

Mechanoreceptors and length of the patellar ligament after Ho-YAG laser treatment

A long-term follow-up in rabbits

Toshiaki Takahashi, Yukihiisa Wada, Masanobu Tanaka, Norio Yamanaka and Hiroshi Yamamoto

Department of Orthopaedic Surgery, Kochi Medical School, Oko-cho, Nankoku, Kochi, Japan, 783-8505. takahast@kochi-ms.ac.jp
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ABSTRACT – We studied the shortening and the number of mechanoreceptors in the patellar ligament up to 18 months after Ho-YAG irradiation of the ligament's surface in 35 rabbits. The ligaments shortened an average 13% immediately after irradiation. After treatment, we divided the rabbits into a mobilized or immobilized group. At 2 weeks and 12 months after treatment both groups showed no shortening of the ligament, as compared to the intact ligament while that in the mobilized group had elongated at 12 months.

Fewer Pacinian and Ruffini corpuscles were found in the irradiated ligaments than in the intact ones at 2 weeks after treatment, but we found no difference between irradiated and intact ligaments at 18 months after treatment.

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Recently, several kinds of laser treatment have been used experimentally and clinically because laser energy is thought to shrink soft tissue effectively (Moreira et al. 1993, Vangsness and Smith 1995, Kilkelly et al. 1996). In clinical work, it has been regarded as a new and less invasive treatment of glenohumeral instability to reduce capsular volume and joint laxity (Thabit 1994, Pullin et al. 1997). The amount of ligament shortening induced by investigators has varied greatly (Hayashi et al. 1995, Naseef et al. 1997, Schaefer et al. 1997). To our knowledge, no long-term follow-up concerning the length of the ligament after irradiation has been reported. It has not been

shown whether shortening after irradiation can be maintained, or if it is affected by immobilization/mobilization of the joint.

Histological studies of laser-treated ligaments have reported different shortening ratios after short follow-up time (Schober et al. 1986, Vangsness and Smith 1995, Naseef et al. 1997). It has been clearly shown that capsules and ligaments contain mechanoreceptors (Wrete 1949, Kennedy et al. 1982, Zimny et al. 1986, Wada et al. 1997), which provide information concerning joint position and interactions between the joint and muscles (Barrack et al. 1988). These receptors increase the proprioception that provides static joint position awareness (Ruffini corpuscles), and detect displacement and speed of movement of the joint (Pacinian corpuscles).

We studied shortening of the patellar ligament in rabbits immediately after irradiation, and 12 months and the effect of immobilization and the number of mechanoreceptors after irradiation.

Animals and methods

In this study, we used 35 5–6-month-old Japanese white rabbits having a mean weight of 3.1 (2.4–3.3) kg. Anesthesia was induced by giving phenobarbital intramuscularly (2–3 mL) and intravenously (0.2–0.3 mL). The rabbits were placed in a supine position and a longitudinal median incision exposed the patellar ligament. Both sides of

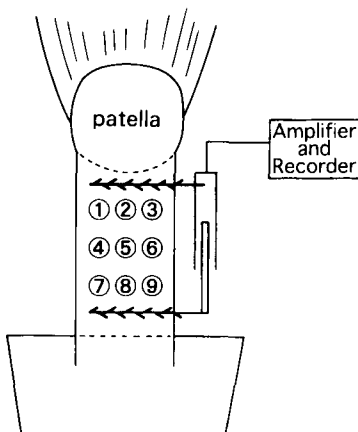


Figure 1. Irradiation spots of the ligament; proximal, middle, and distal rows of 3 spots each (total, 9 spots) at 3 seconds each (total 27 seconds).

the parapatellar ligaments were released.

Ho-YAG Laser (Coherent Co. Ltd, Palo Alto, CA) irradiation was used in a dose of 0.5 J, 5 pulses/sec, 2.5W. A laser probe with a 550 mm fiber and a tip of 0 degrees was placed at a distance of 3 mm from the surface of the ligament. We irradiated the ligament in proximal, middle, and distal rows at 3 spots each (total 9 spots) for 3 seconds each (total 27 seconds) with 70 J (Figure 1).

Length of the ligament

To examine the immediate effect of irradiation, we used biological tissue displacement measurements required for the calculation of strain, employing a differential variable reluctance transducer (DVRT-100; Microstrain, Inc., Burlington, Vermont) (Beynon et al. 1992). The long axis of the DVRT-100 was oriented colinearly from one side and the fixation needles were inserted in the proximal and distal ends of the patellar ligament which had an initial length of 13–14 mm in an extended position of the knee ($n = 28$). We continuously recorded the length of the ligament, using a DVRT-100, starting from before irradiation until the length stopped changing for at least 10 minutes. The ratio of the shrinkage of the ligament before to that after irradiation was assessed quantitatively. The relative changes in displacement of the patellar ligaments recorded by the DVRT were used to calculate the shrinkage ($E = [(L_0 - L) / L_0] \times 100$). L was the length of the DVRT at a particular point, and L_0 was that before irradiation.



Figure 2. Marking wires on the proximal and distal sites of the patella at 2 weeks after operation.

Then, we examined the effect of immobilization after irradiation in a long-term follow-up. Markings were made at the proximal and distal sites of the ligament, using a wire of small diameter. The distances between the markers were measured (14–15 mm), and the skin was then closed. On the contralateral side, a sham operation was performed, with the same incision. The ligament was exposed and marked, as described above. The rabbits (groups I and II) were fed for 18 months after the operation. In group I ($n = 7$), they were allowed to move in a cage without immobilization. In group II ($n = 7$), their irradiated legs were immobilized in a plastic cast at 45 degrees of flexion for 2 weeks.

In both groups, lateral radiographs of the legs were taken at about 90 degrees of flexion over at 2 weeks (Figure 2) and at 12 months after operation. The length of the ligament was measured as the distance between the proximal and distal marking wires, using a micrometer. Radiographic magnification was calculated at 1.1 from comparison with the actual length.

Mechanoreceptors in the ligament

The rabbits were killed at 2 weeks ($n = 7$) or 18 months after surgery ($n = 6$) with an injection of 10 mL phenobarbital intravenously. The patella, patellar ligament, and proximal tibia were harvested en bloc and immediately preserved at -20°C in

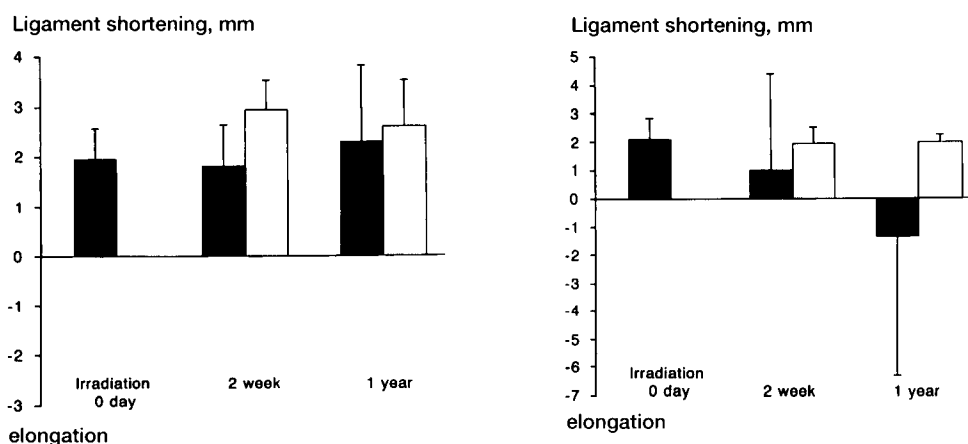


Figure 3. Ligament length after irradiation with (left) or without immobilization (right). ■ irradiated and □ control.

a freezer, pending examination. The preserved limbs were thawed at room temperature, and the soft tissue of the bone and surrounding the patellar ligament were removed.

The middle of the ligaments at 15 mm was stained using a modified gold chloride method (Gairns 1930, Zimny et al. 1986). Specimens were frozen and sectioned with a sliding microtome at 100 μ m. Serial sections were studied with light microscopy. We counted the Pacinian and Ruffini corpuscles and also determined the total number of each type of sensory nerve ending per ligament in the irradiated and control ligaments (Wada et al. 1997).

Statistics

The lengths of the ligaments were compared statistically, with the Student's paired t-test. The Wilcoxon signed-ranks test was used to analyze differences between the irradiated and intact (non-irradiated) ligaments and thereby assess the number of mechanoreceptors. P-values < 0.05 were considered significant.

Results

Lengths of the ligaments

The mean shortening of ligaments was 15.4% (SD 7.4) immediately after radiation, and these recovered by a mean of 2.4% (SD 1.5) within 10 minutes ($n = 28$).

To evaluate changes in the lengths of the ligaments postoperatively, we measured the distance

between two marking wires intraoperatively and postoperatively on lateral radiographs of 17 rabbits. The findings could not be evaluated in 3 of them because 1 rabbit developed an abscess in a knee after treatment, another died of severe diarrhea 5 days after surgery, and a proximal dislocation of the patella occurred in the irradiated ligament occurred still another rabbit.

We compared the lengths of the ligaments in the nonimmobilized group I ($n = 7$) and the immobilized group II ($n = 7$) immediately after surgery, at 2 weeks and again 12 months after treatment. In group I, the irradiated ligament had shortened, on average 2.1 mm in 10 minutes, 1.0 mm in 2 weeks, but had elongated, on average, 1.3 mm in 12 months after the treatment, while the intact ligament had shortened, on average, 1.9 mm in 2 weeks, and 2.0 mm in 12 months. We found no difference between the irradiated and intact ligaments at 2 weeks after the treatment ($p = 0.3$). The nonimmobilized irradiated ligament was longer than the intact ligament at 18 months after the treatment ($p = 0.05$).

In group II, the irradiated immobilized ligament had shortened, on average, 2.0 mm in 10 minutes, 1.8 mm in 2 weeks, 2.3 mm in 18 months after the treatment, while the intact ligament had shortened, on average, 2.9 mm in 2 weeks, and 2.6 mm in 12 months (Figure 3). In the immobilized group, no significant difference in length was seen between the irradiated and intact ligaments at 2 weeks ($p = 0.06$) or 18 months ($p = 0.9$) after the treatment, and irradiation had had no shortening effect.

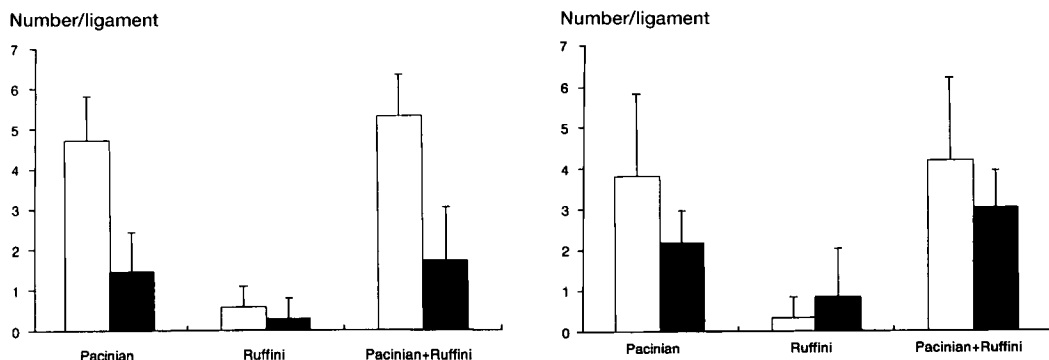


Figure 4. Number of mechanoreceptors in the irradiated ligament at 2 weeks after operation (left) and at 18 months after operation (right). ■ irradiated and □ control.

Mechanoreceptors in the ligament (Figure 4)

2 weeks after treatment ($n = 7$), we found an average of 4.7 Pacinian corpuscles in the intact patellar ligaments, and 1.4 in the irradiated ($p = 0.02$), and of 0.6 Ruffini corpuscles in the intact, and 0.3 in the irradiated ligaments ($p = 0.2$). The total number of Pacinian and Ruffini corpuscles in the irradiated ligaments was less than that in intact ligaments ($p = 0.02$). However, at 18 months, the total number of Pacinian and Ruffini corpuscles averaged 3.0 in the irradiated, and 4.2 in the intact ligaments ($n = 6$). We found no differences between the irradiated and intact ligaments ($p = 0.3$) in the number of Pacinian corpuscles (average 3.8 in the intact, and 2.2 in the irradiated ligaments ($p = 0.2$) or in the number of Ruffini corpuscles (average 0.3 in the intact, and 0.8 in the irradiated ligaments ($p = 0.4$)).

Discussion

Naseef et al. (1997) showed that the use of Ho-YAG laser in vitro caused shrinkage of the bovine calf knee capsule at 60 °C, and correlated with the duration of thermal exposure. Maximal shrinkage of 50% in length occurred at and above 65 °C with thermal exposures of 1 minute or more. They also found that thermal shrinkage correlated with the denaturation of collagen fibers and depended on time and temperature. Capsule shrinkage occurred with maximal shortening along the major axis of collagen fibers anisotropically.

Hayashi et al. (1995) reported that the length of an original rabbit capsular model was reduced by 35% after a dose of 300 J/cm² in an unconstrained

condition, and tissue shrinkage increased with a reduction in tensile stiffness. In contrast, Schaefer et al. (1997) described the length, stiffness, and structures of the connective tissue after irradiation with Ho-YAG of a rabbit patellar tendon model which had not been immobilized postoperatively, and found an average of 6.6% tendon shrinkage. However, at 8 weeks, the length of the ligament had increased beyond its original length and stiffness was 19% less than that on the contralateral side. We found a 13% shortening of the ligament on the day of operation.

There is no report on the length of the ligament with immobilization after irradiation. We found that intact patellar ligaments had shortened at 2 weeks and 12 months after treatment. We think that the release of both parapatellar areas caused the ligament contracture. 12 months after irradiation, the ligament that had not been immobilized had become longer than the intact ligament. Immobilization of the knee prevented the ligament from stretching after irradiation at the long-term follow-up, but had not prevented it from shortening after irradiation. The expected effect of immobilization of the knee after irradiation is the prevention of excessive ligament tension during exercise and a reduction in stress caused by weight bearing. Therefore, we thought that immobilization after irradiation for several weeks was needed to prevent loading that would shrink the capsular ligament if thermal therapy was used.

Hayashi et al. (1999) found, on histological examination, substantial thermal changes in collagen and fibroblasts in irradiated tissues. Capsular tissue removed immediately after irradiation

showed hyalinized collagen and pyknotic cells. At 14 days, they found fibroblastic and capillary responses, and at 3 months, a fibrous joint capsule with a slight increase in cellularity. In our study, fewer mechanoreceptors were seen in the irradiated ligaments than in the intact ones at 2 weeks after treatment, but at 18 months no statistically significant difference could be detected. Therefore, although mechanoreceptors seemed to regenerate after irradiation in a long-term follow-up study, we found no shortening of the ligament even following immobilization of the knee after irradiation.

Recently, some authors have reported cases of spontaneous rupture and disappearance of the cruciate ligament after thermal treatment (Perry and Higgins 2000, Sekiya et al. 2000). Thus, such treatment should not be used to shrink the ligament and we should be wary of the risk of stretching the irradiated ligament. The kind of laser and conditions of irradiation, such as the amount of energy and method of irradiation, should also be evaluated in future studies.

Radiofrequency energy is viewed as a relatively noninvasive approach that can be used to shrink the capsule. Radiofrequency units offer several advantages since they are less expensive, safer and smaller than laser units (Lopez et al. 1998). The effects of this energy on the mechanoreceptors of the capsular ligament should also be studied.

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