THE ACTION OF LOW REACTIVE LEVEL LASER THERAPY (LLLT) ON MAST CELLS:

a possible pain relief mechanism examined

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The tongue of the Swiss mouse is rich in mast cells (MC). Tongues of animals in the experimental group were irradiated using a He-Ne laser (2.4 J/cm², 632 nm), and a second set of unirradiated animals served as the control. Two output powers were used, with the irradiation time adjusted to produce the same energy density. The possible nonselective degranulation of LLLT-irradiated mast cells was examined quantitatively and morphometrically using optic and electron microscopy. By means of radioimmunoassay, the histamine content of pulverized tongue was evaluated, comparing the levels of both experimental and control tissue. The irradiated tongue tissue showed a significantly higher histamine level compared with the control. In addition, the level of histamine in the interstitial cellular medium and the degree of other histological changes such as vasodilation was significantly higher following a single irradiation with the He-Ne laser at the above settings. The observations from this study may serve as a further indication of a bioactive effect of low reactive level laser therapy, a therapeutic process still not fully understood.

KEY WORDS He-Ne laser LLLT Mast cell excretion Bioactivation

Introduction

In previous work,¹ it was reported that low reactive level laser therapy (LLLT) produced vasodilation, and that this phenomenon could be linked to the direct or indirect action of LLLT on mast cells (MC). Vasodilation does not occur immediately after LLLT irradiation, but a few minutes later,² and then continues latently even after the irradiated tissue has cooled. This latent action supports the view that the vasodilation is not linked only to a photothermal reaction. It could indicate that enhanced circulation is related to specific hormonal mechanisms, possibly originating from the excretion of biologically active substances stored and manufactured in the mast cells.³ Table 1 shows a list of these substances.

To confirm the possible nonselective action of LLLT on MC, a study was designed to determine if these cells could be implicated in the interaction between LLLT and tissue.

Materials and Methods

A continuous wave He-Ne laser (632.8 nm) was used, with output powers of 4 mW and 15 mW. Normally-bred, male Swiss laboratory mice of 25 g were used as subjects. There were three groups, 12 animals in each group. Group 1 served as the control. The experimental procedure was performed, but the laser was not actually activated. Group 2 was irradiated with the 15 mW He-Ne laser, irradiated area 1 cm² (power density = 0.015 W/cm²), and an irradiation time of 2 min 40 s. This gave an output energy of 2.4 J, and an energy density of 2.4 J/cm². Group 3 was irradiated with the 4 mW He-Ne laser with the same spot diameter (power density = 0.004 W/cm²), with an irradiation time of 10 min. This gave similar output energy and energy density as for group B.

Irradiation was carried out in the oral cavity, directly on the tongue, exposure area of 1 cm². Immediately after the first irradiation, two animals from each group were killed, their tongues were excised and processed for electron microscopy (inclusion into 2.5% glutaraldehyde and mounted in araldite). Slices (0.5 µ) were prepared and lead citrate contrasting and metachromatic toluidine blue staining were carried out.

The remaining animals received one irradiation every second day to a total of five irradiations. The animals received no special care, and no local or

Table 1. Mast cells granules. Contents

Histamines
Amines
Serotonin (in animals)
Slow reacting substances
Prostaglandins
PGD ₂
Eosinophil chemotactic factor
Neutrophil chemotactic factor
Platelet aggregation factor
Enzymatic system
Mucopolysaccharide acids
Heparin
Hyaluronic acid

general drugs were administered. On the tenth day, after 5 irradiations, all animals were killed. Immediately after death, the tongues of four animals from each group were excised, and all macroscopic characteristics were recorded: the tongues were then fixed in a 10% formol solution. Five micro-incisions were made at different points on each of the tongues, with a similar protocol. Hematoxylin/eosin, Giemsa and toluidine blue staining were carried out.

The remaining tongues were excised, weighed and pulverized in a mortar with 0.1 ml physiological serum, and then frozen at -30°C . Histamine analysis was performed using radioimmunoassay (RIA) with the Immunotech kit.

Results

Histological Studies

Macroscopic and optical microscopy studies. (Figures 1–6). On macroscopic analysis, the tongues of the animals from the experimental groups 2 and 3 were noticeably more reddish-purple than the tongues of control group 1 animals. Microscopic studies revealed good cell maturation in the pavement epithelium of the group 1 animals with a thin keratinized surface layer. Among the striated muscle fibres of the corium, fine arterial and venous vessels were observed. MC with rounded nuclei and granule-rich cytoplasm were observed in quantity grouped around these vessels. Vessels of the corium appeared normal, containing erythrocytes, with no sign of vascular thrombosis. There was no sign of any increase in the number of vessels; hyperplasia and hypertrophy of the endothelial cells were not noted. On the other hand, the vessels of the LLLT-irradiated tongues were noticeably dilated throughout the tongue. Careful study at low magnification with selective metachromatic staining revealed fewer mast cells in LLLT-irradiated tongue tissues from groups 2 and 3 compared with control group 1.

Figure 1. Control tongue. Normal aspect. No vessel dilation

Figure 2. Control tongue. Normal aspect. Observe the MC

Figure 3. Tongue irradiated with 4 mW laser

Figure 4. Observe increase of vessel lumina and decrease of MC, compared with the control sample

Figure 5. Tongue irradiated with 15 mW

Figure 6. Note the greater dilation of the vessels, presenting stasis phenomenon and few MC, compare with control tongue

Electron Microscopic Analysis (Figures 7-9).

Under toluidine blue staining, a definite decrease in the number of mast cells in tongue tissue from groups 2 and 3 could be seen. In addition, the MC were irregularly distributed, compared with control samples. Ultrastructural study of the normal control mast cells showed a normal cytoplasm containing the usual physiopathological products of MC, such as histamine, heparin and serotonin. In the LLLT-irradiated tongue tissues, MC cytoplasm appeared totally or partially clear of granules, some of which could be observed free in the interstitial medium. Vessels were dilated, but with no sign of thrombosis or increase in vessel number. At greater magnification, these phenomena could be confirmed, and some MC could be seen in the act of exocytosing their granules.

Histamine Dosage (Table 2)

Statistical analysis of the results using the Mean Comparison Technique between small populations showed firstly a significant decrease in the number of MC in LLLT-irradiated tissue with a security coefficient of 95%; and secondly no significant difference in histamine levels could be noted between

Figure 7. Normal aspect. Control MC. No degranulation

Figure 8. MC after irradiation. Active degranulation

Figure 9. Electronmicroscopy. Dilated vessels

groups 2 and 3, irradiated with different output powers but with the same dose of 2.4 J.

Discussion

Optical microscopy revealed that the mast cells in LLLT-irradiated tissue from groups 2 and 3 were fewer in number than in control group 1 tissue. This indicates that mast cell degranulation is a function of LLLT irradiation. Control group 1, under simulated irradiation, exhibited no degranulation, and no subsequent vasodilation. A single He-Ne laser dose

Table 2. Mast cells. Histamine dosage

of 2.4 J produced vasodilation in the Swiss mouse tongue, coupled with an active degranulation of the mast cells. The MC decreased in number, and their granules could be observed draining out, and floating free in the interstitial medium.

Following LLLT irradiation, various substances including histamine, heparin and prostaglandin D₂ are released, and either act locally or are systematically carried by the vasculature. The release of histamine which acts on the smooth muscle of the vasculature causes active vasodilation, maintained through successive laser irradiations. This may have a beneficial effect on the rate of tissue repair and wound-healing. Five 2.4 J irradiations produced a significant decrease in the number of MC and an increase in the histamine level in groups 2 and 3, compared with the control group. This was accompanied by vasodilation, one of the principal possible therapeutic actions of LLLT in tissue.

On the other hand, the synthesis of prostaglandin D₂, recently confirmed as a product of MC,⁴ would help to explain the anti-inflammatory effect of LLLT irradiation, due to its intensive action on secondary inflammatory mediators. It must be stressed that neither in this study, nor in any of the reports on LLLT in the literature, have observations of vascular thrombosis or thrombi precursors been made. It may well be that the heparin excreted from the MC is the mediator in this case.

The dose of 2.4 J was delivered faster in group 2 irradiated with the 15 mW laser, and the decrease

in the number of MC was more marked in this group, indicating that the degranulation process was faster. This leads to the possible conclusion that higher power densities with shorter irradiation times may be more efficient in the delivery of LLLT.

The mast cell may act as a unicellular gland, which is stimulated by LLLT irradiation, and may therefore prove worthy of further research into the action and role of the various MC products, of physiopathological importance prior to and after LLLT. It is quite possible that analysis and interpretation of the fluctuation in the level of all MC products following LLLT will help towards a better understanding of the action of LLLT in tissue.

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