EFFECT OF DIODE LASER IRRADIATION ON INTERLEUKIN-8 PRODUCTION BY HUMAN NEUTROPHILS

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Low reactive-level laser therapy (LLLT) is a safe and easy therapeutic modality and now utilized in the treatment of inflammatory conditions. However, the clinical evidence base for such application remains sparse. This study was designed to examine the effects of LLLT on production of interleukin-8 (IL-8) by human neutrophils. Neutrophils, separated from blood drawn from 10 healthy volunteers, received laser irradiation (150 mW/cm²) for 30 or 60 sec at 60 min prior to or just before the stimulation with opsonized zymosan. These conditions had different effects on production of reactive oxygen species by neutrophils. After the incubation at 37 °C for 3 and 9 hours, the concentration of IL-8 was measured by ELISA. The concentration of IL-8 was higher when neutrophils were incubated longer. However, the levels of IL-8 production by irradiated neutrophils did not differ from those of unirradiated neutrophils even under the conditions whereby both the period of irradiation and the duration from irradiation to stimulation were different. IL-8 production by neutrophils may not play a role in the therapeutic effects of LLLT in inflammatory conditions.

Key words: laser irradiation, neutrophil, interleukin-8

Introduction

Chemokines play a very important role as far as white blood cell chemotaxis is concerned, especially chemotaxis to inflammatory lesions⁽¹⁾. Chemokines are classified into four subfamilies from their structural characteristics. In particular, the CXC chemokine subfamily, members of which have a single amino acid between the first two cysteines of the characteristic four cysteine motif, has an very important role in neutrophil-mediated inflammation. One of the best-known CXC chemokines is interleukin-8 (IL-8), which is mobilized from the bone marrow and is activated against neutrophils through discharge of the lysosome enzymes, production of Roicotorien B4 and reactive oxygen species, and the induction of adhesion factors to neutrophils, and plays a very important role in acute and active inflammation.⁽²⁾

Activated neutrophils infiltrate inflamed tissue, and reactive oxygen species (ROS) are thought to be involved with damage to tissue in an

inflammatory state.⁽³⁾ It is known that LLLT irradiation is useful in the treatment of skin ulcers,⁽⁴⁾ oral aphthae⁽⁵⁾ and arthritis.⁽⁶⁾ However, the exact mechanism in these entities remains unknown except for an increasing effect on blood flow.⁽⁷⁾ We have already reported one possible mechanism through which LLLT irradiation suppresses ROS production.⁽⁸⁾

On the other hand, up to the present, no report has examined the relationship between IL-8 and LLLT irradiation. A high rate of production of IL-8 is seen from neutrophils which have infiltrated inflamed tissue. Therefore, we hypothesized that, if LLLT irradiation could decrease neutrophil IL-8 production, the decreased levels of IL-8 would subsequently inhibit inflammation. In this study, we therefore examined how LLLT irradiation affected the IL-8 production of neutrophils.

Materials and Methods

Neutrophil suspension preparation and opsonization of zymosan

Normal pooled human neutrophils were obtained from the peripheral blood of 10 healthy adult male volunteers and separated by Histopaque density gradient centrifugation, according to the method already reported.⁽⁸⁾

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Briefly, human blood was diluted with the same volume of HBSS containing heparin (5 U/ml) and this heparinized diluted blood was then settled onto Histopaque 1077 layered over Histopaque 1119 (Sigma, USA) and then centrifuged at 500 g at 4 °C for 30 min. The neutrophil rich layer was collected and washed twice in HBSS and the neutrophils were resuspended to 2000 cells/ μ l using an automatic blood cell counter (Coulter MD II, Coulter Co. Ltd., Tokyo, Japan).

Opsonized zymosan (OZ) was used to stimulate neutrophil phagocytosis. Zymosan from *Saccharomyces cerevisiae* was employed for opsonized particles. Zymosan A (Sigma, USA) was suspended in Hank's balanced salt solution (HBSS) at a concentration of 5 mg/ml and then opsonization was performed by adding the zymosan suspension to the serum samples to a final concentration of 13% and incubating at 37 °C for 30 min.

Laser irradiation of neutrophils

A GaAlAs semiconductor laser, the Luketron MDL-1000 (Mochida Pharmaceutical Co., Tokyo, Japan), at a wavelength of 830 nm and an output power of 1000 mW in continuous wave was used for the laser irradiation. One of the features of this system is the fiberoptic delivery system which allows homogeneous irradiation of fairly large areas due to the multimode characteristics of the beam.

Wells were irradiated with the laser before and after incubation with an incident irradiance of 150 mW/cm². In a previous study we had ascertained that irradiation 60 min before stimulation of the neutrophils induced the greatest suppression of ROS production,⁽⁸⁾ so that protocol was followed in the current study. Irradiation times of 30 and 60 sec were used, giving incident radiant fluences of 4.5 and 9 J/cm². These exposure times were chosen as being representative of popular clinical parameters. Following incubation, OZ was added to a final density of 1 mg/ml, and incubation was continued at 37°C. A control set of neutrophils was handled in exactly the same way as the irradiated sets including incubation, but did not receive any laser irradiation.

Measurement of IL-8

The samples in the control and experimental wells were removed after 3 or 9 hours, and centrifuged for ten minutes at 1000 g. The IL-8 density in the supernatant was measured with an enzyme-linked immunosorbent assay (ELISA; BioSource Co., Camarillo, CA, USA).

Statistical analysis

All the measurement were given as mean values $\pm$ standard deviation (SD). One-way ANOVA was done to test the differences in IL-8 density among three samples (immediately before irradiation, 60 minutes before irradiation and no irradiation) with post hoc test. Values of less

Fig 1: IL-8 concentration in irradiated and unirradiated neutrophil supernatant 3 hours after stimulation with opsonized zymosan

Fig 2: IL-8 concentration in irradiated and unirradiated neutrophil supernatant 9 hours after stimulation with opsonized zymosan

than P 5% were evaluated as significant.

Results

The IL-8 density from the unirradiated control neutrophils was 89.1 ± 16.7 pg/ml (Figure 1). When the neutrophils were irradiated with the laser immediately before stimulation, the IL-8 density was 88.7 ± 12.4 pg/ml following at 30-second exposure, and 81.0 ± 10.8 pg/ml following a 60-second exposure. Although there was a decreasing trend, there was no significant difference between either of the exposure times and the unirradiated control, or between the two irradiation times themselves.

When the laser was used to irradiate the neutrophils 60 minutes before neutrophil stimulation, the IL-8 density was 83.0 ± 12.2 pg/ml following the 30-second irradiation, and 88.2 ± 15.9 pg/ml following the 60-second irradiation. Once again there were no statistically significant differences between IL-8 density from the irradiated and unirradiated cells. In other words, at the parameters used in the present study IL-8 production from neutrophils did not depend on either the timing or the duration of the laser irradiation.

Next, the IL-8 density after 9 hours of neutrophil stimulation is shown in Figure 2. The IL-8 density was 105.4 ± 12.5 pg/ml in the unirradiated control neutrophils. When the neutrophils were irradiated with the la-

ser immediately before stimulation of the neutrophils, the IL-8 density was 117.5 ± 13.9 pg/ml following the 30-second exposure, and 81.0 ± 10.8 pg/ml for the 60-second irradiation. No significant difference was seen in IL-8 density between the irradiated and unirradiated neutrophils, or between the two doses. When the neutrophils were irradiated 60 minutes before neutrophil stimulation, the IL-8 density was 114.1 ± 17.4 pg/ml for the 30-second exposure time, and 108.5 ± 18.8 pg/ml for the 60-second irradiation. Therefore, IL-8 production from neutrophil did not depend either on the timing or duration of the laser irradiation, though it did depend on the incubation time.

Discussion

LLLTT irradiation may be effective for the treatment of arthritis, as has been suggested in the literature.⁽⁹⁾ In arthritis, neutrophil infiltration into the tissue is seen. Furthermore, in previous reports of an experiment in the rat model, an injection of IL-8 into the inside of the joint capsule induced inflammation similar to chronic arthritis.⁽¹⁰⁾ Such evidence suggests that neutrophil infiltration may play some role in arthritis. Furthermore, it is known that IL-8 is associated with tissue damage via increased ROS production from neutrophils.⁽¹⁾ Indeed, administering anti-IL-8 antibody reduces neutrophil infiltration into the joint capsule and joint swelling.⁽¹¹⁾

These findings mean that IL-8 induces neutrophil infiltration and tissue damage.

ROS from activated neutrophil by such as bacterial lipopolysaccharide should induce not only arthritis but also ulcers of the digestive tract or aphthae of the oral cavity.⁽¹²⁾ It is known that the level of the neutrophil infiltration into tissue is associated with the amount of local IL-8, and it has been suggested that the existence of IL-8 is associated with ulcer formation.^(13,14) Moreover, it has been shown in a recent report that gingival fibroblastoma cell production of IL-1 β is inhibited by irradiation with the GaAlAs laser. This suggests a possible role for LLLT radiation through an anti-inflammatory action via inhibition of cytokine production.⁽¹⁵⁾

In our previous studies, LLLT inhibited the ROS production from neutrophils, suggesting a possible application pain relief or wound healing.⁽⁸⁾ In the current study, when neutrophils were irradiated with LLLT at same level which significantly inhibited the ROS production in our earlier study, IL-8 production showed no change. Therefore, LLLT irradiation did not have any effect on IL-8 production when the neutrophils was stimulated with zymosan (via the CD11b, CD16 receptors⁽¹⁶⁾). However, if a stimulating factor other than zymosan were to be used, IL-8 production might be changed following LLLT irradiation. Furthermore, since there are complex cytokine networks *in vivo*, further study is required in the future.

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