the granulation tissue continued, the number of inflammatory cells decreased, fibroblasts proliferated, and macrophages appeared. The processes of maturation were slightly more intensive in the experimental animals. By the 9th day, in the region of the wound scar the content of acid mucopolysaccharides was greatly reduced, the number of cells in the scar tissue fell sharply, and the new collagen fibers were increasingly fuchsinophilic in their staining properties. In the epidermis covering the wound the difference between the young and old epithelium disappeared and mitoses were extremely rare. On the 11th day the wound edges were completely united throughout its depth in the animals of both groups. However, the scar showed some morphological differences, especially in its lower part. In the experimental animals the scar tissue stained intensively with fuchsin, cells were few, and the tissue differed only a little from the surrounding connective tissue, except that no elastic fibers could be detected in it. In the control animals the scar tissue in the upper parts of the wound was indistinguishable from that in the experimental animals. In the lower parts, however, the regenerating connective tissue was not yet quite mature: foci of infiltration with lymphocytes and histiocytes, accompanied by a few macrophages, were seen and young fibroblasts continued to be converted into fibrocytes. All these observations point to better healing of the wound in the experimental animals, and this was confirmed by the results of tensimetry.

LITERATURE CITED

1. V. I. Bachurin, "On the effect of electromagnetic waves of the shf range on healing of donated areas of skin," Klin. Khir., No. 5, 40 (1968).
2. D. N. Bidenko, "The use of pulsed shf energy for therapeutic purposes," Vrach. Delo, No. 2, 181 (1959).
3. Yu. G. Shaposhnikov and I. F. Yares'ko, "Healing of purulent wounds in animals following chronic exposure to a low-intensity shf field," Eksper. Khir., No. 1, 47 (1974).