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Photoimmunological and Photobiological Action of Infrared Radiation

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While ultraviolet radiation alters various cutaneous cell functions, little is known about photo-immunological and photobiological effects of infrared radiation (IR) on the skin except its local thermal effects. The first part of this study demonstrated that single exposure of mouse skin to near IR (0.7 - 1.3 μm) reversibly suppressed the proliferating activity of the epidermis, the density of Langerhans cells, and the ability of skin to induce contact hypersensitivity reaction. The second part demonstrated that the rate of wound closure was significantly accelerated by repeated exposures in animal models. The production of transforming growth factor-β1 and matrix metalloproteinase-2, which are responsible for the wound healing processes, was significantly upregulated by irradiation, as shown by enzyme immunoassay, zymography, and reverse transcription polymerase chain reaction. Thermal controls were negative. The results suggest that near-IR irradiation can modulate the epidermal proliferation and part of the skin immune system, and stimulate the wound healing processes, presumably by non-thermal effects.

Key words : contact hypersensitivity, epidermal proliferation, infrared radiation, Langerhans cell, metalloproteinase, transforming growth factor, wound healing

INTRODUCTION

Ultraviolet radiation is the main wavelength of sunlight that causes significant alterations to cutaneous immune cells. UV has a variety of photobiological and photo-immunological effects. However, little is known about effects of infrared radiation on the skin, besides that it can increase the skin surface temperature and local blood circulation.

The aim of this study is to examine whether infrared (IR) radiation has any photobiological and photo-immunological effects. In the 1st part (1), we examined IR effects on the epidermal proliferation rate, Langerhans cell counts, and contact hypersensitivity reaction using mouse skin. In the 2nd part (2), we examined IR effects on wound healing by animal and culture studies.

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As to photophysiological properties of IR radiation, IR radiation is subdivided into: near, middle, and far IR according to the range of wavelength. They have different thermal effects: near IR penetrates the deeper portion of the skin, moderately warming the whole layers. On the other hand, energy of middle and far IR is largely absorbed within the uppermost layers, and the skin surface temperature is elevated to as high degrees as causing irritation and a burn.

MATERIALS AND METHODS

Light source. In this study, we used IFRARAY A (Clinical Supply Company) as a light source. The light source is equipped with 16 halogen lamps and a special cut filter that completely eliminates middle and far IR. IFRARAY A selectively delivers energy of near IR in the range of 0.7 to 1.3 μm. The light source is suitable in our study because it does not cause serious damage to mouse skin or cultured cells.

Epidermal proliferation rate and Langerhans cell counts. Groups of BALB/c mice were exposed to near IR at doses

of 30 and 60 J / sq cm. Bromodeoxyuridine (BrdU) pulse labeling was performed at various time intervals after irradiation using different groups of mice. Sham-irradiated mice were used as controls. Ear biopsy samples were stained with BrdU antibodies to see the epidermal proliferation rate. An epidermal sheet was also made and reacted with adenosine 5'-diphosphate (ADPase) in order to visualize Langerhans cells. The skin surface temperature was 27 degrees in average before irradiation and raised up to only 31 degrees after irradiation.

Contact hypersensitivity reaction in BALB/c mice. We examined the effects of near IR on contact hypersensitivity reaction using BALB/c mice, a UV-resistant strain. For local immunosuppression, the shaved abdominal skin was exposed to near IR (30 and 60 J / sq cm), and 3 or 14 days after irradiation, mice were sensitized with 1% dinitrofluorobenzene (DNFB) applied to the irradiated skin. Six days after sensitization, shielded ear lobes were challenged with 0.2% DNFB, and ear swelling response was measured. For systemic immunosuppression, irradiated mice were sensitized on the shielded, remote back skin. Sham-irradiated mice were used as controls.

Contact hypersensitivity reaction and tolerance in C57/BL6 mice. Next, we repeated a similar experiment, using the UV-sensitive strain of mice, to examine near-IR effects on contact hypersensitivity reaction and tolerance. Irradiated mice were sensitized on the irradiated abdominal skin to confirm local immunosuppression. 14 days later, they were re-sensitized on the shielded, remote back skin. Challenge test was performed on the shielded ear lobes.

Animal models for wound healing. A full-thickness incisional wound was created on the shaved back skin of ICR and C57BL-db diabetic mice. The test group of mice was given daily exposure to near-IR (60 J / sq cm) and wound areas were measured. Sham-irradiated mice were used as controls.

Culture studies. Cultured cells, including human foreskin Epipak keratinocytes, microvascular endothelial cells, and fibroblasts, were exposed to near-IR at the doses of between 36 and 108 J / sq cm. Sham-irradiated cells and thermal controls were also prepared. After irradiation, the viability of the cells was over 80%. The content of transforming growth factor (TGF)-β1 and matrix metalloproteinase (MMP)-2 in the medium was measured by enzyme immunoassay and zymographical techniques, respectively. MMP-2 mRNA was extracted from the cells and the amount was measured by reverse transcription polymerase chain reaction (RT-PCR). Experiments were performed in triplicate.

RESULTS

Near-IR reduces epidermal proliferation rate. The number of BrdU+ cells, expressed as % of basal cells, was significantly reduced between 5 h and 1 day after irradiation at the dose of either 30 or 60 J / sq cm (mean: 3.3 before irradiation *vs* 1.7 at day 1 after 60 J, n=8). The reduction, however, was transient, followed by return to the normal range by 14 days. Histologically, there were neither epidermal cell necrosis nor marked inflammatory cell infiltrate in the dermis.

Near-IR reduces Langerhans cells. The density of ADPase+ cells in 1 sq mm of epidermal sheet was most significantly reduced 3 days after irradiation at the dose of either 30 or 60 J / sq cm (mean: 922 before irradiation *vs* 50 at day 3 after 60 J, n=6). The reduction gradually recovered to the normal range by 14 days. Histologically, the dendritic processes were shortened or disappeared, and the central bodies became an oval or round shape.

Near-IR induces local immunosuppression in BALB/c mice. Compared with non-sensitized, negative controls, contact hypersensitivity reaction was good enough in sham-irradiated, sensitized mice. In near-IR irradiated groups, local immunosuppression was significant in groups of mice that were sensitized 3 days after irradiation at the dose of either 30 or 60 J / sq cm (mean of ear swelling: 0.13 mm in nonirradiated group *vs* 0.074 mm in 60 J-irradiated group, n=6). Local immunosuppression was negative when mice were sensitized 14 days after irradiation. Systemic immunosuppression was not successful.

Near-IR induces local immunosuppression, but not tolerance, in C57/BL6 mice. In this strain of mice, local immunosuppression was confirmed after the 1st sensitization, as shown in BALB/c mice. We further examined whether near IR induces tolerance of immunosuppression after the 2nd sensitization. However, the results were negative.

Near-IR accelerates wound healing. The rate of wound closure in ICR mice was significantly accelerated by daily exposure to near-IR at the dose of 60 J/sq cm, compared with sham-irradiated control group (between day 2 and 14). In C57/BL-db diabetic mice, too, wound healing was accelerated by near-IR (between day 7 and 21).

Near-IR increases the content of TGF-β1. In both Epipak and endothelial cells, the content of TGF-β1 in the medium was significantly increased 48 h after a single exposure to near-IR at doses of between 36 and 108 J /sq cm (mean in Epipak cells: 150% of control value, 48 h after 108 J), compared with sham-irradiated controls.

Near-IR increases the content of MMP-2 in cultured fibroblasts. Zymographical analysis on known amouts of MMP-2 yielded a good, dose-dependent curve, confirming the technical standard. Compared with sham-irradiated controls, the content of MMP-2 in irradiated cells was increased 24 and 48 h after near-IR irradiation at doses of between 54 and 108 J / sq cm (141% of control value, 48 h after 108 J). Effects of thermal controls were negative.

Near-IR upregulates the expression of MMP-2 mRNA. RT-PCR assay demonstrated that compared with sham-irradiated controls, the amount of MMP-2 mRNA was upregulated by near-IR irradiation at 24 and 48 h (155% of control value, 48 h after 54 J).

DISCUSSION

Near IR reversibly decreased the epidermal proliferation rate. The effects are similar to those of UVB radiation. However, unlike UVB, near IR never induced the acceleration phase of DNA synthesis nor inflammatory changes. Mechanisms are unknown, but the suppression does not seem to depend upon DNA damage.

Langerhans cells were sensitive to near IR. There was a positive correlation between the number of Langerhans cells and the ability of near-IR to induce local immuno-suppression. However, unlike UVB, near-IR never induced systemic immunosuppression nor tolerance of suppressed contact hypersensitivity, as far as within our experimental conditions.

Near-IR has the potential of accelerating wound healing in an acute animal model. The findings confirm the beneficial therapeutic effects of near IR on leg ulcers. The effects may partially be based upon upregulation of TGF-β1 and MMP-2, because these cytokine and collagen breakdown enzyme are responsible for the inflammatory and remodeling phases of the wound healing processes.

CONCLUSIONS

Near-infrared irradiation has a variety of photobiological and photoimmunological effects, which may be caused by non-thermal effects. We claim that near-IR should be involved in sunlight-induced skin injuries.

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