Effect of low-power laser irradiation on bony implant sites

Key words: laser, implants, biostimulation

Abstract: This study was designed to examine the effects of low-energy laser irradiation on osteocytes and bone resorption at bony implant sites. Five male baboons with a mean age of 6.5 years were used in the study. Four holes for accommodating implants were drilled in each iliac crest. Sites on the left side were irradiated with a 100 mW low-energy laser (690 nm) for 1 min (6 Joule) immediately after drilling and insertion of four sandblasted and etched (Frialit®-2 Synchro) implants. Five days later, the bone was removed en bloc and was evaluated histomorphometrically. The mean osteocyte count per unit area was 109.8 cells in the irradiated group vs. 94.8 cells in the control group. As intra-individual cell counts varied substantially, osteocyte viability was used for evaluation. In the irradiated group, viable osteocytes were found in 41.7% of the lacuna vs. 34.4% in the non-irradiated group. This difference was statistically significant at $P, 0.027$. The total resorption area, eroded surface, was found to be 24.9% in the control group vs. 24.6% in the irradiated group. This difference was not statistically significant. This study showed that osteocyte viability was significantly higher in the samples that were subjected to laser irradiation immediately after implant site drilling and implant insertion, in comparison to control sites. This may have positive effects on the integration of implants. The bone resorption rate, in contrast, was not affected by laser irradiation.

Osteogenesis and bone healing following injuries are regulated by such factors as growth hormones, proteins and local blood flow (Marks & Popoff 1988). In order to accelerate bone healing, various mechanisms, such as electromagnetic fields (Bassett et al. 1982) and electrical stimulation (Bassett et al. 1981), have been sporadically tried clinically. Low-power laser irradiation appears to be another potential stimulus to improve soft tissue healing [Mester et al. 1973], to re-unite central and peripheral axonal lesions (Schwartz et al. 1987; Rockkind et al. 1988; Assia et al. 1989), and to accelerate post-traumatic skeletal muscle healing [Weiss & Oron 1992; Bibikova & Oron 1993, 1994]. In hard tissues, low-power laser irradiation was shown to speed up vascularization and to increase the number of trabeculae in fractured mouse tibiae (Trelles & Mayayo 1987). In another study in rats, alkaline phosphatase was significantly increased six days after irradiation of standardized bone lesions, which reflected an enhanced osteoblast activity [Barushka et al. 1995]. In an in vitro experiment of rat osteoblasts, three low-power laser irradiations significantly increased the osteoblast count after an interval of eight days [Dörtbudak et al. 2000].

The regulatory mechanism of low-power lasers is not yet clearly understood. It is photochemical in nature, with the energy...
probably being absorbed by intracellular chromophores and converted to metabolic energy, most likely involving the respiratory (cytochrome) chain (Belkin et al. 1988; Karu 1989).

These phenomena that have been reported for low-power laser irradiation shortly after injuries prompted us to study the primary effects, if any, on osteocytes and bone resorption following implant site drilling.

Material and methods

The study protocol was reviewed and approved by the local Ethics Committee.

Five male baboons (mean age 6.5 years) were used in the study. A 5 cm skin incision was made bilaterally to expose the iliac crests. Approximately 4 cm posterior to the superior anterior iliac spine, four holes were drilled to accommodate sandblasted and etched implants (Frialit®-2 Synchro, Friadent GmbH, Germany), according to the manufacturer’s instructions.

After drilling the sites to a width of 5.5 mm and a length of 10 mm, radiation was applied to the holes in the left iliac crest for 1 min with a 100 mW low energy (690 nm) laser. The sandblasted and etched implants were then inserted and the laser was anchored in the implant center with a special device for another 1 min course of irradiation to the peri-implant hard tissues. The contralateral sites in the right iliac crest were not irradiated.

Wound closure was done in layers. Augmentin® was administered at a dose of 2.2 g i.v. intraoperatively and 1 g b.d. for 5 days postoperatively. The animals were sacrificed on day 5, and the implants were removed together with the peri-implant bone en bloc. The bone blocks were fixed in buffered formalin solution, embedded in Technovit 7200 VLC and cut to sections of 30 µm using the technique of Donath (1988). The sections were stained as described by Laczko & Levai (1975) and analyzed histologically. In view of the small width of the iliac crest after site drilling and implant insertion, the bone blocks were ground in an anterior-posterior direction to preclude injuries of the lateral and medial cortical layers. As a result, only the anterior and posterior implant margins were available for analysis.

Viable osteocytes were counted with a Nikon Microphot-FXA microscope at a magnification ×375. Count fields of 235 × 350 µm were placed along the longitudinal aspects of the implants so that areas of suitable width that encompassed the entire length of the implants were available for analysis (Fig. 1).

Within the areas of interest, empty lacunae were distinguished from lacunae that contained staining osteocytes and osteocyte remnants. The percentages of viable osteocytes and of empty lacunae were determined. To quantify bone resorption (Howship’s lacunae), the method reported by Merz (1967), with a magnification ×180, was used. For each implant, a total of eight evenly spaced count fields (8 mm²), four on each longitudinal aspect of the implant, were chosen (Fig. 2). From the counts, the eroded surface area (ES) was calculated as per cent cancellous and cortical surface area of bone resorption, using the method reported by Delling (1975).

Statistical analysis

Osteocyte counts and total resorption areas are listed in Table 1. As intra-individual counts varied substantially, per cent differences in cell counts of samples with and without irradiation are given. The data for osteocytes, percentage viability and the dif-
In the control group, with between-group differences in the eroded surface area (ES) of the resorptive cancellous and cortical bone were significant (Table 1a). The eroded surface area (ES) was 24.6% in the non-irradiated group vs. 24.9% in the control group. This difference was statistically significant (P < 0.027).

The eroded surface area (ES) was 24.6% of the resorptive cancellous and cortical surfaces in the irradiated group vs. 24.9% in the control group, with between-group differences of −4.3% to 7% (Table 1b). Student’s t-test was not significant for the total resorptive area (P > 0.05).

### Results

In four of the five animals, the medial and lateral cortical layers were lost so that the implants were covered by soft tissue only. However, laser irradiation and implant insertion were successful in all instances and the postoperative course was uneventful. Wound healing was also uncomplicated.

In the irradiated group, the mean osteocyte count per unit area was 109.8 cells vs. 94.8 cells in the control group (Table 1a). This difference was not statistically significant. Viable osteocytes were found in 41.7% of the total number of osteocyte lacunae in irradiated samples vs. 34.4% in the non-irradiated samples, with inter-individual percentages ranging from 3% to 14.7%. This difference was statistically significant (P < 0.027).

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### Discussion

In this study, the effects of laser irradiation on osteocyte viability and bone resorption were evaluated. The percentage of viable osteocytes (osteocyte viability) was shown to be significantly higher in the irradiated vs. the non-irradiated group. That more viable osteocytes were present in the immediate vicinity of the injury agrees well with the results of a study by Trellés & Mayayo (1987), who investigated the effects of He-Ne laser irradiation on bone fractures in mice. In that study, 24 joules were administered to one point and the fractures were treated every second day for three weeks. After 24 days, the laser-treated group showed an enhanced osteocyte activity around the fracture area, as well as an increased vascularization and a faster formation of bone tissue with a tighter mesh of trabeculae. In the control group, vascularization was poorer and more soft tissue was present. The osteocyte activity reported in the study was not evaluated in quantitative terms.

There is quantitative evidence in vivo of alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) activities following irradiation, which signals a significant twofold increase in bone repair (Barushka et al. 1995). During laser irradiation on days 5 and 6 after a standardized injury, active osteoblasts or pre-osteoblasts were present histologically at the site of the injury. Their presence was confirmed by an ALP peak on day 10. In the repair area, the ALP activity was twice as high as in the non-irradiated control group. This apparently augmented the cell proliferation rate, which resulted in an enhanced ALP production.

In the present study, the eroded surface area (ES) was 24.9% in the control group vs. 24.6% in the laser-treated group. Thus, resorptive processes were not set in motion in our model, although experimental evidence suggests a drop in TRAP activities at 6 days after injury (Barushka et al. 1995). Apparently, morphologic changes occur later than changes in biochemical parameters. In addition, bone that is exposed to low-power laser irradiation appears to be different biomechanically from non-irradiated bone after fracture healing. This was shown by Luger et al. (1998) who, after loading the irradiated bone, measured the force needed to re-fracture the bone at the same site. They found that, while more callus was formed in the non-irradiated group, the bone was weaker than in the irradiated group. This was explained by a more fibrocartilaginous and less ossified callus in the non-irradiated group and an immediate onset of ossification with faster callus resorption in the irradiated group.

Another special feature of our study was that low-power laser irradiation was applied to the very site of the injury almost at the time of surgery. This is much earlier than in comparable studies, which have addressed the effects of laser irradiation on osteoblasts and osteoclasts rather than the direct effects on cells that are stressed by injuries. After all, laser irradiation affects all cell populations. The potential mechanisms of action include a light-induced enhancement of ATP synthesis, as well as a stimulation of RNA and DNA synthesis (Karu 1989). All of these mechanisms potentially support the repair mechanisms that operate in stressed cells, but the precise regulatory mechanisms of bone repair by laser irradiation are still poorly understood.

Osteoneogenesis is determined both by the available local blood flow and by neovascularization. Various reports have been published that suggest that laser irradiation enhances osteogenesis through increased vascularization. Various reports have been published that suggest that laser irradiation enhances osteogenesis through increased vascularization.

### Table 1a. Osteocyte viability with and without laser irradiation

<table>
<thead>
<tr>
<th>Animal</th>
<th>LASER Side</th>
<th>Empty</th>
<th>Osteocyte</th>
<th>CONTROL % Viable</th>
<th>Side</th>
<th>Empty</th>
<th>Osteocyte</th>
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<td>277</td>
<td>101</td>
<td>26.7</td>
<td>R</td>
<td>236</td>
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<td>111</td>
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<tr>
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<td>80</td>
<td>45.2</td>
<td>R</td>
<td>139</td>
<td>61</td>
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<tr>
<td>No. 5</td>
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<td>200</td>
<td>48.4</td>
<td>R</td>
<td>234</td>
<td>171</td>
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<td>Mean</td>
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<td>41.6</td>
<td></td>
<td></td>
<td>94.8</td>
<td>34.4</td>
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<td>7.2</td>
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<tr>
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<td>10.5</td>
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### Table 1b. Total resorptive area (HT) with and without laser irradiation

<table>
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<th>CONTROL Side</th>
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<td>R</td>
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<tr>
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<td>12.1</td>
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<td>7.0</td>
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has a positive effect on inflammatory areas as well as on wound healing and neoangiogenesis, by promoting the production of endothelial cells [Mester et al. 1989].

In the present study, low-energy laser irradiation was found to increase the number of viable osteocytes in the irradiated bone. This suggests that more vital bone tissue is present in the irradiated area than in the non-irradiated area, and that wound healing can be expected to be accelerated.

In view of our data, low-power laser irradiation appears to produce highly reactive and vital peri-implant bone tissue. It can be expected that this would reduce healing times and speed up osseointegration of the implants. The potential effects of low-power laser irradiation on bone autografts are another important aspect. It may well be that the number of viable osteoblasts and osteocytes of both the host bone and the grafted bone can be increased by laser irradiation, so that the grafts would integrate more easily.

Future studies are needed to show whether low-power laser irradiation is indeed beneficial for implant integration in injured bone.

Résumé
Le but de cette étude a été d’examiner les effets de l’irradiation d’un laser à faible énergie sur les ostéocytes et la résorption osseuse au niveau des sites implantaires. Cinq babouins mâles de 6,5 ans ont été utilisés pour cette étude. Deux cavités ont été créées dans chaque crête iliaque pour la pose des implants. Les sites du côté gauche ont été irradiés par un laser à faible énergie 100 mW (690 nm) durant une minute (6 J) immédiatement après la mise en place des implants. Les sites du côté droit ont été utilisés comme groupes témoins. Les osseointégrations et le potentiel de regénération osseuse ont été examinés par des comptages d’ostéocytes dans l’implantat.

Les résultats indiquent qu’une exposition de 3 minutes à une puissance laser de 3 W permet une intégration osseuse plus rapide et un potentiel de regénération osseuse plus élevé que pour le groupe contrôle. Les sites de contrôle n’ont eu aucune pose des implants dans le groupe contrôle et l’insertion de quatre implants dans les sites implantaires. Les résultats montrant une intégration osseuse et un potentiel de regénération osseuse plus élevé que pour le groupe contrôle indiquent que la pose des implants dans les sites de contrôle a été réalisée.

References


