No effect of mop-ending on ligament healing

Rabbit studies of severed collateral knee ligaments

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We have tested the hypothesis that increasing the surface area of cut ligament ends by the creation of "mop-ends" may alter the mechanical properties of healing medial collateral ligaments. In one group of New Zealand white rabbits, a 4-mm midsubstance segment was removed from the right ligament creating a gap (sharp cut-end healing group). In the other group, a similar gap was created but, in addition, cut ligament ends were split longitudinally across the width of the ligament creating "mop-ends", roughly tripling the total injury surface area. In all animals, the contralateral (left) leg was not operated on and served as an internal control. At least 8 animals from each injury model were killed at 3, 6, 14, and 40 weeks post-operatively. Both histological and mechanical tests showed that sharp-cut and mop-end injuries healed at similar rates with similar material.

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Submitted 91-08-24. Accepted 93-01-24

There is considerable controversy concerning the optimal initial treatment of ligament injuries (Frank et al. 1983, Indelicato 1983, Woo et al. 1987) and it is not clear what effects the configuration of a ligament tear may have on rate, quality, quantity or endpoint of periarticular ligament healing. Ligament injuries in most animal models studied to date, with only a few exceptions (Jack 1950, Walsh and Frank 1988, Weiss et al. 1990), have not simulated the well-described clinical failure condition in which ligament ends become "mopped" after rupture (Kennedy et al. 1976, Matyas and Frank 1990). Instead, experimental ligament injury has usually been induced by a simple transverse scalpel cut (Ogata et al. 1980, Piper and Whiteside 1980, Woo et al. 1987). It was postulated that increasing the ligament cut-end surface area by mop-ending may strengthen the entire healing bone-ligament-bone complex by increasing the area for functional attachments of a scar.

Using a variation of a previously described ligament healing model of the rabbit medial collateral ligament (MCL) (Jack 1950, Frank et al. 1983), we compared healing of a flat end gap to healing of a similar gap, but with the ligament ends surgically "mopped".

Animals and methods

72 mature (12–15 months old) New Zealand white rabbits (4.9 ± 0.7 kg mass) were separated into 2 experimental groups. In 1 group (32 animals), a reproducible gap was created in the right MCL (sharp cut-end healing group), while in the other group (40 animals) a similar gap was created, but the cut ligament ends were then split longitudinally into "mop-ends" across the entire width of the MCL (mop-end healing group) (Figure 1). A gap was created to induce the for-

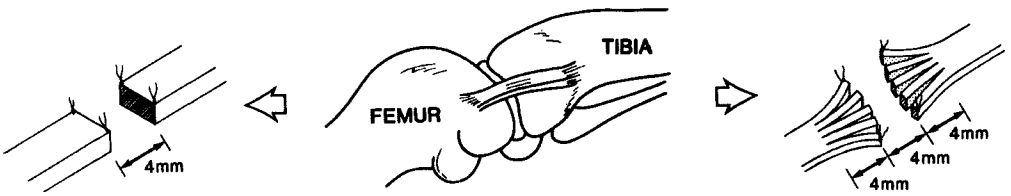

Figure 1. Sharp cut-end and mop-end injury models. While the end-surface area (cross-hatched) remains the same for both models, the longitudinal cuts made in creating a "mop-end" injury produced several new injury surface areas (shaded) along the sides of the fibers.

mation of scar between ligament ends. Results from the sharp cut-end group have been previously published (Chimich et al. 1991). In all animals the contralateral (left) leg was not operated upon: this provided an internal control.

The operations were performed under general anesthesia (halothane 1% and oxygen 1 L/min). The MCL was exposed by an anteromedial incision. For the sharp cut-end injury, a segment of the MCL midsubstance, centred over the joint line, was removed, leaving an initial gap (4.0 ± 0.5 mm) between the opposing ends of the ligament at time zero (Figure 1). The 4 corners of these opposing ends were marked with 6–0 nylon sutures to assist in identifying the original ligament at the time of sacrifice. The mop-end healing group had a similar segment of the MCL removed. The cut ligament ends were split longitudinally within 4 mm on either side of the defect 8 times (0.5 mm apart) across the width of the MCL with parallel razor blade cuts (Figure 1). This controlled mop-end injury approximately triples the total surface area of each cut-end at the time of surgery. As with the gap group, the 4 corners of the mop-end healing MCL were marked with 6–0 nylon suture. Wounds were closed with interrupted nylon skin sutures. Animals were allowed unrestricted cage (0.7 × 0.4 × 0.4 m) activity immediately after surgery.

The 32 animals from the sharp cut-end group were killed in sets of 8 at 3, 6, 14, and 40 weeks post-operatively. The 40 animals in the mop-end group were killed at the same post-operative intervals. 6 pairs of ligaments from the sharp cut-end group and 8 pairs of ligaments from the mop-end group were subject to mechanical testing and the remaining 2 pairs from each group were used for histology.

Mechanical testing

Following killing (Euthanyl, 2.0 mL/4.5 kg), the hind limbs were removed. The right limb was tested immediately and the left limb was placed in an air-tight bag and tested after the right limb, usually within 4 hours post-sacrifice. Legs were dissected, leaving only the menisci, collateral and cruciate ligaments intact. The tibia was cemented into a clamp with polymethylmethacrylate. Regular irrigation with 0.9% phosphate-buffered saline (pH 7.2) prevented the joint complex from drying.

Surface strain measurement was facilitated through the application of dye lines at 2.5 mm intervals along the medial surface of the MCL and perpendicular to the long axis of the ligament. The tibial clamp was mounted in an Instron 1122 material-testing machine, such that the longitudinal axis of the MCL was aligned with the load axis of the machine, with the knee at 70° of flexion. The femur of the specimen was positioned in a second (lower) clamp and then cemented with polymethylmethacrylate. The bone-MCL-bone complex was prepared for testing by sectioning and removing all structures crossing the joint, other than the MCL.

Ligament laxity was determined in a manner similar to that of Chimich et al. (1991) and the cross-sectional area of the mid-substance of the MCL measured with a specially designed instrument (Shrive et al. 1988). Laxity is a low-load measure which can be defined as the distance which the cross-head of the test machine travels, going from joint compression to the point where the MCL first experiences tension (Chimich et al. 1990). This definition of laxity reflects both a change in ligament length and compliance and is different from joint laxity, as defined clinically. The specimen was submerged in phosphate-buffered saline at 35 °C. Surface strain data were recorded on a video recorder for later analysis with a Video Dimension Analyzer system.

Each ligament specimen was subjected to the test sequence described by Chimich et al. (1991). Briefly, the cross-head of the test machine was cycled 30 times from a zero position to a fixed displacement of 0.68 mm at a fixed speed of 10 mm/min. The 0.68 mm displacement produced an average tensile strain of 3% in the average bone-MCL-bone complex. After thirty cycles, a steady state had been achieved, defined as 2 consecutive cycles with peak loads and loading energies within 1% of each other. On the 31st cycle, the cross head was stopped at the 0.68 mm displacement and held for 20 minutes while load relaxation occurred. The cross-head was returned to its zero position and tensile failure of the bone-MCL-bone complex was subsequently induced at a cross head speed of 20 mm/min. Load, deformation and ligament midsubstance strain were recorded during the failure test along with the mode of failure (Chimich et al. 1990).

Histology

Pairs of ligaments from 2 arbitrarily-selected animals in each injury group, at each interval, were macrophotographed for comparison of gross features of healing. All ligaments were analyzed with a standard technique using Hematoxylin and Eosin and examined by light microscopy (Frank et al. 1983). Sections of healing tissue were graded according to alignment, vascularity, defects, and overall similarity to contralateral MCL midsubstance.

Table 1. Ligament cross-sectional areas (mm^2). Mean *SD*

Study interval (weeks)	Sharp	Mop-end	Contralaterals[a]
3	6.8 *2.1*[b]	10.7 *4.2*[b]	4.3 *0.7*
6	8.7 *5.3*	7.4 *1.8*	4.7 *1.2*
14	5.2 *1.3*[b]	9.5 *6.3*[b]	3.9 *1.2*
40	8.8 *2.2*	10.5 *4.9*	4.1 *1.3*

Healing MCL complexes were larger than contralateral ligaments, except for the 14-week sharp cut-end healing group.
[a]Contralaterals from both groups pooled at each interval.
[b]Indicates significant difference between sharp cut-end and mop-end groups ($P < 0.05$).

Table 2. Ligament complex laxity (mm). Mean *SD*

Study interval (weeks)	Sharp MCL	Mop-end MCL	Contralaterals MCL[a]
3	1.6 *0.6*	1.3 *0.4*	0.5 *0.3*
6	0.8 *0.7*	1.0 *0.7*	0.3 *0.2*
14	0.4 *0.2*[b]	0.7 *0.3*[b]	0.2 *0.1*
40[c]	0.9 *0.9*	0.6 *0.3*	0.6 *0.4*

[a]Contralaterals from both injury models pooled at each interval.
[b]Sharp cut-end MCLs different from mop-end MCLs after 14 weeks of healing ($P < 0.05$).
[c]Both injury groups recovered to within contralateral values after 40 weeks of healing.

Table 3. Ligament failure force (N) and stress at failure (MPa). Mean *SD*

	Sharp		Mop-End		Contralaterals	
	N	MPa	N	MPa	N	MPa
3	45 *15*	7 *3*	66 *22*	7 *2*	400 *35*	70 *14*
6	118 *52*	16 *8*	102 *51*	15 *5*	296 *1*	76 *46*
14	135 *30*	26 *6*	143 *87*	17 *7*	305 *59*	82 *28*
40	217 *74*	26 *10*	188 *57*	25 *19*	295 *56*	76 *19*

No differences in failure load or stress at failure were identified between ligaments and both remained well below contralateral values ($P < 0.05$).

Statistics

Statistical analysis of mechanical data consisted of one-way analysis of variance, followed by the Student Neuman-Keul's procedure for multiple comparisons, with significance being set at an alpha level of 0.05. Comparisons were performed within each healing group for all time intervals, and between healing groups for each time interval. Each healing group was also compared with pooled contralaterals at each interval.

Results

Mechanical

Cross-sectional areas of all experimental healing ligaments were increased over contralateral values at all intervals except for the 14-week sharp-cut healing group (Table 1). Mean mop-end healing CSAs were larger than sharp-cut end healing CSAs at 3 out of 4 intervals, significance being observed at 3 and 14 weeks of healing.

Both sharp cut-end and mop-end healing ligaments had greater mean laxities than contralateral MCLs at 3 weeks (Table 2). By 6 weeks and beyond, sharp cut-end healing MCLs had laxities not different from contralateral laxities. Only after 40 weeks of healing had the laxities of the mop-end ligaments become indistinguishable from the contralaterals.

Prefailure viscoelastic behavior was evaluated with cyclic and static load relaxation tests. Cyclic load relaxation was defined as the peak load of the 10th cycle in the cyclic series, expressed as a percentage of the peak load in the first cycle. Healing ligaments were not different from each other at any interval, but were different from pooled contralaterals at all intervals except the 40-week healing interval. Static load relaxation was defined as the difference between the load after 1200 sec of relaxation and the initial load, expressed as percentage of the initial load. Static load relaxation values from both injury groups were different from contralaterals at every interval, but were not different from each other at any interval.

Analysis of bone-ligament-bone complex failure modes reveals little difference between injured complexes. Both models generally failed in the midsubstance region. However, some tibial avulsion failures were observed in both models at 6 weeks and in the sharp cut-end injury group at 14 weeks of healing.

At 3 weeks of healing, ligaments from both injury models had similar failure curves, but were less stiff than contralateral ligaments. At the 40-week healing interval, ligaments from the mop-end injury group showed no significant superiority in stiffness over those from the sharp cut-end injury group and had recovered to within contralateral stiffness values. Contralateral ligament complexes still had higher stiffnesses compared to sharp cut-end healing ligament complexes (Figure 2).

Force at failure was found to be lower for all healing ligaments as compared to contralaterals, but with a trend for the strength of scars to increase over the entire interval studied (Table 3). Sharp cut-end healing ligaments withstood similar failure loads to mop-end healing ligaments at all intervals of healing and no statistical difference was observed between these models

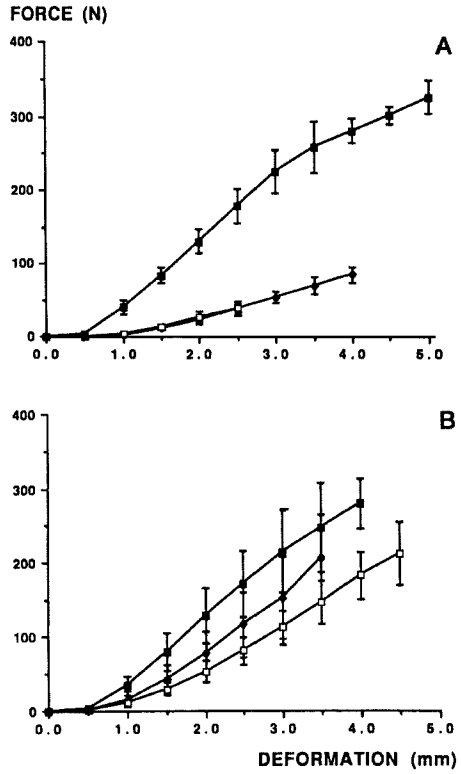

Figure 2. Composite-force deformation curves at (A) 3 weeks and (B) 40 weeks showing the structural behavior of healing and contralateral MCL complexes. No significant differences between injury types (□ sharp, ◆ mop-end) were identifiable and both injury models remained inferior to contralaterals (■) except for the 40-week mop-end healing group (mean values ± SD).

at any interval. A similar pattern was observed for ligament midsubstance stresses at ligament complex failure. Ligaments from both injury types had stresses at failure well below those of contralateral ligaments, with no significant differences between each other at any of the 4 healing intervals investigated.

Histology

Gross and histological appearances suggested that both injury models had deposition and remodeling of scar tissue between and surrounding original cut-ends of the healing MCL. At the earliest healing interval, the old ligament ends were hypercellular and had blended into scars, as previously described for ligament healing (Frank et al. 1983). Ligaments from both healing groups showed improvement with time with better matrix organization and longitudinal alignment of cells. At the 40-week healing interval, as with earlier healing intervals, no clear difference between injury types was identified. However, the cell shapes, sizes and matrix organization of both injury models remained different from those of the contralaterals. No marked differences in scar-"old ligament" junction sites could be identified at any interval between the 2 injury models, using the methods described.

Discussion

Generally, by all parameters studied, both sharp cut- and mop-end injury models appeared to heal at similar rates with similar material. Intervening scar tissue in both models showed similar rates of remodeling over time, with the "contralateral appearance" still not being reached after 40 weeks of healing.

Several mechanical results also suggest that there was no difference between sharp cut- or mop-end models in quality or rate of ligament healing. Pre-failure viscoelastic behaviors of the complexes were not different at any interval and both injury models recovered to within contralateral cyclic relaxation values by 40 weeks. Moreover, both injury models had similar structural and material failure properties. In particular, the modes of failure of the 2 groups were similar, suggesting no additional strength at the scar-"old ligament" junction due to increasing the ligament end surface area in the mop-end group. Interestingly, both groups had a 30–40 percent incidence of tibial avulsion at 6 weeks, either from a "disuse injury" of the insertion (Matyas and Frank 1990) or from some diffuse inflammatory change due to the surgery alone. This weakness of the tibial insertion persisted in a few of the sharp cut MCLs at 14 weeks, while it was not apparent in the mop-end MCLs, for reasons which are not clear.

Thus, only slight differences were noted between the healing of the 2 injury models. For example, ligaments from the mop-end healing group had cross-sectional areas significantly larger than sharp cut-end healing ligaments at both 3- and 14-week intervals. This difference may have been caused by dissection error, particularly at the 3-week interval, where both injury models were difficult to dissect. At the 14-week interval there was greater definition of old ligament ends and dissection was therefore less difficult. However, dissection error clearly affects cross-sectional area results and could induce apparent structural differences between animals and between models. Alternatively, some mop-ends folding away from the intervening gaps could have caused a "true" increase in scar size by increasing scar deposition and an increased tissue mass at the gap boundaries. The only other difference between injury models was observed

with ligament laxity at the 14-week healing interval, where mop-ends were slightly more lax. The reasons for this difference are not apparent and may only be a statistical aberration.

In summary, the results suggest that both sharp cut- and mop-end injury models of the adult rabbit MCL appear to heal at similar rates and by similar healing mechanisms with respect to virtually all of the properties measured. Despite the increased initial end surface area in the mop-end model, there is no important structural or material difference in healing. Mop-ending by the method described, therefore, has had no effect on ligament healing and is an unnecessary addition to any study on the healing of gaps in ligaments.

It can be argued that no effects of mop-ending were found, since "end cross-sectional areas" of the ligaments were actually unchanged: the direct "load bearing area" of new connection between the ends of old collagen fibers and scar collagen was virtually the same in the 2 models (Figure 1). The addition of new area on the sides of old fibers, by splitting the ligament apart, would increase the potential for load transfer by shear, but this did not increase the strength of the complex. The implications are therefore that these are not significant in stimulating scar-ligament healing, that these "side" interfaces are not a site of weakness in the entire healing complex, and that shear transfer is not a major mechanism of load transfer between old ligament and the scar. The scar, however, may be capable of transferring load by shear between 2 overlapping pieces of old ligament, as evidenced by improved mechanical properties in a z-plasty model of healing (Chimich et al. 1991). Overlap and apposition would thus appear to be more important for ligament healing than simply the surface area of the torn ends.

Finally, it should be pointed out that these results clearly do not mean that more "catastrophic" production of mop-ends, causing more diffuse damage in an entire ligament complex, would not alter the processes that we have measured. Such an injury would clearly simulate the clinical condition more realistically, but would unfortunately be much more poorly controlled. The limited production of more injury to the complex, as noted by the results presented here, however, has almost no effect on ligament healing.

Acknowledgements

The authors gratefully acknowledge the financial support of the Arthritis Society of Canada, the Alberta Heritage Foundation for Medical Research, the Medical Research Council of Canada and the Foundation of the Alberta Children's Hospital. We are also grateful to Carolyn Macarthur for typing this manuscript.

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