Ultrasoundographic and histologic evaluation of medial and middle patellar ligaments in exercised horses following injection with ethanolamine oleate and 2% iodine in almond oil

Linda M. Van Hoogmoed, DVM, PhD; Dalen W. Agnew, DVM; MaryBeth Whitcomb, DVM; Dallas W. Hyde, PhD; Melinda H. MacDonald, DVM, PhD; Jack R. Snyder, DVM, PhD

Objective—To describe the ultrasonographic and quantitative histologic effect of injecting 2% iodine in almond oil (IAO) and ethanolamine oleate (EO) in the medial and middle patellar ligaments of horses and to determine whether a difference in response exists between IAO and EO treatment.

Animals—10 healthy horses.

Procedure—In 5 horses, the medial and middle patellar ligaments of 1 limb were injected with EO, whereas IAO was injected in the medial and middle patellar ligaments of another 5 horses. Ultrasonographic evaluation was performed on the experimental and control limb before injection of IAO and EO and prior to euthanasia to determine cross-sectional area and evaluate fiber pattern. The patellar ligaments were harvested 2 weeks after injection and examined histologically to evaluate the inflammatory response, fibroplasia, and chondroid metaplasia.

Results—Injection of the patellar ligaments with IAO resulted in a greater increase in cross-sectional area on ultrasonography than EO. Both agents caused a decrease in echogenicity of the ligament. Histologically, significantly greater infiltration of inflammatory cells and fibroplasia developed after injection with IAO, compared with EO. Both agents resulted in significantly greater fibroplasia relative to control specimens.

Conclusions and Clinical Relevance—Injection of the medial and middle patellar ligaments with IAO induces more severe inflammation and fibroplasia than EO. Maturation of the inflammatory and fibrous response may contribute to resolution or attenuation of upward fixation of the patella by subsequent stiffening of the ligaments. (Am J Vet Res 2002;63:738–743)

Upward fixation of the patella is a condition in which the patella becomes fixed, transiently or more persistently, over the medial trochlear ridge of the distal end of the femur and is not reduced by contraction of the quadriceps. Upward fixation of the patella in horses is thought to result from anatomic abnormalities of the stifle joint, poor confirmation, or improper conditioning. In most instances, fixation is intermittent with the limb briefly locked in extension but quickly released, creating a catch in the stride. Even a brief hesitation in stride can have severe performance-limiting effects. Surgically, horses with upward fixation of the patella have been managed by desmotomy of the medial patellar ligament, although there are concerns this procedure may lead to stifle joint instability or incongruity. A modification of this procedure that involves cutting the aponeurosis of the sartorius and gracilis muscles has also been described. However, surgical correction has been associated with postoperative complications. In a study evaluating the effect of medial patellar ligament desmotomy in clinically normal horses, pathologic changes in the articular cartilage and fragmentation of the apex of the patella were detected 3 months later, supporting the theory that this procedure induces biomechanical changes in the stifle joint. In another study, the surgery resulted in an abnormal position of the patella that was attributed to altered joint stability and led to fracture of the patellar apex. Therefore, when intermittent fixation of the patella is diagnosed, conservative treatment involving conditioning of the quadriceps, especially in young or debilitated horses, is advocated. Because the patellar ligaments are the fibrous insertions of the quadriceps femoris and biceps femoris muscles, increasing the muscular tone via conditioning exercises is thought to subsequently tighten the ligaments and facilitate normal tracking of the patella in the trochlear groove. Injection of irritants in the patellar ligaments has also been recommended in lieu of surgical procedures to increase the fibrosis of the ligaments with subsequent thickening and potential shortening. Clinically, the irritants are injected in multiple sites within the medial and middle patellar ligaments cautiously to ensure the irritant is deposited in the ligament and not subcutaneously or intra-articularly. Of the agents available, 2% iodine in almond oil (IAO) is most commonly used in horses that catch their stifle and is injected into the medial and middle patellar ligaments. A previous investigation in ponies found that 1.9% iodine in peanut oil increased the diameter of the patellar ligaments. Another substance that has been used is ethanolamine oleate (EO), a sclerosing agent primarily used in humans to induce vascular thrombosis by irritating the intimal endothelium. Ultimately, vessel wall fibrosis develops, and the vessel becomes occluded. Although the
injection of patellar ligaments is common in horses, to our knowledge no ultrasonographic and quantitative histologic studies have been performed to evaluate the effect of these substances. The objective of this study was to describe the ultrasonographic and histologic effect of injecting IAO or EO in the medial and middle patellar ligaments of horses and to determine whether a difference in response exists between IAO and EO.

Materials and Methods

Animals—Our study was approved by the University of California-Davis Animal Use and Care Committee. Horses used in our study were randomly selected from a population of horses maintained at the university research facility assigned to an unrelated terminal study. That study involved tissue collection following euthanasia and did not influence the outcome of our investigation. The 10 clinically normal geldings used had a mean age of 8.3 years (range, 2 to 15 years) and represented 2 Warmblood Horses, 1 Arabian, 5 Thoroughbreds, and 2 Quarter Horses. Preference for inclusion in our study was not made for sex, but the pool of available horses was limited to geldings. These horses had no hind limb lameness, no palpable abnormalities in the stifle joint region, and no history of stifle joint injury. In 5 horses, the middle and medial patellar ligaments on 1 limb were injected with IAO, whereas 5 additional horses received EO. The patellar ligaments on the opposite limb were not injected and served as control limbs. Following the injection, horses were lunged 20 minutes in both directions for 2 weeks.

Treatment protocol—For injection of agents, horses were placed in stocks and sedated as needed with detomidine hydrochloride (5 mg/horse) and butorphanol tartrate (5 mg/horse). The right stifle joint region was aseptically prepared by use of standard protocols. For the IOA-treated horses, 2 ml of IOA was deposited in each middle and medial patellar ligament divided into 5 locations, specifically the origin; proximal, middle, and distal aspects; and insertion, to ensure the substance infiltrated the length of the ligament. In 1 horse, the depth was adjusted to 5 cm. The time gain compensation was consistent for all horses, and in most horses, the power to gain setting was 80%. In 1 horse the power to gain setting was 90%. The ligaments were imaged in the transverse and longitudinal planes from the origin on the patella to the insertion on the proximal aspect of the tibia. Images were obtained at 5 levels on each patellar ligament: origin; proximal, middle, and distal aspects; and insertion. Data collected included the cross-sectional area of each transverse section and whether areas of decreased echogenicity and abnormal fiber patterns were found. Cross-sectional areas were obtained by freehand tracing and calculated via the software package in the ultrasound machine. All images were digitized, printed on thermal paper, and stored on videotape.

Histologic evaluation—Following euthanasia using an overdose of pentobarbital, the medial and middle patellar ligaments of the treated and control limbs were immediately dissected free of overlying soft tissues; cross sections were taken from the proximal, middle, and distal aspect of the ligaments. The tissue was placed in neutral-buffered 10% formalin solution and incubated for a minimum of 48 hours. Paraffin-embedded cross sections of the ligaments were cut 5 μm thick and routinely processed by use of Masson trichrome and H&E stains. The specimens for histologic evaluation were interpreted in a randomized manner by a pathologist (DWA) who was blinded regarding EO and IAO treatment and control specimens.

Changes were described and estimated as a fraction of total volume (volume fraction estimation). Three primary changes were recognized in ligament sections: 1) infiltration of inflammatory cells, including macrophages, lymphocytes, plasma cells, eosinophils, and neutrophils; 2) infiltration, separation, and replacement by fibroblasts; increased proteoglycans (basophilic matrix); and new blood vessels; and 3) loss of cellularity, chondroid metaplasia, and mineralization. The volume percentage of each change was estimated by use of a Weibel 2 graticule with 42 points. Five randomly chosen fields were examined, and the character of ligamentous change was assessed at each of 42 points in each field, assigned to 1 of 8 categories, and tabulated. The categories were divided into the following: normal appearing interstitium or ligament, inflammation of interstitium or ligament, fibroplasia and proteoglycan deposition of interstitium or ligament, and chondroid metaplasia of the interstitium or ligament. Those grid points that fell off the edge of the ligament section or fell within artificial spaces between bundles were not counted. Technical difficulties in preparation of tissues, embedding, and sectioning precluded precise objective analysis of fiber size and separation.

Statistical analysis—For histologic analysis, all variables were analyzed by use of commercial computer software with a 1-way ANOVA, and differences between treatment and control specimens were determined by use of a Bonferroni multiple comparison test. For ultrasonographic evaluation, differences in cross-sectional areas of patella ligaments between IAO- and EO-treated horses were analyzed by use of the Mann-Whitney U test. Comparisons between treated limbs and the opposite control limbs were analyzed by use of the paired Student t test. Significance was established as a value of P < 0.05.

Results

None of the horses had clinical signs of any discomfort or lameness in the initial period following the injection of IAO or EO in the patellar ligaments. During the 2-week lunging period, none of the horses had any lameness or clinically apparent stifle joint swelling.

Ultrasonographic evaluation—Injection of the medial and middle patellar ligaments with EO or IAO...
resulted in a significant increase in the cross-sectional area. Prior to injection with IAO, mean (± SD) cross-sectional areas of the middle and medial patellar ligaments were 0.91 ± 0.17 and 0.65 ± 0.21 cm², respectively, compared with 1.04 ± 0.21 and 0.75 ± 0.24 cm², respectively, following injection of IAO. Similarly, prior to injection with EO, mean cross-sectional areas of the middle and medial patellar ligaments were 0.89 ± 0.15 and 0.72 ± 0.18 cm², respectively, compared with 0.96 ± 0.16 and 0.84 ± 0.26 cm², respectively, following injection of EO. A significant (P = 0.14) difference was not found in the cross-sectional area of the medial patellar ligament between IAO- and EO-treated horses. However, the cross-sectional area of the middle patellar ligament was significantly (P = 0.04) larger following injection with IAO, compared with EO.

Following the injection of IAO and EO, there was a consistent decrease in echogenicity of the ligament in transverse views and an increased irregularity of fiber pattern in longitudinal views (Fig 1 and 2). In 2 horses, focal anechoic areas were also observed on the transverse view, which appeared as fiber disruption on the longitudinal axis (Fig 3). Injections of IAO induced a greater quantity of peri-ligamentous edema, compared with injections of EO. This was also apparent grossly when the ligaments were harvested following euthanasia.

Microscopic findings—On microscopic evaluation, cross sections taken from the patellar ligament control specimens were composed of evenly spaced large primary collagen bundles in parallel arrays, with a moderate number of closely applied fibrocytes (ligamentocytes) and small-caliber blood vessels. These bundles were packed into larger secondary bundles dissected by fine interstitial connective tissue septa and larger blood vessels (interstitium). The connective tissue capsule surrounding the whole ligament was considerably more dense and fibrous (Fig 4). Chondroid metaplasia, and to a lesser extent, increased proteoglycans, were common findings in control specimens as well as treatment specimens.

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Figure 1—Ultrasonographic images of the cross-sectional and longitudinal axis of the distal aspect of the middle patellar ligament of a horse before (A) and after (B) the injection of 2% iodine in almond oil (IAO). After injection, the cross-sectional area was larger than the preinjection area. Notice the diffuse decrease in echogenicity in the transverse view (arrowhead) and the irregular fiber pattern on the longitudinal view (arrows).

Figure 2—Ultrasonographic images of the cross-sectional and longitudinal axis of the mid region of the middle patellar ligament of a horse before (A) and after (B) the injection of ethanolamine oleate (EO). After injection, cross-sectional area was larger than the preinjection area. Notice the diffuse decrease in echogenicity in the transverse view (arrowhead) and the irregular fiber pattern on the longitudinal view (arrows).

Figure 3—Ultrasonographic image of the distal region of the middle patellar ligament of a horse before (A) and after (B) the injection of IAO. In addition to an increase in cross-sectional area, notice the focal anechoic regions on the transverse view (arrowhead) and fiber disruption is on the longitudinal view (arrows).
For IAO- and EO-treated horses, 3 primary changes were recognized in patellar ligament treatment specimens: 1) inflammatory cell infiltration composed of macrophages, lymphocytes, plasma cells, and neutrophils; 2) infiltration, separation, and replacement of ligamentous architecture by fibroblasts and increased proteoglycans (basophilic matrix); and 3) loss of cellularity, chondroid metaplasia, and mineralization. On the basis of quantitative assessment, changes were most severe in IAO-treated horses, although in all injected patellar ligaments, the degree and distribution were variable. The interstitium and capsule was expanded by activated fibroblasts and increased amounts of proteoglycans. Secondary ligament bundles were often partially or completely replaced by fibroplasias, and in other areas proteoglycans expanded the spaces between primary collagen fibers, acellularity, and mineralization. Inflammatory cells were also found in many areas of inflammation, abundant eosinophils were also found. In injected patellar ligaments of EO-treated horses, increased accumulation of proteoglycan in the interstitium and between primary collagen bundles was the most prevalent change. Fibroplasia was occasionally found, but inflammatory cells were almost completely lacking. A significant difference was not found in any histologic variable between ligaments, therefore the data analyzed represents the combination of values obtained from the medial and middle patellar ligaments (Fig 7).

Significant differences in the degree of inflammation or ligamentous fibroplasia were not found between control and treatment specimens from EO-treated horses. Compared with control specimens, EO treatment specimens had significantly ($P < 0.001$) greater interstitial fibroplasia and correspondingly less normal appearing interstitial areas. Other variables were not significantly different. For IAO-treated horses, treatment specimens had significantly ($P = 0.001$) greater interstitial and ligamentous fibroplasia ($P = 0.001$) and inflammation ($P = 0.007$), compared with control specimens. A significant difference in chondroid metaplasia was not found between control and corresponding IAO- or EO-treatment specimens. In comparing treatments, injection of IAO resulted in significantly ($P = 0.03$) more ligament fibroplasia and inflammation. Significant differences in the degree of interstitial fibroplasia or chondroid metaplasia were not found between treatments.

Figure 4—Photomicrographs of sections of the middle patellar ligament from a horse prior to injection of the ligament. Tissue sections are stained with H&E (top panel) and Masson trichrome (bottom panel), which stains collagen blue. Notice that primary collagen bundles are packaged into larger secondary bundles separated by fine interstitial connective tissue. Bars = 400 μm.

Figure 5—Photomicrographs of sections of the middle patellar ligament from a horse 2 weeks following injection of the ligament with EO. Tissue sections are stained with H&E (A) and Masson trichrome (B). Notice the extensive fibroplastic infiltration (arrows) of the secondary bundles extending along the interstitium and directly into the bundles. Normally collagen is stained blue in Masson trichrome. Bars = 100 μm.
The novel fibrous tissue observed 2 weeks after injection was in the process of becoming organized as characterized by rather parallel alignment of the collagen fibers and active fibroblasts. In models investigating the healing of severed and damaged ligaments, fibroblasts appear in the first week following injury, proliferate, and become longitudinally organized by 3 weeks.11,12

Because more fibroplasia developed with IAO, this substance initiated a stronger irritant effect than EO. In our study, we also found extensive infiltration of lymphocytes, plasma cells, macrophages, eosinophils, and, to a lesser extent, neutrophils within the interstitial area; occasionally, these cells could also be found within the primary bundles. This finding was expected given that inflammation represents the initial response of type-1 collagenous structures to injury.13 This contrasts with a previous study in which the neutrophils predominated after iodine injection and were not found within the fasciculi at any time. Inflammatory cells are likely to arrive via the blood vessels within the interstitial area, which would account for their accumulation in this area. The finding of inflammatory cells within the bundles in that previous study may have represented the response to more severe disruption of the ligamentous tissue. In contrast, we found a greater degree of fibroplasia in the injected patellar ligaments from IAO-treated horses, which may reflect the fact that our horses received regular postinjection exercise. Tension and exercise in rehabilitation of injured ligaments have been found to increase fibroblast proliferation, migration, and collagen synthesis.11

Chondroid metaplasia and, to a lesser extent, increased proteoglycans were common findings in control specimens as well as treatment specimens from IAO- and EO-treated horses. These are common findings in uninjured ligaments near points of insertion, but they are also commonly seen after chronic injury or stress. In our study, it likely represented normal variation because a significant difference was not found between treatment and control specimens. Further, chondroid metaplasia would likely be seen after active inflammation, and even fibroplasia were resolved.

Ethanolamine oleate is used primarily to thrombose veins when injected directly into the lumen of the vessel. In humans, EO is most commonly used to treat varicosities of the saphenous vein, esophagus, and hemorrhoids.6,14,15 Because EO can rapidly diffuse through the vessel wall, an extravascular inflammatory response also results. This drug is locally caustic and must be administered by direct observation of the vessel to avoid extravasation of the fluid. In our study, it did not appear to have an advantage over IAO, as IAO elicited a greater reaction with increased inflammatory infiltrates of macrophages and lymphocytes and more fibroplasia. Because EO is used to induce fibroplasia in blood vessels, it may have limited efficacy in ligaments that already possess dense connective and fibrous tissue and would potentially require greater volumes or higher concentrations to incite a suitable inflammatory response. Therefore, it is likely that IAO is more caustic to local tissues than EO. Ethanolamine oleate is...
suspended in an aqueous solution of 2% benzyl alcohol, which may diffuse away more rapidly than the almond oil carrier that retains the iodine in the vicinity of the injection longer. This was evident histologically by the identification of macrophages with residual intracellular iodine.

Ultrasound was also useful in describing the effect of injection of IAO or EO on the medial and middle patellar ligaments. The greater irritant effect generated by IAO was evident ultrasonographically by the significant increase in cross-sectional area of both ligaments, compared with EO. Focal areas of disruption in fiber pattern also suggested there was some remodeling developing within the fiber that likely corresponded to the areas of inflammation and fibroplasia. Potentially, the injection itself by use of a 20-gauge needle may have resulted in an inflammatory response and fiber disruption. However, because the changes were more severe in IAO-treated horses, this was more likely a treatment effect. The changes within the ligaments were also diffusely spread along the ligament, which suggested we were successful in depositing EO and IAO uniformly in the ligaments.

Potential limitations of our study may be some variation in the harvesting of ligament specimens. Although every attempt was made to harvest the ligaments in the same location, it is possible they were not collected in exactly the same place. In our study, the horse population used was also limited to only geldings. However, the advantage of this method was that we were able to exclude any confounding variables from hormonal influences. Potentially, mares and stallions might respond differently to injection of these irritants.

In our study, we could not determine whether the injection of EO or IAO induced shortening of the ligaments in addition to thickening. Because the patellar ligaments are actually the fibrous insertions of the quadriceps muscles, it would be logistically difficult to evaluate changes in the ligament length because shortening may be the result of treatment or contracture of the quadriceps. In practice, we are aware that some clinicians also deposit IAO, using a 20-gauge needle in 3 to 4 sites in the quadriceps muscle near their insertion to the patellar ligaments to theoretically facilitate shortening via muscle fibrosis. On the basis of findings in our study, IAO injection induced a greater increase fibroplasia and inflammation relative to EO.

References