Stimulatory effects of low-power laser irradiation on bone formation *in vitro*

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ABSTRACT

The effects of low-power laser irradiation on bone formation *in vitro* were assessed. Osteoblast-like cells were isolated from rat calvariae of 21d rat fetuses. The cultured calvarial cells were irradiated with a low-power laser (830nm, 60mW) one time only or once daily for 21d at various energy doses (10.8-108 J/day). The number and the total area of mineralized bone nodules that had developed in the culture dish on day 21 were evaluated. DNA content, alkaline phosphatase (ALP) activity and the amount of extra-cellular collagen were also measured. Calcium and phosphorus in bone nodules were examined with an X-ray microanalyzer. Laser irradiation significantly increased the number and the total area of bone nodules in a dose-dependent manner. Cell proliferation and ALP activity in the irradiation group were higher in the early and middle culture periods, while the collagen content was higher in the middle and late periods compared with the control. Calcium and phosphorus were both higher in the irradiation group. These findings indicate that laser irradiation may play a principal role in stimulating differentiation of osteoblasts during the early stage of the culture, resulting in increased bone formation through acceleration of bone nodule maturation.

INTRODUCTION

The effect of stimulating bone regeneration with low-power laser irradiation has recently been studied[1-5]. There are some studies showing biostimulatory effects of low-power laser irradiation *in vivo* and *in vitro* such as stimulation of wound healing[6-8], fibroblast proliferation[9-12], chondral proliferation[13], collagen synthesis[14-17], and nerve regeneration[18]. These effects are all related to bone regeneration. However, the possible mechanisms of the laser biostimulatory effects on bone are still not fully understood. Although some experiments have been carried out to examine the effects of laser irradiation on bone regeneration, to date mainly radiological[1,5] and histological methods[3,4] have been used, and these *in vivo* experiments provide only clues about the mechanism, but lack standardized quantitative measurements of bone regeneration. Chen and Zhou[2] used biochemical and quantitative analysis to show that low-power laser irradiation significantly stimulated calcium, phosphorus and hydroxyproline contents in the newly formed bone in an experimental bone defect. However, only a partial estimation of bone regeneration was made in their study and the mechanism of bone regeneration can not be elucidated by these methods. It is therefore necessary to comprehensively study the effect of laser on bone using more convincing methods. Although *in vitro* experiments are generally more suitable for quantitative evaluation than *in vivo* experiments, there have been no suitable experimental models to evaluate bone formation *in vitro*. Recently, an experimental system was established in which rat calvarial cells form bone *in vitro*[19, 20], and this system may enable direct assessment of the action of laser irradiation on bone formation.

In the present study, we used this system to determine and quantify the effects of low-power diode laser irradiation on bone nodule formation from rat calvarial cells *in vitro*. Furthermore, the effects of laser irradiation on cell proliferation, alkaline phosphatase (ALP) activity and collagen content were quantified to determine the mechanism of the stimulatory effects from low-power laser irradiation on bone formation.

MATERIALS AND METHODS

Cell isolation and culture procedures

The cells were enzymatically isolated from calvaria of 21 d Wistar rat fetuses. Calvaria were dissected aseptically and the cells were isolated using sequential digestion with 0.3 % collagenase (Wako, Osaka, Japan). The procedures of the cell isolation and culture have been described by Bellows *et al*[19]. Cells obtained from the four sequential digestions were mixed and plated into T-75 tissue culture flasks (Falcon, Franklin Lakes, NJ) in minimal essential medium (α-MEM; Gibco, Grand Island, NY) containing 15 % FCS and antibiotics comprising 100μg/ml Penicillin G (Sigma Chemical Co., St. Louis, MO), 50 μg/ml gentamicin sulfate (Sigma), and 0.3 μg/ml Fungisone (Flow Laboratories, McLean, VA) supplemented with 50 μg/ml ascorbic acid (Wako), 10 mM Na β-glycerol-phosphate (β-GP, Wako) and 10^{-7} M dexamethasone (Wako). Cultures were maintained in a humidfied atmosphere consisting of 95% air/5% CO_2 at 37°C. After cells were cultured for 3 d in the flasks, they were detached with 0.05% trypsin (Gibco) in phosphate-buffered saline (PBS) for 5 min and