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The biological effects of far-infrared ray (FIR) on whole organisms remain poorly understood. The aim of our study was to investigate not only the hyperthermic effect of the FIR irradiation, but also the biological effects of FIR on wound healing. To evaluate the effect of FIR on a skin wound site, the speed of full-thickness skin wound healing was compared among groups with and without FIR using a rat model. We measured the skin wound area, skin blood flow, and skin temperature before and during FIR irradiation, and we performed histological inspection. Wound healing was significantly more rapid with than without FIR. Skin blood flow and skin temperature did not change significantly before or during FIR irradiation. Histological findings revealed greater collagen regeneration and infiltration of fibroblasts that expressed transforming growth factor-β1 (TGF-β1) in wounds in the FIR group than in the group without FIR. Stimulation of the secretion of TGF-β1 or the activation of fibroblasts may be considered as a possible mechanisms for the promotive effect of FIR on wound healing independent of skin blood flow and skin temperature. Exp Biol Med 228:724–729, 2003

Key words: wound healing; far-infrared ray; transforming growth factor-β1; fibroblast; collagen

Far-infrared ray (FIR) is an electromagnetic wave from the sun, with wavelengths ranging from 5.6 to 25.0 μm. Infrared radiation is arbitrarily subdivided into three categories: near-infrared (0.8–1.5 μm), middle-infrared (1.5–5.6 μm), and FIR radiation (5.6–1000 μm). Infrared radiation is that invisible portion of the electromagnetic spectrum adjacent to the long wavelengths, or red end, of the visible light range that extend up to the microwave range. However, they can be perceived as heat by specialized nerve endings known as thermoreceptors in the skin (1, 2).

The skin functions primarily as a protective barrier against the environment. Injury, illness, or surgery results in the loss of integrity of large portions of the skin and leads to major disturbances of this barrier function. Delayed wound healing and dehiscence of operative wounds are significant clinical problems, and wound care is an important factor in clinical situations. Low-energy lasers, such as helium-neon and argon laser, have been used to treat wounds in several animal models (3–6).

Recently, much attention has been paid to its activities with regard to health and food preservation. Accumulated evidence indicates that FIR is biologically active (7–9). FIR has been reported to inhibit tumor growth in mice and is used for treatment of bedsores in clinical situations (10, 11). However, there are few reports of the scientific analysis of the biological activities of FIR irradiation, with most of these being concerned with the hyperthermic effect of FIR. The biological effects of FIR on whole organisms remain poorly understood.

In this study, our aim was to investigate not only the hyperthermic effect of FIR irradiation, but also the biological effect of FIR on healing of full-thickness skin wounds using an animal model.

Materials and Methods

Equipment for FIR Irradiation. A rack was constructed with the top and two sides containing FIR sources. The top source was approximately 40 cm above the rats, and the side sources were approximately 20 cm from the rats. The FIR sources were constructed from a ceramic-coated sheet of aluminum, and the opposite side of the sheet was heated by an electric heater. FIR emitted from the ceramic-coated sheet ranged from 5.6 to 25 μm with a maximal intensity of 8 to 12 μm. The temperature in the rack was

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approximately 24.0°C to 25.0°C for the control group and 26.5°C to 27.5°C for the two experimental groups. These temperatures were continuously monitored with a sensor placed in the rack. These equipments were supplied by Sagano Co. Ltd. (Kobe, Japan; Fig. 1).

Full-Thickness Skin Wound Preparation and FIR Irradiation. A total of 110 Sprague-Dawley rats aged 6 to 7 weeks were purchased from Charles River Japan (Yokohama, Japan) to be used in this experiment. They weighed between 170 and 190 g. A round section of full-thickness skin (diameter around 15 mm) was resected with scissors on the clipped dorsal skin of all animals under diethylether anesthesia. The wound was not covered with dressing (Fig. 2). The curative effect was expressed as the percentile of wound area compared with that on Day 0 (100%). We calculated the wound area from the product of cross-diameters of the wound site. To evaluate the effect of irradiation of FIR, we compared wound healing among the following three groups: the Control group ($n = 10$), which was kept until the end of the experiment in a normal rack in the absence of FIR at a temperature of 24.0°C to 25.0°C, Group A ($n = 50$), which was constantly exposed to FIR at a temperature of 26.5°C to 27.5°C, and Group B ($n = 50$), which was kept in the rack in the absence of FIR at the same temperature as Group A (26.5°C to 27.5°C). All animals were provided with unrestricted food and water. Ten animals from each group were used to measure the wound area. We measured the wound area on Days 0, 4, 7, 12, and 14. The remaining animals were sacrificed to provide histological inspection.

A Measurement of Skin Blood Flow and Skin Temperature. We measured skin blood flow using a contact laser flowmeter (ALF21N; Advance, Tokyo, Japan) before and during FIR irradiation at a temperature of 26.5°C to 27.5°C in the rack. This device instantaneously measures capillary blood perfusion parameters (blood flow, volume, and velocity). Adult male Sprague-Dawley rats weighing 170 to 190 g were anesthetized with an intraperitoneal injection of 40 mg/kg pentobarbital and were shaved closely on the dorsum. The probe was placed over the dorsal skin. At the same time, a needle thermosensor was placed in the dorsal skin to measure skin temperature.

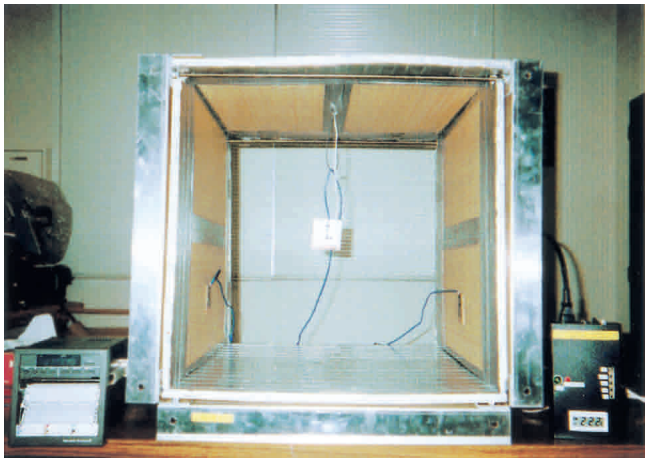

Figure 1. Photograph of the equipment of the FIR source. The FIR sources were constructed from a ceramic-coated sheet of aluminum, and the opposite side of the sheet was heated by an electric heater. FIR emitted from the ceramic-coated sheet ranged from 5.6 to 25 μm with a maximal intensity of 8 to 12 μm. The temperature in the rack was continuously monitored with a sensor placed in the rack.

Figure 2. An area of full-thickness skin of approximately 15 mm was resected with scissors from the dorsal area.

Histological Inspection. After the 10 rats from each group were used to measure the wound area, the remaining rats were sacrificed to provide histological inspection. Ten rats in Groups A and B were sacrificed at 1, 3, 5, and 7 days, and histological specimens were taken from each wound at each day. The wound with surrounding tissues was fixed with 10% formalin, embedded in paraffin, and sectioned. Sections of the wound tissue were stained with hematoxylin-eosin (H&E) and toluidine blue for histological inspection and scoring.

Histological Scoring. Six arbitrary sample areas per section of the wounds in Groups A and B were examined by light microscopy. Morphological findings, including those for epitheliazation, cellular content (neutrophils, macrophages, and fibroblasts), collagen regeneration, and vascularization were scored according to a previously published system (3, 4). These morphological findings were scored as none (0), few (0.5), moderate (1), many (2), and considerable (3). Two independent pathologists performed the histological examination and applied the scoring system in blinded fashion.

Immunohistochemical Staining for Transforming Growth Factor-β1 (TGF-β1). Anti-human TGF-β1 rabbit polyclonal antibody (Yanaihara Ins. Inc., Shizuoka, Japan) was diluted 1:500 in 1% bovine serum albumin (BSA) in 0.05 *M* phosphate-buffered saline (PBS, pH 7.5) as the primary antibody, and biotinylated goat anti-rabbit IgG was diluted 1:1000 in PBS for TGF-β1 staining as the secondary antibody. Dewaxed sections were blocked with normal rabbit serum for 5 min. The sections were incubated with anti-TGF-β1 antibody in 1% BSA in PBS at 4°C overnight. After washing in PBS, the sections were then incubated with biotinylated goat anti-rabbit IgG for 10 min,

washed in PBS, and incubated with avidin horseradish peroxidase complex at a dilution in accordance with the manufacturer's instructions. The chromogenic reaction was performed with 3-3′-diaminobenzidine-4HCL. Positive staining was indicated by a brown color.

Evaluation of Immunostaining. Six samples arbitrarily selected per TGF-β1-stained section of the wounds were examined by light microscopy at ×400 magnification. The number of migrated fibroblasts expressing TGF-β1 in the six fields was counted.

Collagen Determination. The presence of collagen fibers was determined by light microscopy in histological preparations of the skin wound on Day 7 stained with Azan-Mallory. Mallory's collagen fiber staining was completed according to a method described previously. The area of collagen per field in Groups A and B was examined by an imaging system consisting of a computer and microscope (VIDEOMICROMETER VM-30; Olympus, Tokyo, Japan).

Statistical Analyses. Statistical analyses were performed using two-way analysis of variance (ANOVA) and the unpaired Student's *t* test. A *P* value less than 0.05 was considered statistically significant. Data are expressed as means ± SD.

Results

Macroscopic Observations. Wound healing was completed in all rats within the 14-day observation period. The wounds were measured on Days 0, 4, 7, 12, and 14. Figure 3 shows the time course of changes in the relative wound area. Wound healing between the three groups was compared using two-way ANOVA. The wound healed more rapidly by Day 7 in Group A than in the Control Group and Group B. Statistically significant differences were found during the observation period between Group A and both the control group and Group B ($P = 0.0172$), but no significant difference between the Control Group and Group B was shown. To confirm the reproducibility, we repeated the same procedure as described above three times, and we obtained the same results statistically.

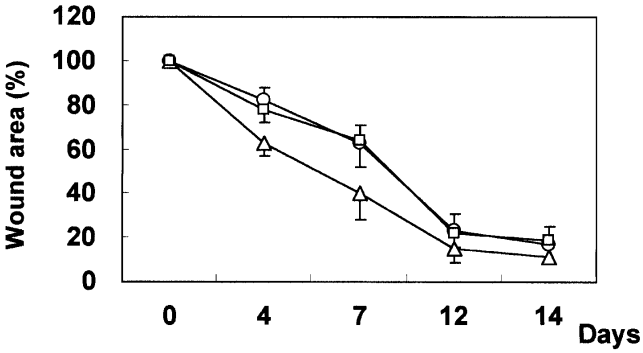

Figure 3. Comparison of changes in wound area of three groups. We compared wound healing among the following three groups: the Control Group ($n = 10$), which was kept until the end of the experiment in a normal rack in the absence of FIR at a temperature of 24.0°C to 25.0°C, Group A ($n = 10$), which was constantly exposed to FIR at a temperature of 26.5°C to 27.5°C, and Group B ($n = 10$), which was kept in the rack in the absence of FIR at the same temperature as Group A (26.5°C to 27.5°C). The wound area was measured on Days 0, 4, 7, 12, and 14. The curative effect is expressed as the percentile of the wound area at the time of measurement compared with the wound area on day 0 (100%). Comparisons for wound healing among the three groups were performed using two-way analysis of variance. The wound rapidly healed on Day 7 in Group A (△), compared with the Control Group (○) and Group B (□). Statistically significant differences were found during the observation period between Group A and both the control group and Group B ($P = 0.0172$). However, there was no significant difference between the Control Group and Group B.

Skin Blood Flow and Skin Temperature. Skin blood flow did not change significantly before and during FIR irradiation. Although skin temperature differed among individual rats, differences before and during FIR irradiation were not noted.

Histological Scorings. Because there was no difference in wound healing between the Control Group and Group B, histological scores of Group A were compared with those of Group B (Table I).

Infiltration of fibroblasts in subcutaneous was significantly greater in Group A than in Group B on Days 1, 5, and 7. There was a statistically significant difference between the two groups using two-way ANOVA ($P = 0.0183$). Abundant collagen regeneration was observed in Group A on Day 7, with collagen regeneration being significantly greater in Group A than in Group B on Day 7 ($P < 0.001$). Epitheliazation, cellular content (neutrophils and macrophages), and vascularization did not differ significantly between Groups A and B on Days 1, 3, 5, and 7.

Evaluation of Immunostaining. To investigate the secretion of TGF-β1, which is a cytokine that is well known to accelerate wound healing, the number of infiltrating fibroblasts expressing TGF-β1 in the six fields was counted (Fig. 4, a and b). The number of migrated fibroblasts expressing TGF-β1 in Group A was significantly greater than in Group B on Days 3, 5, and 7 (Fig. 4c). Two-way ANOVA demonstrated a statistically significant difference between the two groups ($P < 0.001$).

Collagen Determination. The fibers appeared as wavy structures of variable width and intermediate length (Fig. 5, a and b). The area of collagen fibers per field was 56.8% ± 11.3% in Group A and 26.5% ± 18.5% in Group B (means ± SD). There was a significant difference between the two groups ($P = 0.0124$; Fig. 5c).

Discussion

In this study, we investigated the promotive effects of FIR wound healing of normal skin in rats and we found significant differences in the speed of wound healing between groups that were and were not subjected to FIR. However, differences in ambient temperature did not significantly affect acceleration of wound healing. We repeated the same procedure three times, and obtained the same results. Therefore, reproducibility of the effect of FIR on wound healing was thought to be confirmed.

Although increased in skin temperature and blood flow by FIR irradiation have been reported, under the FIR irra-

Table I. Histological Scorings

		Neutrophils	Fibroblasts	Macrophages	Vascularization	Epithelialization	Collagen
Day 1	Group A	0.92 ± 0.39	0.60 ± 0.22[a]	0.28 ± 0.26	0.84 ± 0.55		
	Group B	0.80 ± 0.29	0.50 ± 0.21	0.21 ± 0.26	0.65 ± 0.45		
Day 3	Group A	0.71 ± 0.24	1.30 ± 0.51	0.67 ± 0.31	0.91 ± 0.40	0.37 ± 0.49	
	Group B	0.86 ± 0.35	1.39 ± 0.56	0.61 ± 0.24	0.95 ± 0.49	0.30 ± 0.40	
Day 5	Group A	0.53 ± 0.12	2.06 ± 0.57[a]	0.51 ± 0.11	1.05 ± 0.52	0.73 ± 0.84	
	Group B	0.55 ± 0.16	1.81 ± 0.62	0.61 ± 0.21	0.99 ± 0.48	0.65 ± 0.73	
Day 7	Group A	0.53 ± 0.12	2.06 ± 0.54[b]	0.49 ± 0.06	0.64 ± 0.34	0.86 ± 0.97	1.68 ± 0.59[c]
	Group B	0.57 ± 0.18	1.83 ± 0.41	0.50 ± 0.15	0.67 ± 0.35	0.80 ± 0.87	0.77 ± 0.29

Note. We compared histological scorings among the following two groups: Group A (n = 10), which was constantly exposed to FIR at a temperature of 26.5°C to 27.5°C, and Group B (n = 10), which was kept in the rack in the absence of FIR at the same temperature as Group A.

[a] $P < 0.05$

[b] $P < 0.01$

[c] $P < 0.001$ compared with Group B.

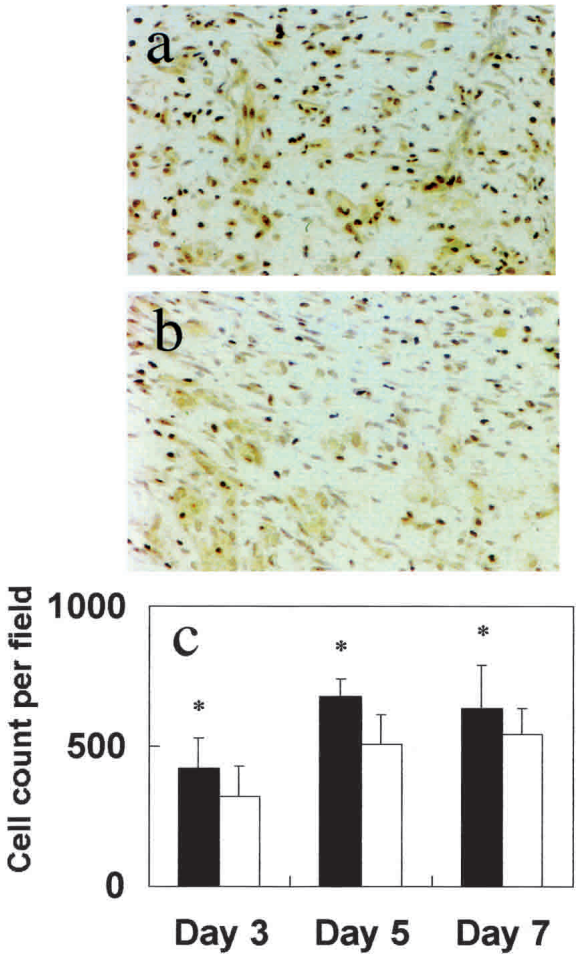

Figure 4. Infiltrating fibroblasts expressing TGF-β1 during wound healing. We compared the number of infiltrating fibroblasts expressing TGF-β1 in the wound among the following two groups: Group A, which was constantly exposed to FIR at a temperature of 26.5°C to 27.5°C, and Group B, which was kept in the rack in the absence of FIR at the same temperature as Group A. Infiltrating fibroblasts expressing TGF-β1 were observed during wound healing (a, Group A; b, Group B; Day 7 wound). Magnification ×400. (c) Number of migrated fibroblasts expressing TGF-β1 of Group A (closed column) and Group B (open column) were counted. Results are means ± SD, n = 10, for each time point and group. *$P < 0.001$ compared with Group B.

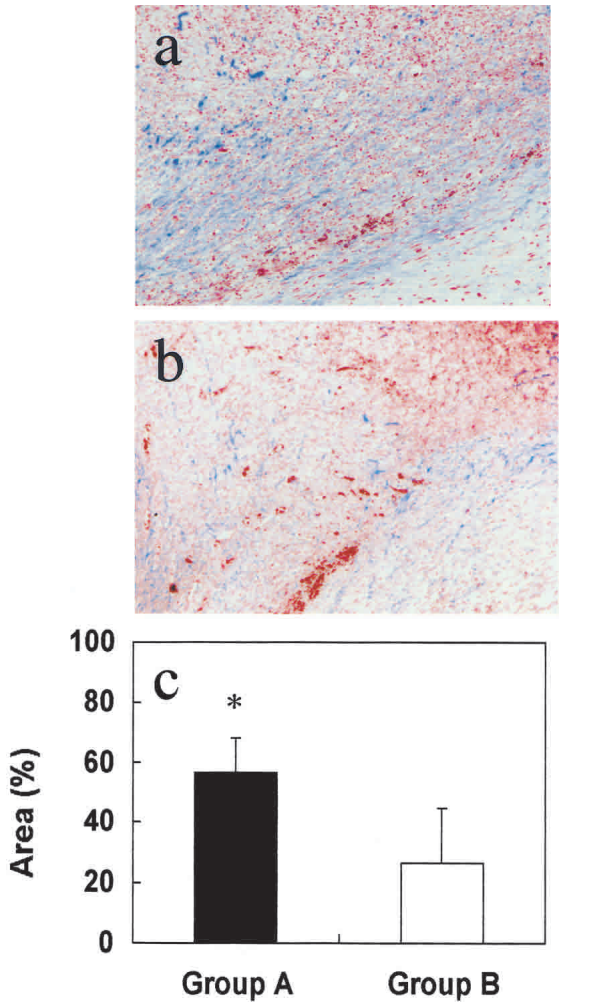

Figure 5. Mallory's collagen fiber staining on Day 7 wound. We compared collagen fiber in the wound among the following two groups: Group A, which was constantly exposed to FIR at a temperature of 26.5°C to 27.5°C, and Group B, which was kept in the rack in the absence of FIR at the same temperature as Group A. Collagen fiber was observed on Day 7 wound (a, Group A; b, Group B). Magnification ×250. (c) Area of collagen fibers per field of Group A (closed column) and Group B (open column) were examined. Results are means ± SD, n = 6, for each group. *$P < 0.05$ compared with Group B.

diation conditions that we used, no such increases were observed (1, 12). Because the blood flow and the skin temperature did not influence the wound healing in our study, it is thought that the ray directly affects the biological process of wound healing such as cellular proliferation or secretion of cytokines independently from the blood flow and the skin temperature.

The wound healing process can be categorized as follows: inflammation, formation of granulated tissue, and tissue remodeling (13, 14). The inflammation response is considered to be a key process during which growth factors, including TGF-β1 and platelet-derived growth factor, are released from macrophages and platelets to initiate granulation of tissue (15–17). TGF-β1 is one of the cytokines that is well known to accelerate wound healing, and induces cell influx in the inflammatory phase (17–19). In the granulation phase, TGF-β1 stimulates fibroblasts to produce extracellular matrix proteins, including collagen and fibronectin, and also facilitates their deposition. TGF-β1 is a potent stimulant of fibroblast migration with effective concentrations below that required for activation of gene transcription (20, 21). Dermal fibroblasts are responsible for the synthesis of new extracellular matrix proteins, primarily Type I and III collagen, that initially make up granulated tissue and form new normal tissue or scar tissue (22). Migration of fibroblasts, which occurs from surrounding unwounded skin toward the provisional matrix of the hemostatic plug, is a crucial step in the wound-healing role of these cells.

Our histological inspection showed that infiltration of fibroblasts in the subcutis was significantly greater in the FIR irradiation group than in the group without FIR irradiation on Days 1, 5, and 7. Furthermore, there was significantly greater collagen regeneration in the FIR group than in the group without FIR on Day 7 in sections with Mallory's staining.

The number of migrated fibroblasts expressing TGF-β1 was significantly greater in the FIR group. It is conceivable that the secretion of TGF-β1 by platelets and macrophages was enhanced by FIR irradiation, resulting in TGF-β1-stimulated fibroblast migration. Another possibility is that the activity of fibroblasts is directly stimulated by FIR irradiation. Consequently, the production of collagen was increased in the FIR group. The production of collagen fibers due to the activation of fibroblasts by FIR irradiation has been considered as a possible mechanism for the promotive effect of FIR irradiation on wound healing.

In addition, there was no histological evidence of remarkable inflammation or burning by FIR irradiation, and each parameter for plasma component levels differed little between the control and any experimental group (data not shown). This suggests that the FIR irradiation at an ambient temperature is safe and causes no harmful thermal effects.

Several physical agents such as low-energy lasers have been used in the treatment of wounds in several animal models. Efficiency has been confirmed by experiments with an animal model similar to ours (3–6). *In vitro* studies demonstrated that near-infrared ray and lasers activate a multitude of wound-healing processes such as collagen synthesis (23), cell proliferation (24), and keratinocyte motility (25). Our light source differs from these lasers. We used FIR with wavelengths ranging from 9 to 12 μm in this study, whereas lasers deliver a specific coherent beam (helium-neon at 632 nm and argon at 488 nm). Although it is thought that further *in vitro* studies of FIR are required, the biostimulatory effects of FIR irradiation might be similar to those of low-energy lasers or near-infrared ray.

In conclusion, our results suggest that FIR might have promotive effects on skin wound healing by the stimulating of the secretion of TGF-β1 or by the activating of fibroblasts independent of skin blood flow and skin temperature. Therefore, FIR irradiation may be clinically useful for wound treatment.

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