EFFECTS OF LASER RADIATIONS ON HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Abstract. Effects of low-laser stimulation, although poorly explained in terms of mechanism, are clinically used for wound healing, joint mobility increasing, pain control and, generally, to accelerate repairing processes, by promoting tissue regeneration and/or angiogenesis. Both visible and infrared radiations exhibit local, but also distant beneficial effects by inducing production of signaling molecule which, in turn, modulate physiological processes of other effector cells acting in immune response (immune modulation). Several cell types were found non-responsive to laser stimulation, while in other cases, only a partial activation was observed.

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Introduction

RATIONAL

OBJECTIVE

MATERIALS & METHODS

Human umbilical vein endothelial cells (HUVEC) from cell line collection (ATCC) were cultured in RPMI 1640 (Biochrom AG), by 48 hours passages. Irradiation was performed when the culture reached 90% confluence.

Irradiation conditions: a laser device with a continuous wave diode (λ = 808nm) at an energy density of 46.8 J/cm^2 was used. IR exposure was from the top, at 8cm of dish bottom.

Leukocytes were isolated from peripheral blood, collected from healthy persons on EDTA-coated vials. Cells were suspended in RPMI 1640 medium supplemented with bovine fetal serum 10%, antibiotic/antimycotic solution 1% and L-glutamine 1% and viable cells were determined by trypan blue exclusion in a Burker-Turk hemocytometer.

Equal number of leukocytes were added in 96 well plates RPMI 1640 medium supplemented or not with different volumes of medium collected from dishes with cultured HUVEC (either irradiated or non-irradiated).

Biochemical assays: LDH (for cell membrane integrity) and MTS (for cell proliferation) measurements were carried out using Cytotox^{96} Non-radioactive Cytotoxicity Assay (Promega, Cat. No. G1781) and CellTiter^{96} Aqueous Non-radioactive Cell Proliferation Assay (Promega, Cat. No. G5421) kits, respectively.

Fig. 1. LDH release by cultured HUVEC, just after irradiation (A), and 24 hours post irradiation (B). C – non-irradiated cultured HUVEC, I – irradiated cultured HUVEC

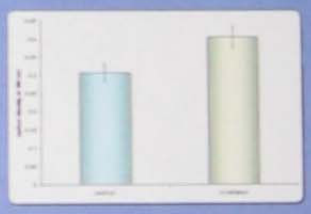

Fig. 2. HUVEC cell proliferation 24 hours after irradiation; C – non irradiated cultured HUVEC, I – irradiated cultured HUVEC

Fig. 3. LDH release by cultured leukocytes in medium supplemented or not with HUVEC culture medium just after (A), or 24 hours post irradiation (B); C – no HUVEC culture medium, M – non-irradiated HUVEC culture medium, I – irradiated HUVEC culture medium. bars represent M or I absorption values normalized to C values.
*p<0.1, **p<0.05, ***p<0.01

Fig. 4. Leukocyte proliferation after HUVEC culture medium treatment. (A): leukocytes treated with culture medium just after HUVEC irradiation; (B): leukocytes treated with HUVEC culture medium 24 hours post-irradiation; C – no HUVEC culture medium, M – non-irradiated HUVEC culture medium; I – irradiated HUVEC culture medium; bars represent M or I absorption values normalized to C values.

HUVEK LEUKOCYTES

CONCLUSIONS

- Laser irradiation does not affect the viability of HUVEC in culture, but increases cell proliferation.
- Medium of cultured HUVEC protects leukocytes, and stimulates their proliferation in a higher manner after endothelial cell irradiation by 808nm laser.
- The effects are only noted for leukocytes treated with HUVEC medium just after irradiation, suggesting a release of short time acting factors.

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