EFFECT OF LOW LEVEL CARBON DIOXIDE LASER RADIATION ON BIOCHEMICAL METABOLISM OF RABBIT MANDIBULAR BONE CALLUS

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Low-level Laser Therapy (LLLT) has been suggested as an effective adjuvant in the healing of bone fractures. However, the mechanisms of the effects of laser bioactivation on tissue are still not completely clear. The present study was designed and performed to investigate the effect of LLLT doses of CO_2 laser irradiation on the bone healing of rabbits that had undergone bilateral mandibular hole-like osteotomies. The calcium, phosphorus and collagen hydroxyproline contents in the bone callus were determined respectively on different postoperative days. After exposure to a CO_2 laser beam at a power density of 225 mW/cm^2, it was demonstrated that the calcium, phosphorus and hydroxyproline quantities in the irradiated side bone callus were significantly higher than the contralateral unirradiated control callus. This may partially explain the bioeffects of LLLT on the speed and quality of bone repair. It is concluded that LLLT using the CO_2 laser has the potential of promoting metabolism and mineralization of bone callus, thus accelerating bone healing.

Key words: Laser bioactivation, LLLT, CO_2 laser, bone healing, calcium, Phosphorus, hydroxyproline

Introduction

Mandibular fractures and osteotomies generally require 6 to 8 weeks of intermaxillary fixation, a modified diet, and restrictions on normal activities. Oral surgeons have been searching for some methods to shorten the fixation time so that the patient can return to normal activities earlier. Many treatments have been tested to accelerate bone healing (vitamins, minerals, hormones, electrical stimuli, and so on).[1-4] To date, electrical stimulation has been thought to be the most effective, but this method requires the implantation of electrodes and the associated risk of infection. Low-level laser therapy (LLLT) might be an alternative technique for accelerating bone healing.

Several LLLT sources have been used in therapeutics to act as stimulators, both analgesic and anti-inflammatory, including the CO_2, 1aser.[5-8] Further, LLLT is now in clinical use for the treatment of mandibular fractures,[9-11] and animal studies have clearly shown the potential for LLLT's increasing the mandibular fracture healing rate.[12,13] However, what are the possible mechanisms of laser bioactivation on bone repair?

Although a series of experiments has been carried out to examine the influence of laser radiation on the mandibular bone healing process,[9,10,12,13] to date mainly descriptive, morphological and histological methods have been used to evaluate the effect of laser irradiation on bone healing. Most experiments *in vivo* provide only clues about the mechanism, but lack standardized quantitative measurement of bone healing. The available statistical data are unreliable. It was therefore felt necessary to study the laser effect on bone healing with more convincing methods. A controlled animal model was developed, and the contents of the calcium, phosphorus and collagen hydroxyproline in LLLT-irradiated and unirradiated rabbit bone callus were determined in the hope of finding possible mechanisms of CO_2 laser LLLT hastening bone healing.

Materials and Methods

Animal Model

Bilateral mandibular osteotomies were performed on 24 New Zealand rabbits of both sexes, 8 – 12 months old, weighing 1.5 to 2 kg each. After adequate anaesthetics were administered to the rabbits (intraperitoneal Pentobarbital, 30 mg/kg), the area of the mandibular angle was shaved and prepared separately with 2% tincture of iodine and 75% alcohol. A submandibular incision was made, exposing the masseter and medial pterygoid muscles. A sharp incision was made on the inferior border of the ramus of the mandible and the masseter and medial pterygoid muscles reflected from the surface of the mandibular ramus. A hole-like osteotomy of 3 mm diameter was made in the ramus of the mandible, approximately 5 mm from the posterior border of the ramus, and 5 mm from the inferior border of the mandible (Figure 1). Copious irrigation was used

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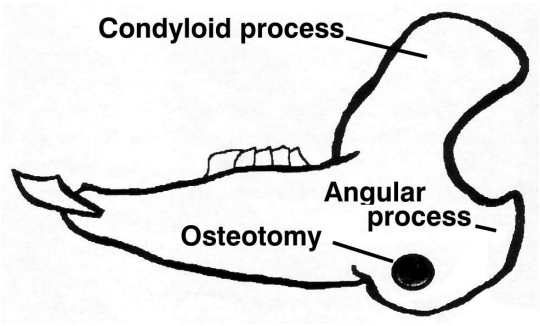


Fig 1: Rabbit lower jaw showing osteotomy site.

at the time of the drilling. Closure was accomplished in layers with 4 – 0 sutures.

Laser Irradiation

The rabbits were divided into four equal groups, matched as far as possible in weight and sex. The irradiated side for each rabbit was chosen at random, and the opposite side osteotomy was unirradiated, serving as the control. Apart from the laser irradiation, both sides were treated exactly the same. Irradiation was carried out with an output of 2 W (wavelength 10600 nm) using a model JG-6 CO_2 laser system, aiming the defocused beam at the region of the osteotomy from a distance of 10 cm. This gave an irradiated area of approximately 2.5 cm^2, and a power density of 255 mW/cm^2. The treatment was carried out every day, 10 mm per session, for seven consecutive days. The control contralateral area was given simulated laser treatment (i.e. the laser was set up and aimed, but never actually turned on). The first LLLT session was given on the first day following the osteotomy operations.

Biochemical Assay

The four groups of animals were killed (intravenous air) on the 7th, 14th, 21st, and 28th postoperative days respectively. The mandible was separated, and the soft tissues removed, exposing the bone callus. The callus was resected from the osteotomy site with a hole punch and labelled. After rinsing and defatting with 1:1 acetone/ether solution, stoving and weighing, the calluses were placed in 10% trichloroacetic acid solution to demineralize for 48 h. The demineralized solution was used for determination of calcium and phosphorus. Calcium content was determined basically according to Connerty's method,[14] and phosphorus content determined with the routine ammonium molybdate method.

The demineralized bone calluses were treated with acid hydrolysis (6N hydrochloric acid, 12 h, 100°C under pressure of 352 gm/cm^2). The hydroxyproline content was determined according to the method of Firschein.[15] Hydroxyproline in living organisms is held to be confined almost exclusively to connective tissue scleroproteins, collagen and elastin.[16] Because of this restricted distribution, hydroxyproline has been widely used as an indicator of both the presence and metabolism of collagen. Hydroxyproline content was therefore selected as an index reflecting the production of bone collagen.

Statistical Methods

A two-tailed t-test for paired samples was used to compare the data from the laser-irradiated calluses and the contralateral unirradiated controls.

Results

Calcium and Phosphorus

According to the determination, the calcium content of the LLLT-irradiated calluses was found to be significantly higher than that of the contralateral control calluses on the 14th, 21st and 28th postoperative days, but not on the 7th day ($p < 0.05 – 0.01$). The phosphorus content of the irradiated bone calluses was also found to be higher than that of the contralateral control bones on the 14th, 21st and 28th postoperative days, but not on the 7th day ($p < 0.02$-0.01) (see Tables 1 and 2). Figures 2 and 3 present a graphical comparison reflecting the difference in the calcium and phosphorus content between irradiated and contralateral control callus material, demonstrating that calcium and phosphorus in bone callus can be significantly increased by laser irradiation.

Hydroxyproline

The hydroxyproline determination revealed a significant collagen production difference between the irradiated side and the contralateral control calluses (Figure 4). Table 3 demonstrates that hydroxyproline content in the irradiated side callus was significantly higher than that of the contralateral control callus within 28 days ($p < 0.05 – 0.01$), indicating that LLLT dosage stimulates bone collagen production.

Discussion

It is well known that active synthesis of collagen and other connective tissue components is necessary for optimal wound healing during the early periods of repair. Bone healing, however, involves a more complex physiologic and biochemical process which includes collagen synthesis, inorganic salts deposition and osteogenesis. Thus, increasing collagen production in order to form a better bone matrix and enhancing inorganic metabolism of bone callus are important steps for speeding up bone repair.

Collagen synthesis is normally enhanced in a reparative process. It is evident now that it can be further stimulated by low-level laser therapy. Mester and Kana have proved respectively that LLLT can activate and increase the collagen synthesis in soft tissue wound healing.[17,18] Abergel *et al.* examined the effects of laser on human fibroblasts in vitro and also showed that laser irradiation of LLLT dosage could significantly increase

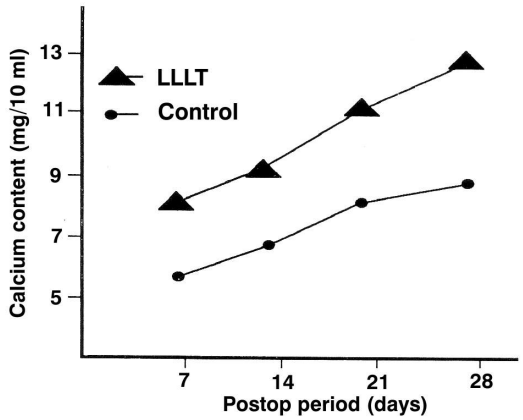


Fig 2: Comparison of calcium content of LLLT-irradiated and unirradiated bone callus.

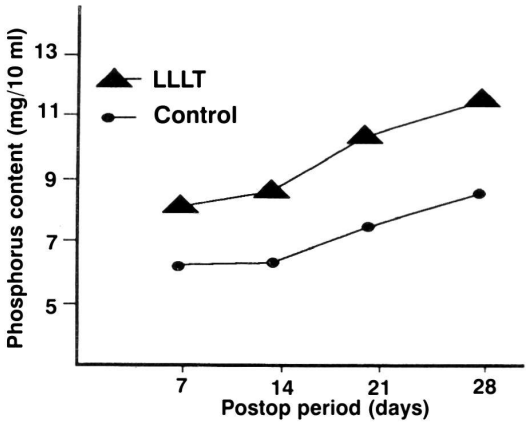


Fig 3: Comparison of phosphorus content of LLLT-irradiated and unirradiated bone callus,

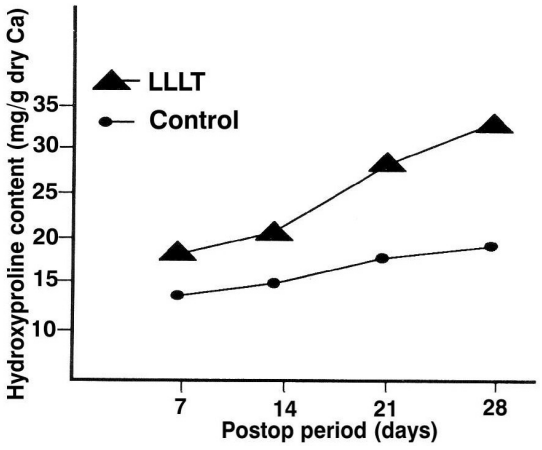


Fig 4: Comparison of hydroxyproline content of LLLT-irradiated and unirradiated bone callus.

collagen production.[19] The present study result coincides with the studies mentioned above, and showed that CO_2 LLLT similarly stimulated the production of bone collagen. According to the study determination,

Table 1: Statistical data on difference between irradiated and unirradiated bone callus calcium content (d = mean of difference value)

Days	d	S.D.	*t*	p
7	1.78	0.574	2.054	>0.05
14	2.85	0.758	3.72	>0.02
21	1.82	0.603	3.02	<0.05
28	3.45	0.64	5.38	<0.01

Table 2: Statistical data on the difference between irradiated and unirradiated bone callus phosphorus content (d = mean of difference value)

Days	d	S.D.	*t*	p
7	0.76	0.82	2.14	>0.05
14	1.16	1.03	3.82	<0.02
21	1.996	0.33	6.13	<0.01
28	1.395	0.28	4.98	>0.01

Table 3: Statistical data on difference between irradiated and unirradiated bone callus hydroxyproline content (d = mean of difference value)

Days	d	S.D.	*t*	p
7	2.265	0.849	2.8	<0.05
14	2.38	0.516	4.38	<0.01
21	10.41	3.014	3.452	<0.01
28	7.125	1.715	4.155	<0.01

the collagen hydroxyproline content of the LLLT-irradiated callus was higher than the unirradiated contralateral control, from an early stage of healing (i.e. 7th postoperative day). The longer the postoperative period became (14th, 21st and 28th days), the more significant the increase in the hydroxyproline content became. This indicates that CO_2 LLLT activated collagen production in the collagenous phase of bone repair, and sustained the effect in later stages.

Laser-enhanced bone consolidation and laser-promoted inorganic salts deposition have been demonstrated *in vivo*.[20,21] In other experiences, there has been evidence that non-destructive laser energy exposure of fracture regions could result in an earlier mineralization of bone callus.[9,22] The P32 metabolic test proved that LLLT irradiation could increase the deposition of inorganic salts in the region of a bone fracture, suggesting that a nondestructive laser beam enhances the metabolism of inorganic salts by means of improving the local blood circulation.[21] The present study also presented supportive results. A significant increase of calcium and phosphorus content in the irradiated callus material was observed over the contralateral control, which was reflected in the active inorganic salts metabolism, and the earlier mineralization of the irradiated callus.

In view of these and other authors' results, it can be proposed that LLLT possesses a potential for stimu-

lating the bone repair processes. Upon laser irradiation of a bone osteotomy region, the resorption of haematoma could be activated because of the stimulation of the blood flow. The abundant blood flow in turn would bring a large number of inflammatory-mediated cells, sufficient oxygen, nutritious substances and inorganic salts to the osteotomy site. This would certainly contribute to the earlier onset of bone healing. Of particular importance is the finding that there was a significant increase in the collagen production in the irradiated calluses. If it is agreed that the enhanced inorganic salts metabolism is due to the stimulated regional blood flow, then the increase of collagen production in bone callus is a result of the laser bioactivation of fibroblasts and other osteogenic cell components, which mature earlier following LLLT exposure.[22] These mature cells synthesize abundant bone collagen protein, which in turn forms a better bone matrix for bone repair. Inorganic salts are then liberally deposited in the bone matrix, leading to the earlier mineralization of the callus.

Besides its effects on collagen production and inorganic salts metabolism, LLLT may also have a special effect on the cells necessary for osteosynthesis, stimulating their differentiation and proliferation, thereby enhancing their functions.[12] According to High,[23] the use of prostaglandin E_2 activates the healing process of fractures, which may also help to explain the effect LLLT produces on the speeding of bone healing. Mester also noted an increase in PGE_2 after laser irradiation.[24]

The improved blood circulation and stimulated vessel growth may be the most important effects of LLLT on the healing process. During the period of bone healing, osteogenesis chiefly depends on the blood circulation, which directly relates to the formation of bone matrix and its mineralization. Bone develops better in a well-vascularized environment: it would therefore be reasonable to achieve this faster healing using LLLT on the target area. Naturally, not only the isolated factors as demonstrated in this study may be responsible for laser-accelerated bone healing: multiple factors may be involved. It can be inferred that through laser energy's photochemical, photoelectric, photomagnetic and photoenzymatic actions, i.e. laser photoactivation, LLLT influences the links in the chain of the bone repair process, improving the environment and the situation of bone healing. In the present trial, only the LLLT effect on bone collagen synthesis and mineralization were examined. Other related mechanisms deserve to be further studied. The experimental results have convinced the authors to recommend the clinical use of CO_2 LLLT to stimulate the healing process of mandibular fractures.

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