Effect of low-power helium-neon laser irradiation on 13-week immobilized articular cartilage of rabbits

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Influence of low-power (632.8 nm, Helium-Neon, 13 J/cm², three times a week) laser on 13-week immobilized articular cartilage was examined with rabbits knee model. Number of chondrocytes and depth of articular cartilage of experimental group were significantly higher than those of sham irradiated group. Surface morphology of sham-irradiated group had rough prominences, fibrillation and lacunae but surface morphology of experimental group had more similarities to control group than to sham irradiated group. There were marked differences between ultrastructure features of control group and experimental group in comparison with sham irradiated group. Low-power Helium-Neon laser irradiation on 13-week immobilized knee joints of rabbits neutralized adverse effects of immobilization on articular cartilage.

Keywords: Low-power Laser, Helium-neon laser, Immobilized articular cartilage, Laser irradiation

Diarthrodial joints form the most exceptional system, enabling both the movement and weight bearing of the limbs. The articular cartilage, covering the surfaces of bone ends, is composed of chondrocytes embedded in a matrix consisting of highly anionic, hydrated proteoglycans, and a network of collagen fibrils. However, immobilization of injured and inflamed joints has been the predominant treatment of choice in medical history. It is also part of the initial postoperative management for fractures and many surgical procedures of limbs. Although necessary to participate in management of diseases, immobilization often causes complications including joint stiffness and contracture, loss of tissue extensibility, muscle weakness and atrophy. Complications of long-term immobilization on articular cartilage have been reported. Akai et al. studied laser effect on cartilage change induced by joint immobilization. They reported that soft laser treatment has a possibility for prevention of biomechanical changes by short-term immobilization.

The aim of present study was to evaluate the effect of low-power He–Ne laser irradiation on the 13-week immobilized rabbit knee articular cartilage by scanning and transmission electron and light microscopic methods.

Materials and Methods

Adult male Dutch white rabbits (15) aged 16-20 week at the beginning of the experiment were used. They were fed a standard diet and tap water ad libitum. All rabbits were randomly assigned into experimental (Group E), sham–irradiated (Group S), and control (Group C) groups. Rabbits of group C were allowed to walk on four limbs without any casting and used for baseline studies. Rabbits of groups E and S were anaesthetized with ketamine hydrochloride (50 mg/kg, im) and diazepam (5mg/kg, im) and their right hind limbs were immobilized in 90° hip and knee flexion and full dorsiflexion at ankle joint by plaster of Paris. An open window was left anterior to the right knee joint. The casts were tied to the trunk, which prevented the weight bearing and restricted movements of the knee joint. All procedures were approved by Institutional Animal Use and Care Committee and Medical Ethic Committee. Rabbits of groups E and S were kept free for 1 week after

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immobilization. There were not any differences between groups S and E except low-power laser irradiation. Immobilized femoral condyles of rabbits of group E were exposed to low-power laser irradiation for the following 12 weeks. The laser (IR-2000, Iranian Atomic Energy Organization) used He-Ne laser type. (Laser source: 10 mW He-Ne laser tube; Wavelength: 632.8 nm; Frequency: continuous; Timing: 21.66 min/cm²/3 times a week; Spot area of the laser beam: 3.14 mm²).

One day after immobilization, rabbits of groups E, and S adapted to condition and walked on three limbs. All the rabbits were killed after 13 weeks by inhalation of chloroform in a closed space. Right femoral condyles of all groups were harvested and separated from each other by using a fine saw.

The medial condyles were chosen for light microscopic study and after fixation in formaline saline were decalcified by EDTA for six weeks. The condyles were embedded as in anatomical position in paraffin. The plane of sectioning was in parasagittal plane. Sections were stained with hematoxylin and eosine for chondrocytes counting, and alcian blue for calculating eva luated morphometrically in depth of articular cartilage. The condyles were evaluated morphometrically in 90 fields of view using a calibrated ocular on a Nikon light microscope adjusted to a magnification of 400 times. The edge of the calibration grid was 20 mm. The grid included 400 squares. The distance between the edges of neighbouring squares was 12.5μm. Distance from the surface of the articular cartilage to mineralized cartilage zone or (IV zone) (lower border of increased staining in Alcian blue slides) in 100 serial points with 10μ distance from each other were calculated.

Student’s t test was used to evaluate the differences between groups C and S, groups C and E, groups E, and S. Probability < 0.05 was significant.

The lateral condyles were from three rabbits chosen for scanning electron microscopic (SEM) study. The samples were rinsed in isotonic solution to remove blood and synovial fluid. The samples were then immersed in 2.5% glutaraldehyde in phosphate buffer (pH 7.3) and again immersed in 1% osmium tetroxid. Dehydration was carried out gradually in ascending concentrations of acetone. Final drying was accomplished using an Edward (England) freeze drier. All samples were then mounted on aluminum freeze stubs and sputter coated with gold. A defined territory of surface area of each condyle was examined and photographed using a ZEISS DSM-940 A SEM.

In order to study the ultrastructure of chondrocytes, 2 decalcified samples of lateral condyles were fixed and post fixed such as SEM method and were deca lified by EDTA. The samples were dehydrated by acetone and were embedded in TAB resin. The ultrathin sections were stained with uranyl acetate and lead citrate and examined in a ZEISS 900 A transmission electron microscope (TEM).

Data of group S were compared with group C in order to reveal probable effects of immobilization on articular cartilage. Data of group E were compared with groups C and S in order to indicate probable effects of low–power laser on articular cartilage.

Results

There were significant differences between number of chondrocytes of control and sham-irradiated group and number of chondrocytes of sham-irradiated and experimental groups (Table I). There were also significant differences between depths of articular cartilage of sham irradiated and control groups and depth of articular cartilage of sham-irradiated and experimental groups (Table I).

Crests and spines were observed on the surface of articular cartilage of the control group, and between them another fine process occupied the surface of articular cartilage (Figs 1a and 2a). There were tubercles and hemispheres like prominences, and between them many fine crests and lacunae existed in the same region of articular cartilage of sham-irradiated group (Figs 1b and 2b). Surface of articular cartilage was not smooth and there were many processes such as non straight crests and tubercles of experimental group, but they were relatively smaller than the processes of sham-irradiated group (Figs 1c and 2c).

<table>
<thead>
<tr>
<th>Table 1—Number of chondrocytes and depth (μm) of articular cartilage of study groups</th>
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<tbody>
<tr>
<td>[Values are mean ± SD]</td>
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<tr>
<td>Number of chondrocytes:</td>
</tr>
<tr>
<td>Control: 107.6±7.8b</td>
</tr>
<tr>
<td>Sham-irradiated: 92.5±4</td>
</tr>
<tr>
<td>Experimental: 117.8±8.5b</td>
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<tr>
<td>Depth of articular cartilage:</td>
</tr>
<tr>
<td>Control: 283.7±12.5b</td>
</tr>
<tr>
<td>Sham-irradiated: 258.7±15</td>
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<tr>
<td>Experimental: 308.7±25b</td>
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</tbody>
</table>

P values: *< 0.01 between number of chondrocytes of the control group and sham-irradiated group, †< 0.001 between number of chondrocytes of sham-irradiated group and experimental group, *< 0.01 between depth of articular cartilage of control group and sham irradiated group, †< 0.05 between depth of articular cartilage of sham irradiated group and experimental group.
Figs 1, 2—Scanning electron micrographs of surface of articular cartilage of femoral condyle of (a) control, (b) sham-irradiated, and (c) experimental groups [C=crest, Sp=spine, Sm=small prominence, Tu=tubercle like prominence, F=fine processes, He=hemisphere like prominence, L=lacuna, T=tubercle. Figs 1a, b, c x 500, Figs 2a, b, c x 3000]
The TEM findings in chondrocytes of deep zone of control (Fig. 3a) and experimental group (Fig. 3c) were: marked prominent filopodia in cell membrane; and euchromatin nucleus. Filopodia of sham-irradiated group (Fig. 3b) was less prominent and was fewer than other groups, at the same time nucleus of sham-irradiated group was heterochromatin.

Discussion

The present results showed that immobilization of the limb significantly decreased depth of immobilized articular cartilage of femoral condyle and the number of its chondrocytes. Olsen et al. reported that immobilization of articular cartilage showed thinning of cartilage. The probable reason for decreased number of chondrocytes and depth of articular cartilage in present study are decreased weight bearing and restricted movements of immobilized knee joint.

Haapala et al. reported that immobilization significantly decreased the thickness of uncalcified articular cartilage and safranin O staining intensity of the uncalcified articular cartilage. Haapala et al. concluded that when applying these data to human clinical practice, it seems to be important to reduce duration of the immobilization periods as much as possible. On the other hand the present study showed that low-power He-Ne laser irradiation to immobilized articular cartilage of femoral condyles during immobilization period restored the number of chondrocytes and depth of articular cartilage as compared to the control group. Vanwanseele et al. reported that knee cartilage of spinal cord-injured patients displays progressive thinning in the absence of normal joint loading and movement. It is recommended to apply low-power laser in a situation such as above-mentioned case in order to prevent thinning the articular cartilage.

The effect of low-power laser on glycosaminoglycans of articular cartilage is unknown, further investigations will define it. Present study showed many tubercles, hemisphere like prominences, lacunae and fine ridges on the surface of immobilized articular cartilage of knee joint. Fine ridges may reflect fibrillation of the articular cartilage. Burr et al. examined tibial articular cartilage from cast-immobilized rabbits whose quadriceps were electrically stimulated were compared with those from cast-immobilized rabbits without muscle stimulation. Cartilage from non-
stimulated rabbits showed evidence of deep fibrillation and loss of safranin o metachromasia and large area of cavitation and cartilage erosions. Cartilage from cast-immobilized muscle-stimulated rabbits appeared most similar to normal cartilage.

Burr et al. described that intermittent joint loading stimulates the formation and circulation of nutrient-rich synovial and interstitial fluids, which both nourish and lubricate the cartilage and which may transmit to the chondrocyte hydrostatic pressures important for cell function. TEM preparation of the chondrocytes revealed that there were no marked differences between control chondrocytes and laser treated chondrocytes; which confirmed biostimulatory effect of laser on immobilized chondrocytes. In view of positive effects of low-power laser on fracture healing and immobilized knee articular cartilage of rat for four weeks, it seems that low-power laser irradiation reinforces formation and circulation of synovial and interstitial fluid and the improved chondrocyte nourishment and lubrication in addition to direct biostimulatory effect of the low-power laser on chondrocytes. Further, cellular, biochemical and molecular biology research with low-power lasers in vivo and in vitro is needed to clarify exact mechanism of action of low-power lasers.

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References