doi: 10.56761/EFRE2022.S4-P-012701

Comparison of the effects of exposure to nanosecond pulsed microwaves on a burn injury depending on the pulse repetition frequency

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Abstract. The effect of nanosecond repetitive pulsed microwaves (RPMs 10 GHz, 100 ns pulse duration, 8 and 13 Hz pulse repetition rate, 140 W/cm^2 peak power flux density (pPFD)) on the regeneration of burn wounds in rats was studied. It is established that after 4-fold local irradiation of wounds with intensity of 140 W/cm^2 at pulse repetition rates of 8 and 13 Hz stimulates the healing of burn wounds. A comparison of the obtained results allows us to state that nanosecond low-intensity RPMs of 140 W/cm^2 accelerate wound healing processes. At the same time, the impact with a pulse repetition rate of 8 Hz turned out to be more effective in comparison with a pulse repetition frequency of 13 Hz. Histological analysis of rat skin showed an increase in the rate of wound healing due to the accelerated formation of granulation tissue, a decrease in the thickness of eschar and scarless healing.
Keywords: nanosecond pulsed microwaves, burn wound, rats.

1. Introduction

Effective restoration of human skin covers damaged as result of various thermal injuries is urgent medical and biological problem [1]. One of the promising and original methods of recovery of burn injuries can be the use of low-intensity electromagnetic factors. In this connection the data on the wound-healing effect of pulsed radiofrequency radiation [2, 3], in particular, nanosecond repetitive pulsed microwave radiation (RPMs) which under certain exposure parameters can stimulate reparative regeneration of full-layer skin wound in laboratory mice are of some interest [4].

The aim of the present work is to study the possibilities of stimulation of regeneration of skin burn wounds in laboratory rats using nanosecond repetitive pulsed microwave radiation with different parameters.

2. Materials and methods

1.1. Animals

The experimental study has been performed on 30 mature female Wistar rats with mass of 230–250 g. The animals were kept in standard vivarium conditions under natural light regime and on a standard diet with free access to water and food. All procedures with animals were performed at the same time (from 9:00 to 11:00). The study was performed in accordance with the ethical standards of work with laboratory animals (ETS No.123, 2007) and the sanitary rules for the design, equipment and maintenance of experimental biological clinics (Rules of Laboratory Practice in the Russian Federation).

Experimental animals have been randomly divided into three groups of 10 individuals:

1. The control group ($n = 10$) – the rats which after modeling the thermal burn were kept in standard vivarium conditions and not exposed to RPMR;

2. The experimental group No.1 ($n = 10$) – the rats which after modeling the thermal burn were subjected once in 4 days to local exposure of nanosecond RPMR with pPFD of 140 W/cm^2 and pulse repetition rate of 8 Hz.

3. The experimental group No.2 (n = 10) – the rats which after modeling the thermal burn were subjected once in 4 days to local exposure of nanosecond RPMR with pPFD of 140 W/cm^2 and pulse repetition rate of 13 Hz.

The choice of exposure modes (duration, pPFD, repetition frequency) is based on the results of the previous study as most effectively affecting the rate of reparative regeneration of full-layer skin wounds [4].

1.2. Modeling thermal burns

One day before modeling burn on the dorsal surface of rat's body the skin area was depilated by shaving with an electric veterinary machine. Shaving and burn modeling were performed under CO_2 anesthesia. The thermal wound was created by applying a metal rod with diameter of 2 cm heated up to 100 °C to the skin surface in the interscapular region without effort for 30 seconds. The damage area in the control and experimental groups was in average 340±18 mm^2 which corresponded to 8–9% of body surface area.

In the time of the whole experiment the rats were kept in pairs in the special cages separated by a transparent partition to exclude contact of animals with each other and mutual influence on the process of healing burn wound. Observation of the process of healing wound after 4 fold irradiation was performed in dynamics for the control and experimental rats up to the formation of a keloid scar. Dynamics of healing thermal burns was evaluated according to reduction of the damaged skin area using an electronic caliper [4], that was registered with the Sony-DSC-F717 camera (Japan) with subsequent analysis of photos (Imageanalyzer software). The obtained results of measuring the surface area of burn wound of the irradiated animals were compared with similar indicators in the control group of rats.

1.3. Irradiation of thermal burns

In 5 hours after formation of burn the laboratory rats were exposed daily for 4 subsequent days to single exposure of nanosecond RPM (4000 pulses per session/day) with intensity of 140 W/cm^2 at pulse repetition rates of 8 and 13 Hz. For local exposure to thermal wound and elimination of the possibility of irradiation of the entire organism the rest part of the animal's body was covered with a radio-absorbing material. The duration of single irradiation with the specified exposure parameters was 8 minutes. The pulsed laboratory generator based on the MI-505 magnetron (an item of serial production of JSC "Tantalus", Russia, carrier frequency of 10 GHz, output peak power of 180 kW, pulse duration at half power level of 100 ns) was used as the source of nanosecond RPM. The used peak intensity (140 W/cm^2) was recorded in accordance with the standard methodology based on antenna measurements and calorimetric calibrations. During the exposure, animals were placed in the special plastic containers with diameter of 10 cm and length of 30 cm at distance of 20 cm from the horn of the generator antenna, in the zone of the formed RPM wave. Radiofrequency electromagnetic radiation may cause warming of tissues and, accordingly, increase of the irradiated tissue temperature. Therefore, in the time of exposure temperature control was carried out using the fiber-optic thermometer MT-4MO-1 (Russia). At the used RPM intensity the temperature of tissue warming in the wound area did not exceed 0.03–0.05° at 140 W/cm^2.

1.4. Statistical analysis of data

The wound area was calculated by the method of point counting, by measuring the minimum and maximum diameter of the wound and finding their half-sum. Statistical processing of the obtained results was carried out using capabilities of the "Statistica 8.0 for Windows" program. The obtained results were presented as the arithmetic average value and the standard error of the arithmetic average (M±m) for all groups of the experimental animals in dynamics. The significance of differences in values between the control and irradiated indicators was determined using the nonparametric Mann-Whitney U–test. The critical level of difference significance p when testing statistical hypotheses was assumed equal 0.05.

3. Results

In the performed experiments, after thermal exposure in laboratory rats, a burn injury was formed, corresponding to a third-degree burn in humans. In the control group of animals not exposed to nanosecond RPM, a monotonous decrease in the area of burn wounds was recorded. The healing process proceeded gradually from days 1 to 32 of the study. It was accompanied by a long-term preservation of the scab, which completely fell off only on the 16th day of the experiment, and partial epithelialization was observed from the 28th day. In rats of experimental group No.1, subjected to 4-fold local irradiation with RPM with intensity of 140 W/cm^2 at pulse repetition rate of 8 Hz, beginning from the 19th day, a statistically significant decrease has been recorded in the area of the wound in comparison with the control group (Fig.1). At the same time, the discharge of the formed scab began on the 12th day of the experiment, and epithelialization occurred on the 24th day with complete healing of burns in all animals on the 28th day. In rats from experimental group No.2, irradiated with nanosecond RPMs with intensity of 140 W/cm^2 at pulse repetition rate of 13 Hz, beginning from the 5th day of the study, a statistically significant decrease has been observed in the area of the wound in relation to both the control group and experimental group No.1. However, a further decrease in the area of wounds in experimental group No.2 was monotonous and did not differ significantly from that in the control group. The scab was fully formed and began to recede only on the 14th day of the experiment, and on the 28th day of the study, partial epithelization of the wounds was observed.

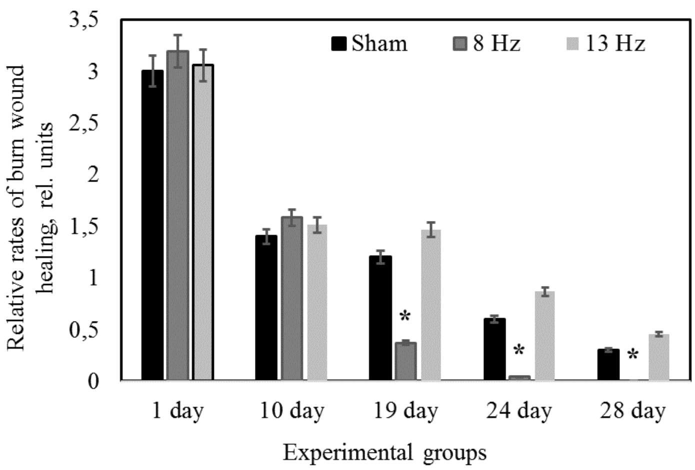

Fig.1. Dynamics of reduction in the surface of burn wounds in rats irradiated with nanosecond RPM with a pulse repetition rate of 8 and 13 Hz (pPFD 140 W/cm^2).

Histological analysis showed that on the 30th day of the experiment, burn wounds in all the studied groups of animals were in the regeneration phase (Fig.2). In the control group, complete epithelialization of the wound was observed in 33% of cases. At the same time, rats of this group with incomplete epithelization of the wound did not differ from those with complete epithelization either in the relative area of the granulation tissue or in the thickness of the newly formed epidermis.

In animals irradiated with a repetition frequency of 8 Hz, complete epithelialization of burn wounds was revealed in 67% of cases (Fig.2). In this group, rats with incomplete wound epithelialization were statistically different from those with completely epithelialized wounds in terms of the thickness of the newly formed epidermis. This parameter was 3.88 times less with incomplete epithelialization of the wound.

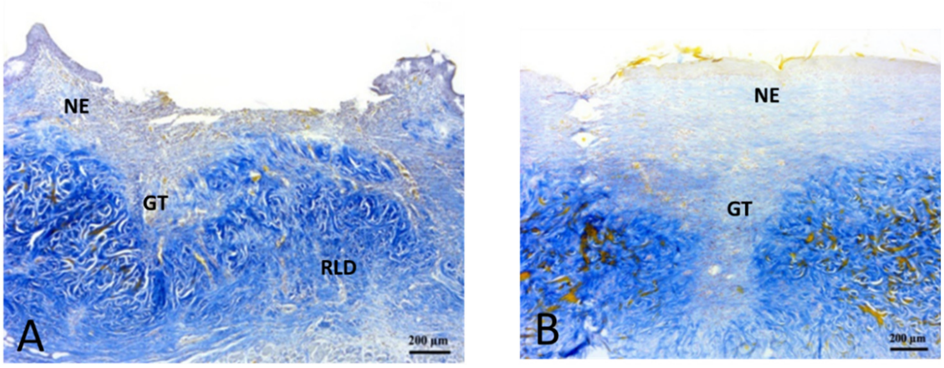

Fig.2. Cross sections of the central region of burn wounds of the skin of control (A) and irradiated rats (B, pulse repetition rate of 8 Hz, pPFD of 140 W/cm^2) on the 30th day of healing. Staining with the modified azan method. Note: NE – newly formed epidermis, GT – granulation tissue, RLD – reticular layer of the dermis.

4. Conclusion

Thus, a comparison of the obtained results allows us to state that nanosecond low-intensity RPMs of 140 W/cm^2 accelerate wound healing processes. At the same time, the impact with a pulse repetition rate of 8 Hz turned out to be more effective in comparison with a pulse repetition frequency of 13 Hz. The less intense action has the more pronounced wound healing effect. Earlier, Adey`s works [5] showed the existence of certain “frequency energy windows”. In accordance with this, radio frequency radiation with pulse repetition frequencies in the range of 6–16 Hz and the very specific optimal intensity has the greatest biological effect. At higher or lower intensities, the effect is significantly reduced or completely disappears. Apparently, pPFD of 140 W/cm^2, which was used in this work at pulse repetition rate of 8 Hz, could be closest to the optimal exposure mode, therefore the observed effect was more pronounced. According to the Adey model, processes mediated by Ca2+ ions are subject to the biological action of electromagnetic radiation.

Acceleration of healing of burn wounds by means of nanosecond microwave pulses, observed in the performed experimental work can become the basis of a new promising technology in cosmetic and therapeutic practice. To successfully use this approach special clinical studies are required to clarify the parameters of the influencing factor (pPFD, pulse repetition rate, number of pulses and repeated irradiation sessions), as well as elaboration of technical requirements necessary for the production of physiotherapy equipment.

5. References

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