THE ACTIVATORY EFFECT OF LOW INCIDENT ENERGY HE-NE LASER RADIATION ON HYDROXYAPATITE IMPLANTS IN RABBIT MANDIBULAR BONE

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Hydroxyapatite (HAP) is a well-accepted biocompatible material used in the repair of bony defects of the oral cavity and jawbone. Low reactive-level laser therapy (LLLT) has been reported as accelerating the repair of bone fractures, and in controlling side-effects from the inflammatory reaction such as oedema. The present study examines the effects of hemilateral He-Ne laser irradiation (632.8 nm, 6 mW, 10 min, daily for 4 days) on HAP implants in bilateral artificially-created defects in the rabbit mandible. The contralateral unirradiated side served as control. At 21 days postimplantation macroscopically and microscopically the irradiated implants showed better bonding with the surrounding bone margins, and a greater degree of osteogenesis than the unirradiated implants, which tended to be loose, or to have totally dislodged. It was concluded that, with pathways and mechanisms as yet not fully understood, LLLT controlled the inflammatory response to the HAP implant resulting in better implant bonding and osteogenesis at the implant borders.

KEY WORDS He-Ne LLLT Osteogenesis HAP

Introduction

In the region of the oral cavity, partial defects of the jawbone occur due to tumours, cysts, and inflammation. Hydroxyapatite (HAP), which has excellent biocompatibility, is widely used as a prosthetic material in the repair of such defects.^{1,2}

It has been reported previously that irradiation with low-output power lasers has bone-repair-promoting effects.³⁻⁵ Therefore, this study was performed to determine if new bone formation could occur at an early stage and if the fusion conditions of the HAP with the bone would be more effective if HAP were implanted in the bone and the low-output laser irradiation were performed, compared to similar implants which were unirradiated.

Materials and Methods

The experimental animals were four white rabbits weighing 2.5–3.0 kg. An experimental osseous deficiency of 2 × 4 mm was created in the bilateral

lower mandibular margin by the use of an ultrasonic saw developed and designed by the authors, with a 0.3 mm thick blade, and HAP block was packed into the entire deficient portion.⁶ The HAP used is a material of high purity, supplied in granular, pore-filled blocks of 2 × 4 × 6 mm with the pore sizes ranging from 20 to 50 µm. The practical and useful application of HAP has been recognized clinically and experimentally.

The laser used was a He-Ne laser made by WLISA (SOFT LASER® 2H-5LM10), and the hemilateral experimental areas were irradiated daily for 4 days from immediately after the insertion of the HAP, output power of 6 mW, a spot size of 1 cm² and an exposure time of 10 min. The contralateral areas were unirradiated, and served as controls.

The animals were sacrificed at 21 days following implantation, and a sample was prepared by extracting the mandible including the area of HAP insertion. The contralateral side was used as the unirradiated control for comparative assessment. After fixation, haematoxylin and eosin and Mallory staining were carried out and light microscopy were performed.

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Table 1. Macroscopic findings 21 days after HAP implant in LLLT-irradiated and control osteotomies

Group	Bonded	Loosened	Dislodged
LLL (n = 4)	3	1	0
Control (n = 4)	1	2	1

Results

Macroscopically, the HAP blocks were found to be present at the time of resection in all of the cavities in the irradiated group, but they had fallen out in some cases in the control group (Table 1).

Histopathologically, granulation had occurred between the HAP and the residual bone with a comparatively smooth border in the control group (Figure 1a), and new bone formation was found at irregular intervals in the granulation (Figure 1b). The granulation showed a high degree of cellularity, consisting mainly of fibroblasts, but osteoblasts were arranged at the margin of the newly formed bone (Figure 1c).

In the laser-irradiated group, the HAP and newly formed bone surface were directly connected in many places, and newly formed bone was also in close linear contact (Figure 2a). Many more osteoblasts were present than in the control group and there was almost no infiltration of inflammatory cells (Figure 2b).

Figure 1. Histological features of control group (see text for details). G—granulation; NB—new bone; B—bone. (Haematoxylin and eosin, original magnification, 1a, $\times 30$; 1c $\times 30$; Mallory, 1b $\times 30$)

Figure 2. Histological features of laser-irradiated group (see text for details). NB—new bone; OB—osteoblasts; B—bone. (Haematoxylin and eosin, original magnification, 2a, $\times 50$, 2b, $\times 100$)

Discussion

HAP is recognized as a superior bone-filling material because of its high *in vivo* affinity for living bone. However, there are occasions when multiple dehiscence of the wound occurs, partly exposing the underlying bone at an early postoperative stage, and severe reactive inflammatory responses such as swelling and redness are also pointed out as possible defects of this material in bone repair.¹

On the other hand, regarding the mechanism of activity following low reactive-level laser therapy, reports have been already made on enhanced synthesis of collagen, accelerated enzymatic activity in LLLT-irradiated tissue, enhanced regeneration of the blood vessels, improved blood flow and activated cells all coming from the photoactivating effect of the laser, although there are still many unknown points regarding specific LLLT pathways and mechanisms. Regarding the influence of LLLT on osseous tissue, it has been reported in the literature that there is an effect of promoting healing of fracture at the initial phase and an activity of accelerating osteogenesis.⁷

To create the bony defects in this experiment, the authors used a prototype supersonic saw manufactured by themselves which has a very thin (0.3 mm) saw blade, and which therefore does not generate much heat in the bone.⁶ This is essential to provide the most stable conditions for the insertion of the HAP into the defect, avoiding as much exogenous stimulation of the bone as possible, such as heat, and keeping surgical trauma to a minimum.

Daily irradiation with the He-Ne laser (632.8 nm) at an output power of 6 mW was carried out for 10 min per session till the 4th day of the inflammatory reactive period in the first phase of the wound-healing. The He-Ne was used as it is one the most generally-applied laser systems for LLLT and bioactivation. Its reported effects on periosteum activities are remarkable and promotion of osseous formation is seen clearly.⁸

The histopathological findings in the irradiated group revealed that inflammatory cellular infiltration in the periphery of the implant is considerably less, and also by the macroscopic findings, most parts of the HAP block adhered solidly to the existing bone, compared with the unirradiated group, in whom deviated and loosened HAP blocks were frequently seen. These findings of favourable operative course obtained agree with the authors' reports made elsewhere.⁹ It is agreed so far that low reactive-level laser therapy brings about the effect of promoting healing by reducing oedema in the initial phase of the process of inflammation due to the increased vascular drainage. In addition, anti-inflammatory effects have been reported, such as photobiochemical alleviation of oedema by carra-

geenin demonstrated in an animal experiment,¹⁰ and it was suggested also that low incident doses of laser energy are involved more directly in osteogenesis as seen in the increase of osteoblasts around and in the LLLT-treated HAP implants than in the unirradiated control sites.

Conclusions

In conclusion, the laser irradiated group showed the following differences compared with the non-irradiated control group:

- (1) There was little granulation between the HAP and residual bone, and the HAP and newly formed bone were in direct contact in many places.
- (2) There was abundant osteoblast activity and more new bone formation.
- (3) Little infiltration of inflammatory cells was observed. From the above results, the specific action of the He-Ne LLLT was not clear, but it appeared to involve cellular and tissue photobioactivation at several levels following LLLT, and it was evident that the He-Ne LLLT suppressed inflammation in response to the HAP implant.

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