Observations on the Healing Process of the Medial Patellar Ligament after Desmotomy in Cattle

Yoshiharu OKAMOTO, Kanjuro OTOMO, and Toshio KOIKE

Department of Veterinary Surgery, Faculty of Veterinary Medicine, Hokkaido University, Kita-18, Nisi-9, Kita-ku, Sapporo, Hokkaido 060, Japan

(Received 4 September 1985/Accepted 25 June 1986)

ABSTRACT. Macroscopic and microscopic observations were made on the healing process of cut ends in the medial patellar ligaments during a period from 7 to 201 days after desmotomy in 24 normal cattle. Lameness was not seen in any of the cattle after the operation. Adherence at the severed site was not observed in any of the samples. The connective tissue already had bound up the cut ends 7 days after the operation. The repair tissue was grossly distinguishable from the ligament and surrounding connective tissue in all samples. On the basis of gross observations, the authors divided the repair period into three stages. In the early stage, there was no significant increase in the distance between the ends of the cut ligament since the operation, in the intermediate stage the distance tended to increase, and in the last stage the distance decreased. The mean distances observed in each of the stages were 19 mm ± S.D. (range 17 to 23 mm), 23 mm ± S.D. (range 13 to 33 mm) and 14 mm ± S.D. (range 7 to 27 mm), respectively. Microscopic observations, demonstrated loose connective tissues in the gap during the early stage were loose connective tissues. Thereafter, fibroblasts closely aligned in longitudinal direction in the area closest to the cut ends. This arrangement progressed gradually towards the center of the gap. In the sample taken 201 days after operation, however, the cell density in the gap was identical to that of the ligament tissue. These results suggested that connective tissues in the gap gradually changed to ligament tissue. —KEY WORDS: cattle, healing process, patellar ligament.

In clinical veterinary medicine, desmotomy of the medial patellar ligament has generally been used for the treatment of the upward dislocation of patella. There are several reports based on clinical observations describing recovery process in treated animals. The process of healing and adherence at the cut sites, however, have not been reported.

Reports on tendon and ligament healing can be classified according to the origin of the reparative tissues. One theory is that the tendon and ligament are repaired by reparative cellular growth derived directly from the ends of the tendon and ligament [1, 2, 3, 4, 10, 13, 14, 15]. Another theory is that the tendon and the ligament are repaired solely by cellular activity of the surrounding tissues [9, 16, 17, 18, 20, 23]. Recently, the intrinsic capacity of the tendon for repair has been confirmed by experimental studies in vivo [6, 21] and in vitro [8, 11, 12, 19]. Furthermore, early mobilization after operation has been reported to promote this intrinsic capacity and also to decrease adherence [3, 5, 7]. However, these theories are based mainly on observations on sutured wounds of tendons and ligaments within the tendon sheath during a short period of time. This paper describes the effects of medial patellar desmotomy in cattle, and gives insights into the repair process of the ligaments on the basis of a long term study.

MATERIALS AND METHODS

Animals: Twenty-four cattle (one bull and 23 cows) were used. Of these, 22 were
Holstein, while two were Gernsey.

Procedure for examination: Desmotomy of the medial patellar ligament was performed under a local anesthesia on each cattle. Immediately after the desmotomy, the ligaments retracted, leaving a gap of about 2 cm which could be pulped over the skin after the completion of the operation. The animals were kept for different periods postoperatively and then were sacrificed to obtain samples of the cut ligaments. Seven cattle were kept for 7 to 24 days, ten for 27 to 42 days, and another group of seven for 44 to 201 days.

Macroscopic observations: Macroscopic observations were made on each sample. The distance between the ends of the cut ligament were measured and the longitudinal sections were observed.

Microscopic observations: Following macroscopic observation, 23 samples were fixed in 10% formalin. Longitudinal sections of the fixed ligament were embedded in paraffin, sectioned at 4-5 μm in thickness and stained with hematoxylin and eosin (HE). The tissue between the severed ends of the ligament were examined histoplanimetrically using an ocular plotting micrometer.

RESULTS

Clinical findings: Immediately after the patellar ligament had been severed, there was no visible change in the gaits. Lameness was not seen thereafter, and general conditions were normal.

Distance between the ends of the cut ligament: The results are shown in Fig. 1. The mean distance was 19 mm ± S.D. (range 7 to 33 mm). The distance of the gap of the seven samples taken 7 to 24 days after operation did not change since the operation, and this period was designated as the early stage. With regard to ten samples taken 27 to 42 days after operation, there was a tendency for the distance of the gap to increase and this period was designated as intermediate stage. The distance of the gap in the seven samples taken 44 to 201 days after operation shortened, and this period
Table 1. Histological findings

<table>
<thead>
<tr>
<th>No.</th>
<th>Days</th>
<th>Vascula-</th>
<th>Fatty</th>
<th>Eosinophils</th>
<th>Neutrophils</th>
<th>Histiocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>75-122</td>
<td>7</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>75-114</td>
<td>7</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>73-131</td>
<td>16</td>
<td>+</td>
<td>++</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>67-022</td>
<td>24</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>70-028</td>
<td>7</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>72-017</td>
<td>7</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>83-049</td>
<td>7</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>68-018</td>
<td>27</td>
<td>++</td>
<td>-</td>
<td>±</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>81-054</td>
<td>28</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>82-046</td>
<td>31</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>83-053</td>
<td>31</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>78-059</td>
<td>32</td>
<td>++</td>
<td>++</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>78-060</td>
<td>35</td>
<td>++</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>71-014</td>
<td>38</td>
<td>++</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>80-048</td>
<td>40</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>81-057</td>
<td>41</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>80-049</td>
<td>42</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>66-023</td>
<td>44</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>82-047</td>
<td>7</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>71-018</td>
<td>64</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>69-016</td>
<td>67</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>76-175-4</td>
<td>91</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>69-013</td>
<td>201</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 2. The nuclei of fibroblasts are oval or circular in shape, and are not uniform in their size. Fatty cells, fibroblasts and collagen fibers arranged randomly. HE stain. ×600.
was designated as the last stage. The mean distance of the gaps in each stage was 19 mm ± S.D. (range 17 to 23 mm), 23 mm ± S.D. (range 13 to 33 mm) and 14 mm ± S.D. (range 7 to 27 mm), in the early, intermediate and last, respectively.

Macroscopic findings: 7 days after operation, connective tissues had already invaded the space between the cut ends of the ligament and bound up the severed ends. In the early stage, the ends of the cut sections were distinct and the repair tissue was distinguishable from the ligament and also from the surrounding connective tissue. In the intermediate and last stages, the ends of the cut sections became progressively indistinct, but the repair tissue remained clearly distinguishable from the surrounding con-
connective tissue. In all samples, there was no adherence between the repair tissue and the surrounding tissue. The connective tissue fibers of the repair tissue arranged in a longitudinal direction. Particularly in the sample taken 201 days after operation, the repair tissue was macroscopically distinguished from the ligament tissue, but the arrangement and density of the connective tissue fiber was similar to that of the ligament tissue.

**Microscopic findings:** Light microscopic findings are shown in Table 1. In the early stage, connective tissues containing many fatty cells were seen in the space between the ends of the cut ligament. Vascularization, eosinophils and histiocytes were also observed. The nuclei of the fibroblasts in these connective tissues were oval or circular in shape, and their size was not uniform (Fig. 2). Vascularization was seen remarkably in the samples taken 27 to 38 days after operation, but decreased gradually with time. Fatty cells, eosinophils and histiocytes also gradually decreased. In the intermediate and last stages, fibroblasts in the connective tissue between the severed ends of the ligament gradually aligned in a longitudinal
direction. The shape of the cell nuclei elongated gradually in the same direction and became oval shaped (Fig. 3). Especially in the sample taken 201 days after operation, vascular density was small, and fatty cells, eosinophils, neutrophils and histiocytes were no longer seen. However, collagen fibers increased. In this sample, the connective tissue in the gap resembled the ligament tissue as to the shape and density of the cells as well as to the arrangement of collagen. These two tissues were not easily distinguished from each other (Fig. 4).

In most of the samples, fibroblasts in the connective tissue near the cut ends aligned up closely in a longitudinal direction. In the early stage, this particular arrangement of fibroblasts was first observed in the region closest to the cut ends, and gradually progressed towards the center of the tissues between the gap. In 13 samples, in which longitudinally aligned fibroblasts were distinct, the length and cell density of the region with longitudinally aligned fibroblasts was measured using an ocular plotting micrometer. The field visible under the ocular plotting micrometer was 0.0625 mm².

The length of the region with longitudinally aligned fibroblasts is shown in Fig. 5. The average length in the early, intermediate and last stages was 1.4 mm ± S.D. (range 0 to 3.5 mm), 3.8 mm ± S.D. (range 3.3 to 4.5 mm) and 4.3 mm ± S.D. (range 3.8 to 4.5 mm), respectively.

Typical examples of cell density in each stage are shown in Fig. 6. The density was identical in both of the cut ends. In the early stage, the cell density was highest in the area nearest to the cut end, but gradually decreased towards the center of the gap. The highest cell density was not greater than 100 cells/0.0625 mm². In the intermediate stage, the cell density increased from the area nearest to the cut end towards the center of the gap, and then suddenly decreased. The highest cell density was greater than 200
cells/0.0625 mm². In the last stage, the cell density was generally the same throughout the region, but was smaller than that in the intermediate stage.

DISCUSSION

Ever since the medial patellar desmotomy was used for the treatment of upward dislocation of the patella, the clinical usefulness of this technique has been reported. However, cattle with no major disturbances of motility were easily claimed healed because cattle are not required to move, and healing process of this technique was not explored.

A considerable amount of research work has been carried out to examine the healing process of injured tendon and ligament as well as adherence. Recently, the intrinsic capacity of the tendon for repair has been confirmed. Mobilization has been pointed out by many investigators to be useful in the healing process [1, 3, 5, 6, 7]. These theories, however, are based mainly on sutured wounds of tendons and ligaments within the tendon sheath during a short observation period.

In this study, we examined the effect of medial patellar desmotomy in the cattle. In addition, we made observations on the healing process of the ligament cut and left apart for a long period as a model to investigate the repair mechanism of the tendon and ligament.

After the operation, the gait of the cattle did not show any visible changes. This finding was compatible to previous reports [22]. In all samples, there was no adherence between the repair tissues and the surrounding tissues.

According to gross observations, the author divided the repair period into three stages. In the early stage, the distance between the ends of the cut ligament did not change much compared with that shortly after the operation. In the intermediate stage, the distance tended to increase. In the last stage the distance shortened. The extension of the distance in the intermediate stage was due to tension from movement. The shortening of the distance in the last stage suggested newly formed fine collagen fibers which changed to tissues similar to the ligament tissue at the cut sites.

In interpreting the above results, it is unlikely that the loose connective tissue in the gap during the early stage were derived only from cellular proliferation from the endotenon and epitelenon of the ligament. Bridging of the cut ends, tight binding of the loose tissue to the ends of the sections, and distinct division between the surrounding tissue and loose tissue were observed in the sample obtained as early as 7 days after the operation. These findings suggest that the surrounding tissues invaded the gap and that the gap was constantly under mobilization.

From microscopic observations, the tissues in the gap were found to be loose connective tissues in the early stage, containing randomly arranged fatty cells, fibroblasts and collagen fibers. Thereafter, fibroblasts closely aligned in a longitudinal direction, starting at the region closest to the cut ends and progressed gradually to the center of the tissue in the gap. This observation was supported by a gradual increase in the length of the region consisting of longitudinally aligned fibroblasts. The difference in the density of the fibroblasts suggested that the cells actively divided during the early to intermediate stages, and then activity gradually decreased in the last stage. The shape of the fibroblast nuclei in the gap changed gradually to an elongated oval shape in the longitudinal direction. Particularly, in the sample taken 201 days after operation, cell density of the connective tissue in the gap was identical to that of the ligament tissue.

In the present study, it became apparent
that the gap which was about 2 cm long immediately after the ligament had been severed, repaired without adherence. We concluded that the distance shortened to 7 mm in the sample taken 201 days after operation, because the connective tissues in the gap gradually changed to ligament tissues at the cut sites. These findings suggested that medial patellar desmotomy which is a treatment of upward dislocation of the patella in cattle does not cause functional disturbance. It is estimated that the distance will become still short in further observation.

We expect that these observations will serve as basics when improving suturing techniques of tendons and ligaments as well as when treating those injured structures in the clinical veterinary medicine.

ACKNOWLEDGEMENTS. We are indebted to Professor Y. Fujimoto and Associate Professor H. Sato of Hokkaido University for their advice in this experiment.

REFERENCES

要約

牛の内側膝蓋靭帯切断術後の治癒過程の観察：岡本芳晴・大内秀一郎・小池寿男（北海道大学獣医学部家畜外科学講座）—24頭の正常牛の内側膝蓋靭帯を切断し、その切端間隔を術後7〜201日の期間、肉眼的および組織学的に観察した。手術直後から跛行を示さず、全例において切断部位の壊着はみられなかった。肉眼的観察から、修復時期を3期に区分すると、初期には切端間隔がほとんど一定（19mm±S.D.（17〜23mm））で、中期には間隔が伸長する傾向（23mm±S.D.（13〜33mm））がみられ、後期には短縮（14mm±S.D.（7〜27mm））した。切断端間を満たす組織は、初期では粗鬆な結合組織であったが、その後切端端部で線維芽細胞が長軸方向に密に配列し、次に切断端間中央方向へ進行した。術後201日には、間隔を埋める組織は細胞密度、線維線維の走行などにおいて靭帯組織と同様であった。