Cytological and immunohistochemical studies of the effect of bioresonance therapy (BRT) on the healing of mature wounds rats

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Relevance

In connection with the development of science and technology, the introduction of new devices into medicine that use low-intensity radiation for therapeutic purposes, qualitatively new possibilities for the use of physical factors of low intensity in the treatment of various diseases are opening up. Experimental and clinical studies are being conducted to study ways to create optimal conditions for wound healing.

Thus, according to the literature [1–6], studies are carried out, and possible mechanisms of the effect of electromagnetic radiation of low-intensity waves on biological objects are discussed. The prerequisite for this study was literature data indicating the possibility of the effect of low-intensity electromagnetic radiation on

tissue regeneration processes.

Disclosure of the mechanisms of regulation of bioenergetic processes, occurring in the cellular structures of the body under influence electromagnetic radiation of low intensity will undoubtedly contribute to the development of clear indications for appropriate therapy for various pathological conditions.

In this work, we studied the effect of bioresonance therapy on wound healing processes, in particular on the process of tissue regeneration in postoperative wounds, which is of great importance for clinical practice. A study of the healing of skin wounds in laboratory animals (rats) exposed to bioresonance therapy was carried out.

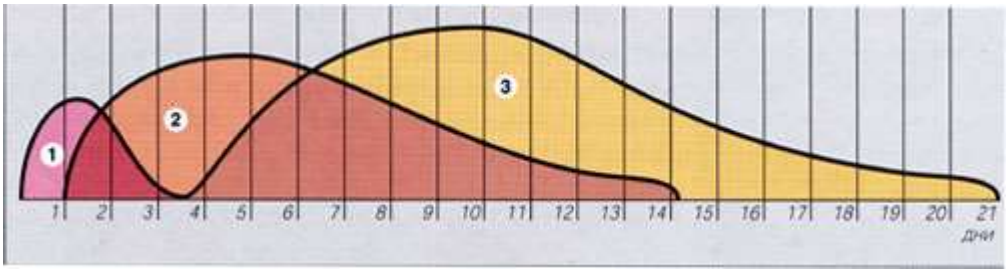
Bioresonance therapy, as a method of treatment, refers to physical factors of low intensity. And its effect can be both private and general (influence on the immune, nervous system, etc.) [7].

Our earlier experimental studies [8] studied the effect of BRT on the healing of wounds in experimental animals. Thus, in particular, it was shown that a one-hour exposure to BRT for 7 days on animals with an excised skin flap accelerates the processes of regenerative growth (in the specific case of the skin).

When evaluating histological preparations (the material was taken at an early stage of regenerative growth, 48–72 h), it was found that in the control group of animals, wound healing proceeds in accordance with the regularities characteristic of normal skin regeneration in case of deep damage. The recovery process in experimental rats also begins with epithelialization. However, to assess the proliferative activity of individual types of skin cells of animals in the control and experimental groups, it is advisable to conduct a study simultaneously by several methods.

Wound healing is a complex biological process that includes phases

inflammation, re-epithelialization, formation of granulation tissue and reorganization of the extracellular matrix. Normally, these processes proceed synchronously and lead to a fairly rapid wound healing. The allocation of individual phases and stages of the inflammatory process is conditional, since it is impossible to draw a strict line between the end of one and the beginning of the other (IV Davydovsky, 1969) (see Fig. 1)



Rice. one.Schematic representation of the temporal relationship of the wound healing phases:

1. Phase of inflammation;
2. The phase of regeneration and proliferation;
3. The phase of scar reorganization and epithelialization.

The aim of our study was to conduct cytological and immunohistochemical studies of the effect of bioresonance therapy (BRT) on the healing of wounds in white rats. For this purpose, an assessment of mitotic activity (colchicine mitotic index-MI), proliferative activity was carried out by determiningRabbit monoclonal [SP6] to Ki67, as well as morphological assessment of the activity of a specific marker protein of keratinocytes (pan Cytokeratin antibody, PCK-26, ab6401) skin cells in the area of the wound of white sexually mature rats.

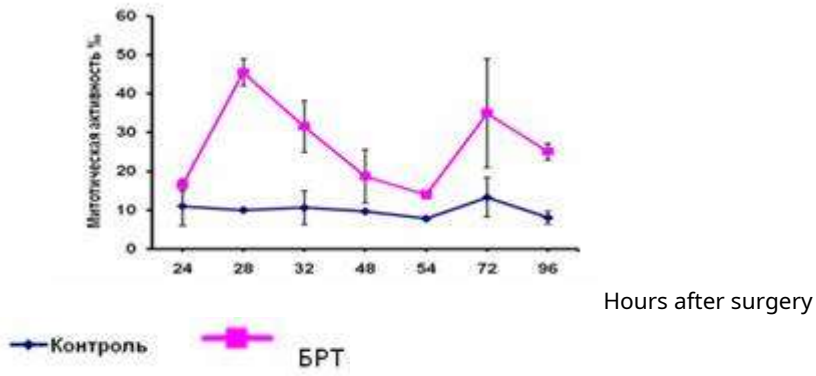
Objects and research methods

In the experiments, we used adult white rats weighing 130–140 g (n = 200). The animals were divided into two groups: Group I - animals with an excised skin flap (2 x 2 cm) (control group); Group II - animals with an excised skin flap (2 x 2 cm), which with the help of the IMEDISFALL APC had BRT sessions (20 min.) According to the 4th strategy every other day for two weeks - the experimental group). Animals of both the first and second groups were subdivided into two subgroups: Ia, Ib, IIa, IIb. To assess the mitotic (MI) index of skin cells, animals of subgroups Ib and IIb were injected intraperitoneally with a solution of colchicine (1 μg / g weight) three hours before sacrifice. The material (pieces of skin tissue from the wound area) was fixed in a 4% formalin solution prepared in Na, K-phosphate buffer (pH 7.2–7.4). Fixed pieces of fabric were embedded in paraffin, sections with a thickness of 5–7 μm were prepared, which were then stained with hematoxylin-eosin. The preparations were examined under a LOMO light microscope (magnification 90 × 10). Individual sections were processed by the immunohistochemical method using monoclonal antibodies to Ki67 proteins and cytokeratin. The killing of animals was carried out under ether anesthesia. The reliability of the data obtained was assessed by the Student's test.

results

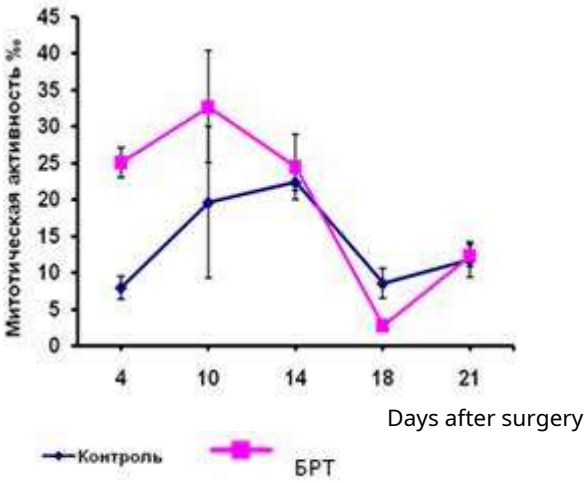
As a result of the studies, it was found that BRT sessions according to the 4th strategy, which were performed on animals with an excised skin flap, stimulate the mitotic activity of cells in the wound area. A burst of mitosis in animals of group IIb is observed already 24 hours after the operation (Fig. 2a). In addition, as can be seen from Fig. 2a, already by the 28th hour, mitotic activity reaches its peak (the first peak of mitotic activity) and is almost 45 ppm (Fig. 2a).

Fig. It also follows that after the first burst, as expected, a decrease in mitotic activity is observed. The second, however, a relatively small burst of mitosis is again detected three days after the operation (Fig. 2a). It should be noted that the mitotic activity of cells in the area of wounds of animals of group Ib does not change within 4 days and averages 10 ppm.



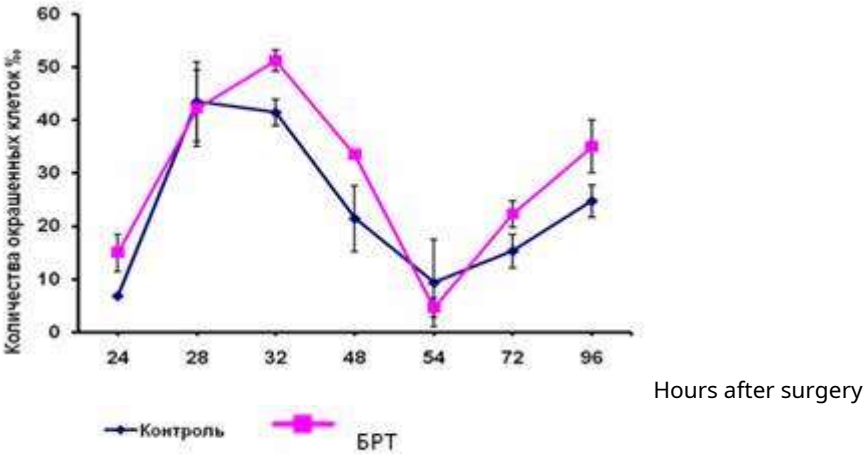
Rice. 2a.Influence of BRT on the change in MI (mitotic index) of skin cells of white sexually mature rats in dynamics (the first four days after surgery)

In fig. 2b, the results of MI changes from the fourth to the 21st day after surgery are presented. It follows from the figure that the difference between the MI indices of animals of groups Ib and IIb is not significant, from which it follows that ten days after the operation, the effect of BRT sessions on the proliferative activity of cells is not observed (Fig. 2b).



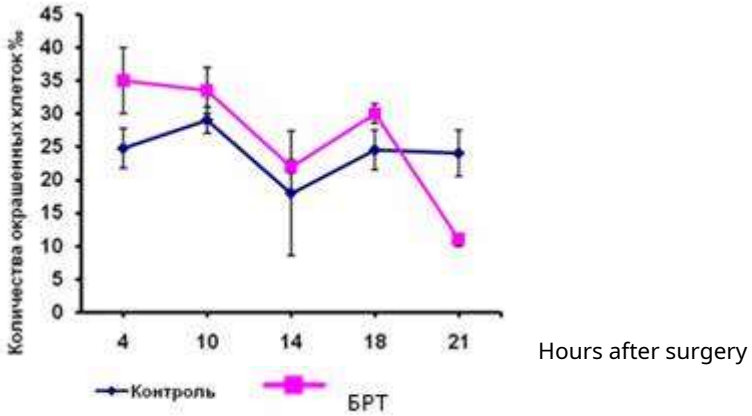
Rice. 2b.Influence of BRT on the change in MI (mitotic index) of skin cells of white sexually mature rats in dynamics (from the fourth to the 21st day after the operation)

In parallel with this, we evaluated the proliferative activity of skin cells by determining the activity of the Ki67 marker protein (Rabbit monoclonal [SP6] to Ki67) in the area of the wound of adult white rats The marker protein of proliferation Ki67 is manifested in the interphase cell only in the nucleus, while during mitosis it is found on the chromosomes. In fig. Figures 3a and 3b show curves reflecting the number of cells stained for the marker protein of proliferation in dynamics (Fig. 3a and 3b).



Rice. 3a.Influence of BRT on the change in the number of skin cells stained for the marker protein of proliferation (Ki67) from the wound area of adult white rats in dynamics (from the fourth to the fourth day after surgery)

Fig. it follows that the number of skin cells stained for the marker protein of proliferation Ki67 from the area of wounds of white mature rats of the II (experimental) group during the first day significantly exceeds the corresponding indicator of the animals of the I (control) group. When comparing the curves shown in Figures 2a, 2b, 3a and 3b, it can be seen that in both cases two peaks appear - the corresponding numbers of stained cells (Fig. 3a and b) and mitotic cells (Fig. 2a and b). These curves, as noted above, reflect proliferative activity cells in the wound area.



Rice. 3b.The effect of BRT on the change in the amount of stained on the marker

protein of proliferation (Ki67) of skin cells of white sexually mature rats in dynamics (from the fourth to the 21st day after the operation)

To assess the epithelialization process, we used antibodies to a specific marker protein of keratinocytes (pan Cytokeratin antibody, PCK-26, ab6401). Studies have shown that cells stratified squamous epithelium move from the edges of the wound, i.e. there is a normal epithelialization process. By the 48th hour, epithelialization in the area of the wound has not yet been observed in any group of animals.

Based on the experimental studies carried out on adult white rats to study the effect of BRT sessions according to the 4th strategy, by cytological and immunohistochemical methods, it can be concluded:

1. BRT according to the 4th strategy stimulates the process of wound healing in whites sexually mature rats, with a cut out skin flap (2 x 2 cm).
2. BRT sessions on the 4th strategy stimulate both the epithelialization process and and cell proliferation in the area of the wound.
3. A burst of mitosis in animals of the experimental group is observed already after 24 hours after surgery, reaching its peak by 28 hours, and is almost 45 ppm.

The results of the studies carried out indicate that bioresonance therapy accelerates the rehabilitation processes in the body.

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