



Low Level Laser Therapy & Immune Responses clinical research

Effects of low-power laser radiation on mice immunity.

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Background/purpose: Because of large interest in biological effects of laser radiation used in laser therapy, the effect of extremely low-level red laser light intensity on the immune cell activity has been studied in the animal model with well-characterized macrophage and T cell populations as responder cells producing cytokines, protective proteins, active oxygen, and nitric compounds. To study of the possible side effects of laser immunotherapy we monitored the productions of cytokines, nitric oxide (NO), and heat shock protein 70 (Hsp70) in mice subjected to a periodic laser exposure for 1 month.

Methods: Helium-neon laser radiation with the power of 0.2 mW/cm² and wavelength of 632.8 nm was applied on two different mouse skin surfaces, i.e. a thymus projection area or a hind limb. Healthy NMRI male mice were irradiated repeatedly with laser light for 1 min with 48-h intervals for 30 days. The animals were divided into three groups of 25 mice. The first and the second groups were exposed to laser light, on the thymus and hind limb area, respectively. The third, sham-irradiated group served as a control. Early and prolonged effects of laser radiation on the levels of NO (by Griess assay), Hsp70 (by Western blot assay), tumor necrosis factors (TNF-alpha and TNF-beta) (by cytotoxic assay using L929 cells as targets), and interleukin-2 (IL-2) (by ELISA assay) were determined.

Results: The dynamics of immune responses to low-power laser light intensity was shown to be dependent on two factors, i.e. the cumulative dose and the localization of the irradiated surface. Besides, various populations of cells demonstrated different sensitivity to laser radiation, with T cells being more responsive among examined populations of the cells. Low intensity laser light induced an immune cell activity when the exposure duration did not exceed 10 days, while a more prolonged period of treatment generated more severe changes in the immune system, up to immunosuppression. The treatment of the thymus zone resulted in more pronounced changes in the cytokine production as well as in NO and Hsp70 synthesis.

Conclusion: Low-power laser irradiation showed more effective immunomodulatory effects when applied on the thymus projection area. The rise in IL-2 and Hsp70 production related to a short-term effect of laser application may be reversed after repeating laser treatment. We suggest that for the support of immune system stability, the prolonged laser therapy should be accompanied by supplementary methods.

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Immunomodulatory effects of low-intensity near-infrared laser irradiation ON contact hypersensitivity reaction.

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BACKGROUND/PURPOSE: Contact hypersensitivity (CHS) reaction is a useful model for studying the skin immune system and inflammatory reactions in the skin. In this study, an experimental model of CHS reaction was employed to assess immunomodulatory effects of near-infrared (near-IR) low-intensity laser (LIL) irradiation, which is used as adjuvant therapy in dermatology, physical medicine, rheumatology, etc., because of its declared anti-inflammatory, biostimulative and analgesic effects. **METHODS:** The effects of near-IR LIL irradiation ($\lambda=904$ nm, irradiance 60 mW/cm², fluence 3.6 J/cm²) on CHS reaction to 1-chloro-2,4-dinitrochlorobenzene (DNCB) in Albino Oxford rats were examined by irradiating experimental groups of animals before the induction phase of CHS reaction, while nonirradiated animals and animals that received vehicle instead of hapten served as controls. Ear-swelling assay, histopathological examination of H&E preparations of ear skin, computer-assisted image analysis of dermal infiltrate, ear skin organ culture with the determination of cutaneous production of tumour necrosis factor-alpha (by ELISA assay) and nitric oxide (by Griess' assay) were used for measuring the effects of LIL in the elicitation phase of CHS reaction. Cellularity, dendritic cell content, flow cytometry and proliferation assays (spontaneous and in the presence of IL-2 and concanavalin A) of the draining lymph node cells (DLNC) were performed for the assessment of LIL irradiation effects in the induction phase. **RESULTS:** In the irradiated group of animals, ear swelling was significantly diminished compared to control animals (101 \pm 11.5% vs. 58 \pm 11.6%, $P<0.01$). This was accompanied by a highly significant decrease in the density of dermal infiltrate (22 \pm 0.81 vs. 14.2 \pm 1.75 cells per unit area, $P<0.01$) and a significant decrease in nitrite levels in the medium conditioned by organ-cultured ear skin (17.63 \pm 1.91 vs. 3.16 \pm 1.69 μ M NaNO₂; $P<0.01$), while TNF-alpha concentration was not changed. Cellularity and dendritic cell content in DLNC population, as well as the expression of TCR-alpha, CD4, CD8 and CD25, were not changed between irradiated and nonirradiated animals. Proliferation rates of DLNC cultured for 72 h were significantly lower in irradiated animals (17.3 \pm 4.1 vs. 13.9 \pm 0.9 $\times 10^3$ c.p.m.; $P<0.01$). In cultures of DLNC with added rIL-2 or 0.5 μ g/ml of concanavalin A, proliferation rates were also significantly decreased in irradiated animals (34.7 \pm 3.5 vs. 31.2 \pm 2.0 c.p.m. in IL-2-supplemented culture, $P<0.01$; 70.9 \pm 6.4 vs. 58.3 \pm 9.1 $\times 10^3$ c.p.m. in concanavalin A-supplemented culture, $P<0.01$). However, this effect was overcome in the presence of the higher concentration of concanavalin A (2.5 μ g/ml) (nonirradiated 38.7 \pm 3.1, irradiated 123.1 \pm 7.3 $\times 10^3$ c.p.m., $P<0.01$). **CONCLUSION:** LIL irradiation showed a systemic immunomodulatory effect on CHS reaction to DNCB in rats. Decreased ear swelling observed in the elicitation phase was associated with diminished proliferative responses of the DLNC in the induction phase of CHS reaction. Further experimental work is needed to examine the possible mechanisms of these effects.

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