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FAR-INFRARED RAYS' BENEFICIAL EFFECTS ON RATS' FULL-THICKNESS SKIN WOUND HEALING

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Abstract:

It is still unclear how far-infrared radiation (FIR) affects entire organisms biologically. Our study's objectives were to look at the biological impacts of FIR radiation on wound healing in addition to the hyperthermic effect of the radiation. Using a rat model, the rate of full-thickness skin wound healing was compared between groups with and without FIR in order to assess the impact of FIR on a skin wound site. Prior to and during FIR irradiation, we examined the skin's temperature, blood flow, and wound area. We also carried out a histological inspection. With FIR, wound healing occurred noticeably faster than it did without it. Neither before nor during FIR irradiation, there was a discernible change in skin temperature or blood flow. Histological results showed that in wounds from the FIR group, there was more collagen regeneration and infiltration of fibroblasts that expressed transforming growth factor-B1 (TGF-β1). Depending on skin temperature and blood flow, stimulation of TGF-B1 production or fibroblast activation may be one of the potential mechanisms for the beneficial effect of FIR on wound healing. Exp Biol Med 2000; 228:724–729

Key words: collagen, fibroblast, transforming growth factor-61, wound healing, and far-infrared radiation.

Introduction:

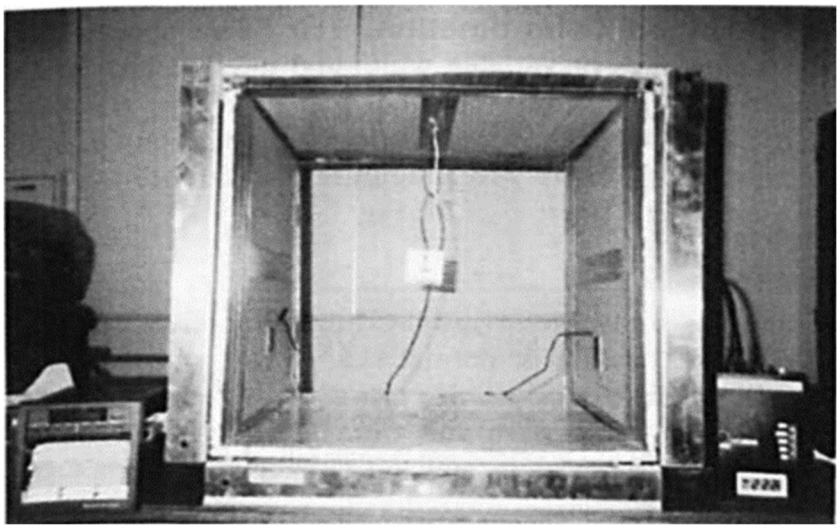
The term "mar-infrared ray" (FIR) refers to an electromagnetic wave that travels from H to 25.0 um. Three categories are used to arbitrarily separate infrared radiation: near-infrared (0.8-1.5 μm), middle-infrared (1.5-5.6 μm), and far-infrared (5.6-1000 μm). The invisible region of the electromag spectrum that lies next to the visible light range's long wavelengths, or red end, and reaches the microwave range is known as infrared radiation. On the other hand, certain nerve endings are able to sense them as heat. The skin's thermoreceptors (1, 2).

The primary protective barrier that the skin provides against the environment is its function.

Large areas of the skin lose its integrity as a result of disease, injury, or surgery, which significantly disrupts the skin's barrier function. Dehiscence of operating wounds and delayed wound healing are important. clinical issues, and wound care plays a crucial role in clinical settings. Several animal models have undergone wound treatment using low-energy lasers, including argon and helium-neon (3-6).
Lately, its health and food preservation initiatives have received a lot of attention. The body of research suggests that FIR has biological activity (7-9). There are a few reports of scientific analyses of the biological activities of FIR irradiation, and the majority of these are concerned with the hypothermic effect of FIR. FIR has been shown to inhibit tumor growth in mice and is used to treat bedsores in clinical situations (10, 11). It is yet unclear how FIR affects an organism's entire biology. Our goal in this study was to look into not only the FIR irradiation's hypothermic effect as well as its biological effects on the healing of full-thickness skin wounds in an animal model

Supplies and Procedures apparatus for FIR radiation. A rack was stolen.

structed with FIR sources on the top and two sides. The side sources were about 20 cm apart from the rats, while the top source was around 40 cm above them. An electric heater was used to heat the aluminum sheet that had been covered with ceramic to produce the FIR sources. The ceramic-coated sheet released FIR with a maximum intensity of 8 to 12 μm, ranging from 5.6 to 25 μm. For the control group, the rack temperature ranged from 24,0°C to 25.0°C, while the two experimental groups experienced temperatures between 26.5°C and 27.5°C. A sensor installed in the rack allowed for continual monitoring of these temperatures. The supplier of these items was Sagano Co. Ltd. (Fig. 1, Kobe, Japan).



Preparing a full-thickness skin wound and applying FIR radiation. For this experiment, 110 Sprague-Dawley rats, ages 6 to 7 weeks, were acquired from Charles River Japan (Yokohama, Japan). Their weight ranged from 170 to 190 g. Under diethylether anesthesia, a circular segment

of full-thickness skin (diameter approximately 15 mm) was excised using scissors from the clipped dorsal skin of each animal. There was no dressing on the wound (Fig. 2). The percentage of wound area compared to Day 0 (100%), which represents the curative impact, was used to express the effect. By multiplying the wound site's cross-diameters, we were able to determine the wound area. We compared the rate of wound healing in the following three groups to assess the impact of FIR irradiation: the Control group (n = 10), which was maintained in a normal rack without FIR at a temperature between 24.0°C and 25.0°C until the end of the experiment; Group A (n = 50), which was continuously exposed to FIR at a temperature between 26.5°C and 27.5°C; and Group B (n = 50), which was maintained in the rack without FIR at the same temperature as Group A (26.5°C to 27.5°C). every animal. were given unlimited access to food and water. To measure the area of the wound, ten ani- mals from each group were employed. On Days 0, 4, 7, 12, and 14, we measured the extent of the wound. The remaining animals were euthanized to allow for a histological examination. A Skin Blood Flow and Skin Measurement.

The degree of warmth. A compact laser flowmeter (ALF21N; Advance, Tokyo, Japan) was used to quantify the blood flow through the skin both before and after FIR irradiation, with the temperature in the rack being between 26.5°C and 27.5°C. The capillary blood perfusion parameters (blood flow, volume, and velocity) are measured instantaneously using this apparatus. Male adult Sprague-Dawley rats weighing between 170 and 190 g were closely shaved on the dorsum and given an intraperitoneal injection of 40 mg/kg pentobarbital to induce unconsciousness. The dorsal skin was covered by the probe. In order to measure skin temperature, a needle thermosensor was inserted into the dorsal skin at the same moment.

Histopathological Examination. Following the measurement of the wound area by 10 rats from each group, the remaining animals were euthanized to allow for histological examination. At 1, 3, 5, and 7 days after the ten rats in Groups A and B were slaughtered, histology samples were extracted from each wound. 10% formalin was used to fix the wound, which was then embedded in paraffin and sectioned together with the surrounding tissue. Hematoxylin-cosin (H&E) and toluidine blue were used to stain and score sections of the wound tissue for histopathological analysis.

The scoring system for histology. Each part of the wounds in Groups A and B had six randomly selected sample locations examined under light microscopy. A previously published approach was used to grade morphological findings, including those for epitheliazation, cellular content (fibroblasts, neutrophils, and macrophages), collagen regeneration, and vascularization (3, 4). These morphological observations received scores of 0.5 for few, moderate, many, (2), and significant (3). The scoring system was employed and the histological investigation was carried out by two separate pathologists. blindly. Staining for Transforming Growth Factor-B1 using immunohistochemistry. TGF-B1 anti-human The rabbit polyclonal antibody (Yanaihara Ins. Inc., Shizuoka, Japan) was used as the primary antibody and was diluted 1:500 in 1% bovine serum

albumin (BSA) in 0.05 M phosphate-buffered saline (PBS, pH 7.5). The secondary antibody used for TGF-B1 staining was biotinylated goat anti-rabbit IgG, which was diluted 1:1000 in PBS. For five minutes, dewaxed sections were blocked with regular rabbit serum. The parts underwent incubation. 1% BSA in PBS with anti-TGF-B1 antibody, and let it sit overnight at 4°C. The sections were first cleaned in PBS, then incubated for 10 minutes with goat anti-rabbit IgG that had beenbiotinylated. Next, they were again cleaned in PBS and incubated with avidin horseradish peroxidase complex at a dilution as directed by the manufacturer. 3-(2-dinirinobenzidine)-4HCL was used to create the chromogenic reaction. Browning served as a positive staining indicator.

An assessment of immune staining. Six randomly chosen samples from each TGF-B1-stained segment of the wounds were subjected to x400 magnification light microscopy examination. In each of the six fields, the number of migrating fibroblasts expressing TGF-B1 was counted. Collagen Identification. The collagen content. fibers in histological preparations of the skin wound on Day 7 stained with Azan-Mallory were identified by light microscopy. Mallory's collagen fiber staining was finished using the previously mentioned technique. Using an imaging system that included a computer, a microscope (VIDEOMICROMETER VM-30; Olympus, Tokyo, Japan), and statistical analyses, the area of collagen in each field in Groups A and B was assessed. The unpaired Student's test and two-way analysis of variance (ANOVA) were used to perform statistical analyses. A statistically significant P value was defined as one that was less than 0.05. The means SD are used to express data.

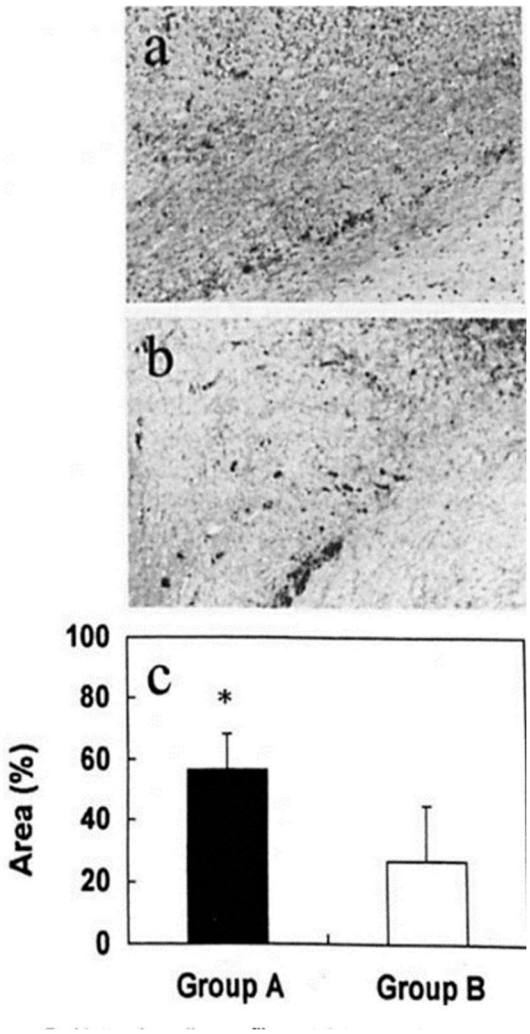
Outcomes

Large-scale observations. All rats had healed their wounds at the end of the 14-day observation period. Measurements were taken of the wounds on Days 0, 4, 7, 12, and 14. The relative wound area changes over time are depicted in Figure 3. Two-way ANOVA was used to compare the wound healing rates of the three groups. Statistically significant differences were found during the observation period between Group A and both the control group and Group B (P0.0172), but no significant difference was shown between the Control Group and Group B. By Day 7, Group A's wound had healed more quickly than those of the Control Group and Group B. We repeated the above-described process three times to ensure reproducibility, and the findings were statistically identical each time. Skin Temperature and Skin Blood Flow. There was no discernible change in skin blood flow prior to or during FIR irradiation. Rats' skin temperatures varied, but these variations were not observed before or during FIR radiation. Scores for Histology. The histology scores of Group A and Group B were compared because there was no difference in wound healing between the two groups.

On Days 1, 5, and 7, Group A had a significantly higher subcutaneous fibroblast infiltration than Group B. Using two-way ANOVA, there was a statistically significant difference (P = 0.0183) between the two groups. On Day 7, Group A showed signs of abundant collagen

regeneration, with a considerable increase. on Day 7, higher in Group A than in Group B ($P<0.001$). On Days 1, 3, and 5, there were no appreciable differences in epithelization, vascularization, or cellular content (neutrophils and macrophages) between Groups A and B. and 7.

An assessment of immune staining. To examine the release of TGF-B1, a cytokine that is widely recognized. The number of invading fibrocytes expressing TGF-B1 in each of the six fields was quantified in order to speed up wound healing (Fig. 4, a and b). On Days 3, 5, and 7, Group A had a considerably higher number of migrating fibroblasts pressing TGF-B1 than Group B ,A statistically significant difference ($P<0.001$) was found between the two groups using two-way ANOVA.



Collagen Identification. The filaments showed up as wavy formations (Fig. 5, a and b) with variable width and intermediate length. Group A had 56.8% ±11.3% of collagen fibers per field,

while Group B had 26.5% ± 18.5% (means SD). Between the two groups, there was a significant difference (P0.0124; Fig. 5c).Talk The present investigation examined the wound healing effects of fractional impulse radiation (FIR) on rats with normal skin. The results indicated notable variations in the rate of wound healing between the FIR-treated and non-treated groups. Nonetheless, the rapidity of wound healing was not significantly impacted by variations in the surrounding temperature. We got the same outcomes after carrying out the identical process three times. As a result, it was believed that the impact of FIR on wound healing was reproducible.

While reports of increased skin temperature and blood flow due to FIR irradiation exist, none of these increases were seen in the FIR irradiation circumstances that we employed (1, 12). It is believed that the beam directly influences the biological process because our study's results on wound healing did not show any influence from blood flow or skin temperature. of wound healing, such as cytokine production or cellular proliferation, that happen independently of skin temperature and blood flow.

The following can be used to categorize the wound healing process: inflammation, granulation tissue development, and tissue remodeling (13, 14). One important process that growth factors are thought to play during is the inflanunation response. To start the granulation of tissue, macrophages and platelets produce several factors, such as TGF-B1 and platelet-derived growth factor (15–17). One cytokine that is widely known for quickening wound healing is TGF-B1, which also stimulates cell infiltration during the inflammatory stage (17–19). TGF-B1 promotes the production of extracellular matrix proteins by fibroblasts, including as collagen and fibronectin, and aids in their deposition during the gratulation phase. With effective concentrations below those needed to activate gene transcription, TGF-B1 is a strong inducer of fibroblast migration (20, 21). Dermal fibroblasts are responsible for the synthesis of new extracellular matrix proteins, mainly Type 1 and III collagen, which form new normal or scar tissue after initially constituting granulated tissue (22–23). An important step in the wound-healing function of these cells is the migration of fibroblasts from surrounding unwounded skin toward the provisional matrix of the hemostatic plug.

On Days 1, 5, and 7, our histological examination revealed that the FIR imadiation group had a considerably higher infiltration of fibroblasts in the subcutis than did the group that did not receive FIR imadiation. Additionally, on Day 7, in sections stained with Mallory's stain, the FIR group showed much higher collagen regeneration than the group without FIR.

The FIR group had a statistically significant increase in the quantity of migrating fibroblasts expressing TGF-81. The possibility exists that platelets and macrophages secrete TGF-B1. was boosted by FIR irradiation, leading to fibroblast migration induced by TGF-B1. There is also a chance that FIR-radiation directly stimulates fibroblast activity. As a result, the FIR group experienced an increase in collagen production. the process of collagen fiber formation. because

FIR irradiation has been thought to activate fibroblasts, which may be a mechanism for the promotive FIR irradiation's impact on wound healing. Furthermore, histological evidence of remarkably marked inflammation or burning caused by FIR irradiation was absent.

The control group and the experimental groups did not significantly vary in any of the plasma component level parameters (data not shown). This implies that there are no dangerous thermal effects and that FIR irradiation at room temperature is safe. Numerous physical agents, including low-energy lasers, have been applied to wounds in a variety of animal models. Experiments with have confirmed efficiency. In vitro studies have shown that near-infrared radiation and lasers trigger a variety of wound-healing processes, including collagen synthesis (23), cell proliferation (24), and keratinocyte motility (25), using an animal paradigm that is comparable to ours (3-6). Unlike these lasers, our light source is different. In this work, we employed FIR at wavelengths between 9 and 12 μm, whereas lasers provide a particular coherent beam (argon at 488 nm and helium-neon at 632 am). The biostimulatory effects of FIR irradiation may be comparable to those of low-energy lasers or near-infrared radiation, notwithstanding the belief that more in vitro research on FIR is necessary.

Conclusion:

Ultimately, our findings imply that FIR may promote skin wound healing through its stimulating properties. of TGF-B1 secretion or by fibroblast activation that is unaffected by skin temperature or blood flow. For the treatment of wounds, FIR irradiation may therefore be clinically beneficial.

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