CONTINUOUS WAVE VISIBLE RED (633 nm) LASER ENERGY ENHANCES THE DEGRANULATION OF MAST CELLS: A POSSIBLE PHOTOMEDIATED WOUND HEALING MECHANISM EXAMINED *IN VIVO*

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The mast cell in an interesting repostory of a number of pro- and anti-inflammatory mediators, and plays an important role in the inflammatory stage of the wound healing process, and in allergic reactions. The present study was designed to elicit the effect of visible light radiation at 632.8 nm (visible red) on mast cells *in vivo* using light and transmission electron microscopy. The tongue of the Swiss mouse is rich in mast cells (MCs). Tongues of animals in two experimental groups were irradiated using a HeNe laser (15 mW & 4 mW, 2.4 J/cm^2,for both output powers, λ = 632.8 nm), and a third set of unirradiated animals served as the control. The 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, and the histamine levels in both experimental and control tissue were compared. 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 HeNe laser at the above settings. The observations from this study may serve as a further indication of the bioactivative effect of low reactive level laser therapy on mast cells, which are an important component of the wound healing process and inflammation control.

Key words: HeNe laser, LLLT, phototherapy, mast cell degranulation, cytokines, photobioactivation

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 (MCs). Vasodilation did not occur immediately after LLLT irradiation, but a few minutes later,[2] and then continued latently some time after irradiation. This latent action supports the view that the vasodilation was not linked only to a photothermal reaction. It could indicate that enhanced circulation associated with LLLT irradiation is related to specific hormonal mechanisms, possibly originating from the excellulation of biologically active substances stored and manufactured in the mast cells[3] as listed in Table 1.

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 helium-neon (HeNe) laser (632.8 nm) was used, with output powers of 4 mW and 15 mW. Normally-bred, male Swiss laboratory mice weighing 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 HeNe laser, irradiated area 1 cm^2 (power density 0.015 W/cm^2), and an irradiation

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Table 1: Mast cells granules

Contents
Histamines
Amines
Serotonin (in animals)
Slow reacting substances
Prostaglandins
PG D_2
Sodium dismutase (SOD)
Factors
Eosinophil chemotactic factor
Neutrophil chemotactic factor
Platelet aggregation factor
Enzymatic system
Mucopolysaccharide acids
Heparin
Hyaluronic acid

time of 2 min 40 s. This gave an output energy of 2.4 J, and an energy density of 2.4 J/cm^2. Group 3 was irradiated with the 4 mW HeNe laser with the same spot diameter (power density = 0.004 W/cm^2), with an irradiation time of 10 min. This gave a similar output energy and energy density as for group B. The laser used was developed and constructed in the IMV laser laboratory, and energy was delivered from the laser to the target by a flexible fibreoptic light guide.

Irradiation was carried out in the oral cavity, directly on the tongue, exposure area of 1 cm^2. Immediately after the first irradiation, two animals from each group were killed, their tongues were excised and processed for electron microscopy (immersion in 2.5% gluteraldehyde and embedded in araldite). Ultrathin slices (0.5 μm) were prepared and processed for metachromatic toluidine blue staining with lead citrate contrasting.

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 systemic 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% formalin solution. Five 2 mm biopsies were taken at different points on each of the tongues, with a similar protocol. Haematoxylon 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.

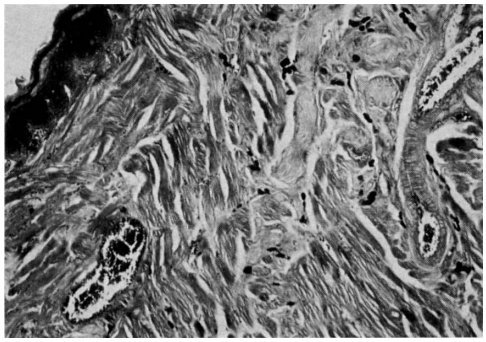

Fig 1: Control tongue under light microscopy. Normal aspect. No vessel dilation.

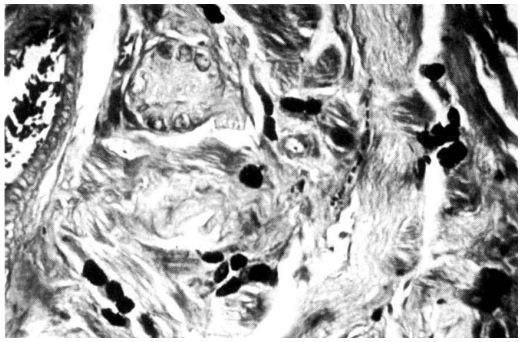

Fig 2: Control tongue under light microscopy. Normal aspect. Observe the MCs.

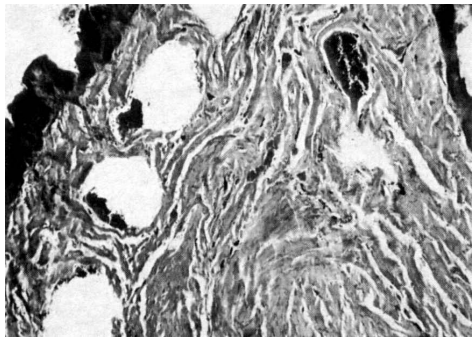

Fig 3: Tongue irradiated with 4 mW HeNe laser. (Light microscopy).

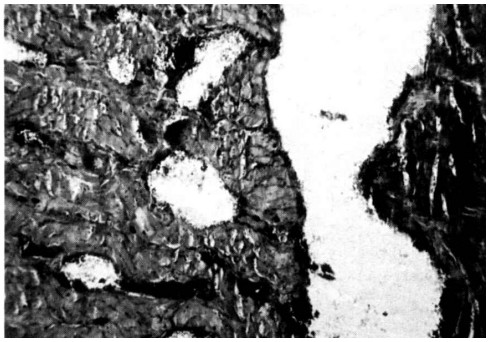

Fig 4: Observe the dilated vessel lumen and the decrease in MCs, compared with the control sample. (Light microscopy).

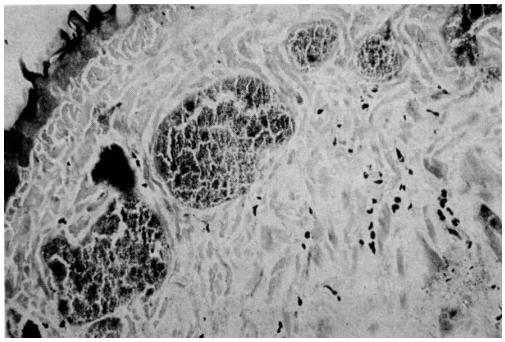

Fig 5: Tongue irradiated with 15 mW HeNe laser. (Light microscopy).

Fig 6: Note the greater dilation of the vessels, presenting the stasis phenomenon, and fewer MCs compared with the control tongue. (Light microscopy).

Results

Histological Studies

Macroscopic and optical microscopy studies. 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 (Figures 1, 2). MCs 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 (Figures 3-6).

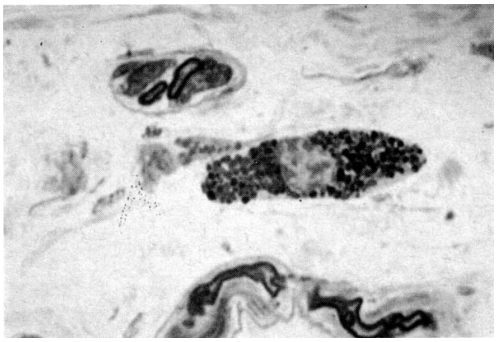

Fig 7: Normal aspect. Control MC. No degranulation. (Transmission electron microscopy).

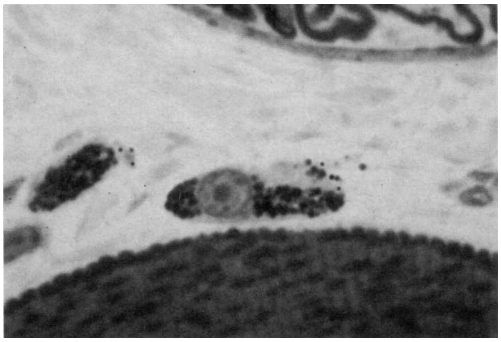

Fig 8: MC after irradiation. Active degranulation, stasis phenomenon and few MC. compared with control tongue. (Transmission electron microscopy).

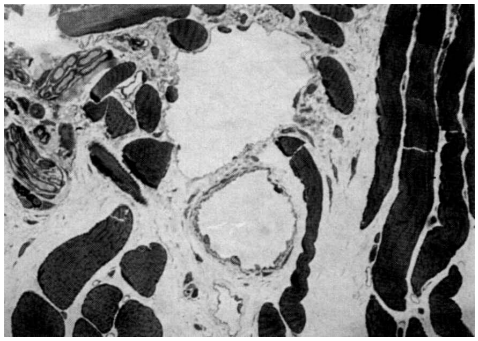

Fig 9: Dilated vessels under transmission electron microscopy.

Transmission electron microscopic analysis (Figures 7 – 9)

Under toluidine blue staining, a definite decrease in the number of mast cells in the tongue tissue from groups 2 and 3 could be seen, more so in group 2 (15 mW) compared with group 3 (4 mW). In addition, the MCs were irregularly distributed, compared with control samples. Ultrastructural study of the normal control mast cells showed a normal cytoplasm containing the usual physiopathological granular products of MCs, such as histamine. heparin and seratonin. In the LLLT

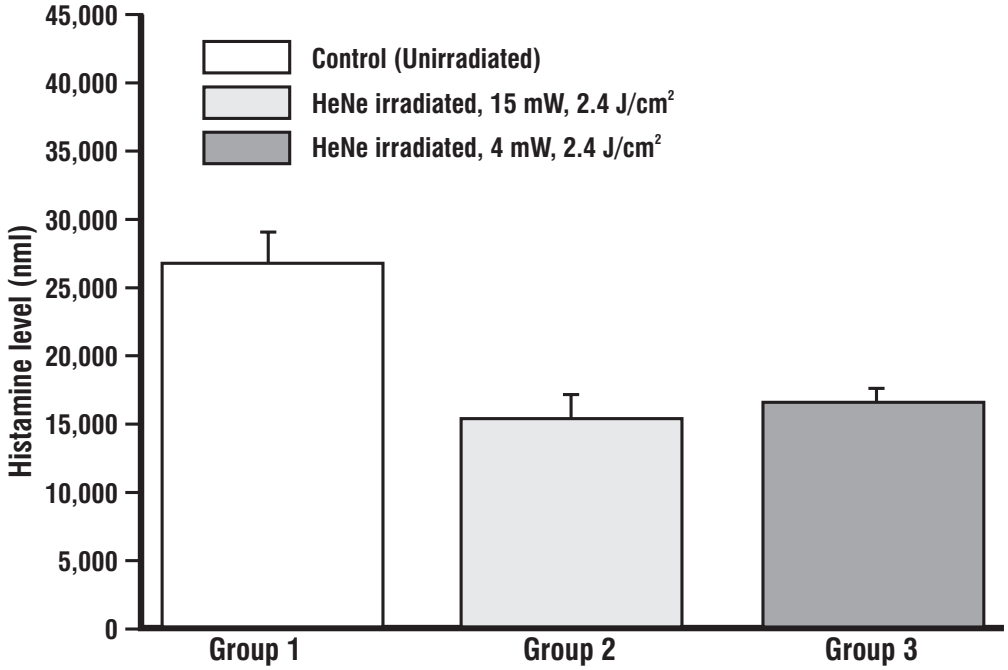

Fig 10: Number of mast cells compared for irradiated (15 mW, 2.4 J/cm^2; 4 mW, 2.4 J/cm^2) and unirradiated control tongue.

irradiated tongue tissues, the 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 MCs could be seen in the act of excellulating their granules (Figure 8).

Histamine levels in unirradiated and irradiated tongues

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% compared with the unirradiated controls; and secondly, although a trend could be seen for a slightly lower level of histamine was seen in group 2 (15 mW / 2.4 J/cm^2) tongues compared with group 3 (4 mW/2.4 J/cm^2) tongues, the difference was not statistically significant.

Discussion

Optical microscopy revealed that the MCs tn 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 HeNe laser dose of 2.4 J/cm^2 produced vasodilation in the Swiss mouse tongue, coupled with an active degranulation of the mast cells. The MCs decreased in number, and excellulation of their granules could be observed, with granules seen floating free in the interstitial medium.

Following LLLT irradiation, various substances including histamine, heparin and prostaglandin D_2 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 treatment sessions of 2.4 J/cm^2 produced a significant decrease in the number of MCs 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 D_2, recently confirmed as a later product of MCs,[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 excellulated from the MCs is the mediator in this case.

One of the later products of degranulation is superoxide dismutase (SOD), one of the most powerful endogenous antioxidants. This may well have a beneficial effect on controlling levels of reactive oxygen spe-

cies (ROS) produced from activated neutrophils and macrophages. beneficial levels of ROS are necessary to maintain tissue homeostasis, but excess levels are deleterious to normal tissue.

The dose of 2.4 J/cm^2 was delivered over a shorter time in group 2 irradiated with the 15 mW laser, and the decrease in the number of MCs was more marked in this group, compared with the control sample, 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.

Conclusion

The mast cell may act as a unicellular gland, which is stimulated by low incident levels of continuous wave visible red photon energy, and may therefore prove worthy of further research into the action and role of the various MC products, and of their pathophysiological importance prior to and after phototherapy. It is quite possible that analysis and interpretation of the fluctuation in the level of all MC products following phototherapy may well help towards a better understanding of the beneficial actions of phototherapy in living tissue.

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