Collagen repair not improved by fibrin adhesive

Cruciate ligament ruptures studied in dogs

Djordje Lazovic[1] and Karola Messner[2]

The anterior cruciate ligament in 30 dogs was transected and repaired by simple suture. In every other dog, fibrin adhesive (Tisseel Kit, Immuno AG, Vienna, Austria) was applied to the transection area before suturing. The proportion of organized versus unorganized and inflammatory tissue formation was assessed histologically. At 3 weeks, the amount of normal organized collagenous tissue was reduced to 20 percent both without and with fibrin adhesive. After 6 weeks, a substantial increase of organized collagenous tissue was observed after suture only, which at 12 weeks reached about 70 percent of the total area. In contrast, repair with fibrin adhesive had at 12 weeks only 30 percent of normal collagenous tissue.

[1]Department of Orthopedics, University Hospital, Annastift e. V., 3000 Hannover 61, D-Germany; and [2]Department of Orthopedics and Sports Medicine, University Hospital, S-581 85 Linköping, Sweden
Correspondence: Dr. Karola Messner, Department of Orthopedics and Sports Medicine, University Hospital, S-581 85 Linköping, Sweden. Tel +46–13 222000. Fax –13 224434
Submitted 92-10-28. Accepted 93-05-02

Fibrin adhesive has been reported to improve repair of osteochondral fractures and ruptured tendons (Braun et al. 1980, Wruhs et al. 1980). Therefore, it was postulated that the use of fibrin adhesive may also improve ACL healing. To test this hypothesis, we studied sutured ACL repair with and without the addition of fibrin adhesive.

Animals and methods

We used 30 skeletally mature 13–21 kg mongrel dogs. Anesthesia was induced with an intravenous infusion of sodium pentobarbital. Each animal was intubated and the knee was draped aseptically. In all right knees the anterior cruciate ligament was exposed through an anteromedial incision and transected transversely 1 cm above its tibial insertion. Damage to major blood vessels supplying the ligament was avoided. The ligament ends were approximated using 4 Dexon sutures.

In half of the animals, 0.1–0.3 mL of fibrin adhesive was applied to the transection area before tightening the sutures. The fibrin adhesive (Tisseel Kit®, Immuno AG, Vienna, Austria) consists of 2 components of bovine origin which are mixed immediately before use: 1) 1 mL of protein concentrate (70–110 mg fibrinogen, 2–9 mg plasmafibronectin, 10–50 U factor XIII and 40–120 μg plasminogen) dissolved in aprotinin solution (3000 KIU/mL), and 2) 1 mL of a low concentration Thrombin 4 (4 IU) dissolved in calcium chloride (40 mmol $CaCl_2$/L). The adhesive hardens 3 minutes after mixing the components. Excessive adhesive was carefully removed.

The Hoffa's fat pad was partly resected in all dogs to exclude contact with the healing ligament which later may influence the histologic appearance of the tissue. An external fixator was applied to the operated limb with the knee joint in about 100° of flexion. In each dog the left knee served as non-operated control. After surgery the dogs were kept separately in cages. 5 dogs of each group were killed at 3, 6, and 12 weeks. The fixator was removed at death or at 6 weeks.

Directly after death the ligament was removed from both its attachments in 1 piece. The sample was fixed on a plate oriented as to the femoral and tibial attachments and kept in 8 percent formaldehyde solution until testing.

For histology, 10 longitudinal 5 μm thick sections at 10 μm intervals were prepared from each specimen, and stained with hematoxylin-eosin and elastica van Gieson. In each specimen, with the help of a calibrating grid (area 10 mm^2), the histologic sample with the largest longitudinal transection area was chosen for further evaluation. A qualitative classification of different repair tissues was established: 1) organized collagenous tissue (Figure 1), 2) vascularized, unorganized collagenous repair tissue (Figure 2), 3) hypercellular inflammatory tissue, and 4) extra-ligamentous vascularized connective tissue of synovial character. The sample was first evaluated with high magnifica-

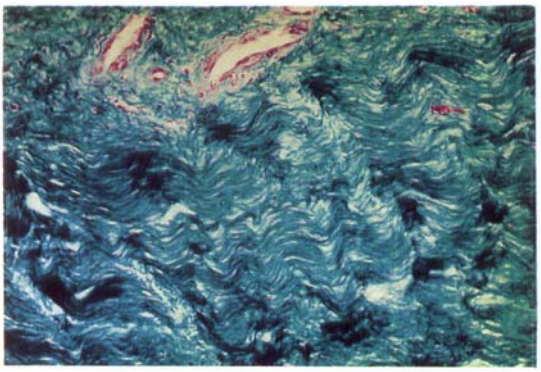

Figure 1. A control specimen shows organized mature collagenous tissue (OC) (van Gieson, ×40).

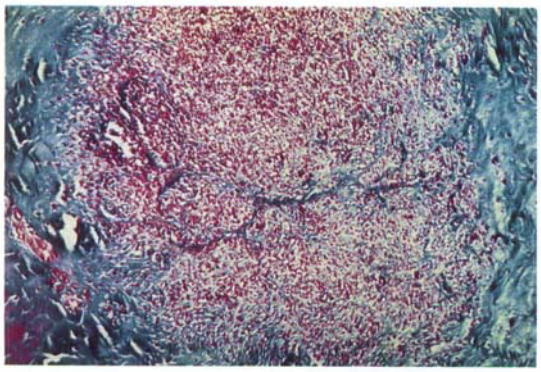

Figure 2. A 6-week-specimen with fibrin adhesive administration. The total area shows vascularized, unorganized collagenous repair tissue, the ligament's ends are clearly distinguishable (UC) (van Gieson, ×40).

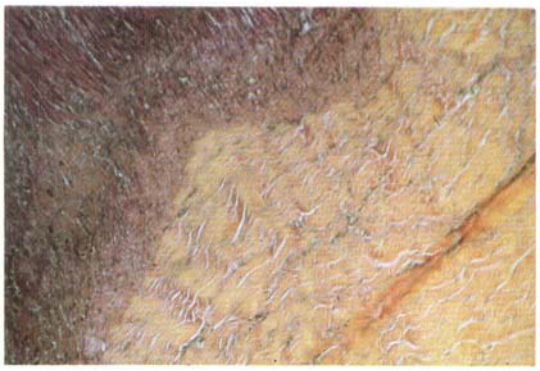

Figure 3. A 12-week-specimen with simple sutured repair. Organized collagenous tissue has formed on the right side while the rest of the sample shows mostly unorganized collagenous tissue (OC) (van Gieson, half polarized, ×40).

tions (×40, ×100) to establish the character of the different tissues. For the semiquantitative evalution a 10× magnification was chosen; 1 unit of the calibrating grid was approximately 1–2 percent of the sample length. The total area of the sample was consecutively covered with the grid, and 1 mm^2 units of the different tissues were quantitated. For each sample, the absolute values and the percentages of the different tissues were calculated. In these calculations, extra-ligamentous tissue was ignored.

All ligaments could be evaluated. All ligaments had healed macroscopically without atrophy or gap formation.

Each sample was compared to its contralateral control knee using the Wilcoxon signed rank test on a 5 percent significance level. For comparisons between different animals and time-intervals, the Kruskal-Wallis ANOVA was used on the same significance level.

Results

Inflammatory response to the ligament transection and repair was rare, and only present in the 3-week groups without differences between the treatments. The longitudinal cross-sectional areas of the repaired ligaments tended to be larger than their controls. This was especially pronounced in the fibrin adhesive group which had a 50 percent increase of the cross-sectional area compared to their controls ($P < 0.001$). Within the control groups, no size differences in cross- sectional areas were noted at the various time-intervals.

For both treatments the percentage of organized collagenous tissue was around 20 percent at 3 weeks and tended to increase with time, notably after simple suture ($P < 0.02$) where around 70 percent of the ligament had a normal collagenous structure at 12 weeks (Table 1, Figures 2–4). At 6 and 12 weeks the percentage of organized and aligned collagenous tissue, compared with unorganized or inflammatory tissue, was higher for simple suture than for repair with fibrin adhesive ($P < 0.03$) (Table 1, Figure 4). In ligaments with fibrin adhesive, only about 20 percent of the repaired ligament reached normal collagenous structure during the first 6 weeks, with an insignificant increase to around 30 percent at 12 weeks (Figures 2 and 4). Within the control groups the absolute values for the different tissues varied, since the dogs were of various sizes, but the distribution of the different tissues was rather similar at all time intervals (SD 5–10 percent). The percentage of organized collagenous tissue was usually over 80 percent. In the repaired ligaments both the absolute values and the percentages varied widely between different animals (SD 20–50 percent).

Table 1. Tissue distribution (mm^2) in repaired and control ligaments. Mean *SD*

Tissue	Group	3 weeks S	3 weeks F	6 weeks S	6 weeks F	12 weeks S	12 weeks F
Organized collagenous	Repaired	17 *9*	21 *11*	33 *12*	14 *4*	38 *18*	18 *3*
	Control	49 *23*	34 *12*	42 *12*	44 *6*	54 *6*	47 *15*
Unorganized collagenous	Repaired	54 *14*	40 *18*	37 *34*	63 *24*	14 *4*	45 *17*
	Control	4 *3*	5 *3*	10 *9*	3 *2*	4 *3*	2 *1*
Inflammatory	Repaired	3 *3*	4 *6*	0	0	1 2	0
	Control	0	0	0	0	0	0

S Simple sutured repair, F Repair with fibrin adhesive. Differences in outcome between the treatments are mentioned in the text.

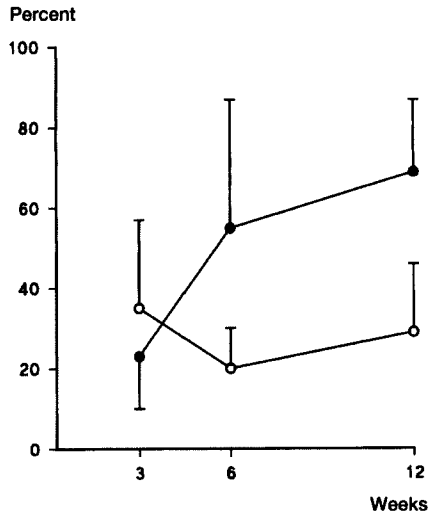

Figure 4. The percentage of organized collagenous tissue (1 SD) in the simple suture group (closed circles) and the group with fibrin adhesive (open circles). The amount of organized tissue increased significantly over time (x-axis) in the sutured group ($P < 0.02$), but not to the same extent in the fibrin adhesive group.

Neither of the 2 alternative repair methods achieved a normal collagen alignment at 12 weeks ($P < 0.004$).

Discussion

Histologic samples are used to provide a qualitative distinction between different types of repair tissue. However, the quality of ligament repair is reflected by the quantity of normally-orientated collagenous formation. Recently, histomorphometry has been introduced to give such a quantitation of different tissue types (Boynton et al. 1992), and Frank et al. (1991) introduced a quantitative assessment of collagen fibril alignment, based on automated image processing of electron microscopic scans. However, both techniques are time-consuming and demand expensive equipment. Our inexpensive semiquantitative technique, based on the assessment of a preselected representative sample, could distinguish between 2 repair treatments at different time intervals, and an increase of tissue organization was noted during the chosen time period. Furthermore, the standard deviations within control ligaments were rather small, which may favor our technique.

In our study, ligament healing showed a high variation in tissue distribution even within 1 treatment group. As the intact control ligament showed only small variations, the variable tissue after anterior cruciate ligament repair seems to be genuine and not associated with choice of animal. After both treatments, only 20 percent of normal collagenous alignment was found at 3 weeks. This indicates that transection of the anterior cruciate ligament, even without damage to the blood supply, led to a disorganization of the entire structure, as noted by Amiel et al. (1982, 1983, 1989) after transection of the anterior cruciate ligament in rabbit knees who found a rapid tissue degeneration with loss of cellularity and matrix. End-to-end adaption of the transected ligament seemed to initiate the healing process after some time, with increase in organized collagenous tissue from 3 to 12 weeks. This seems to be in contrast to the clinical experience, where simple repair without augmentation led to total atrophy and dysfunction of the ligament (Warren 1983, Kohn 1986, Sommerlath et al. 1991). However, in the present experiment only the early healing phase with ligament protection and intact vascularization was investigated, and the results are, therefore, not applicable to clinical long-term evaluations. Furthermore, the mechanical quality of the repair tissue was not tested.

In contrast to our expectations, fibrin adhesive induced massive proliferation of unorganized tissue. This is in accordance with findings of Boynton et al. (1992), who used exogenous local fibronectin to pro-

mote healing of rabbit collateral ligaments. He observed an early dramatic healing response and an increased amount of tissue, compared to simple sutured repair, but the tissue was structurally more disorganized, as in our experiment, and was biomechanically weaker than that after repair without fibronectin. Since Boynton et al. (1992) used autologous fibronectin in his experiment, the massive tissue proliferation after repair with fibrin adhesive cannot solely be explained by antigenic reaction against the bovine protein. It may be speculated that fibronectin and fibrin adhesive chemically enhance cell proliferation which tends to form unorganized inferior tissue.

Acknowledgements

Prof. W. Lang, Department of Pathology, University of Hannover, 3000 Hannover 61, Germany, evaluated the histologic samples. The study was supported by a grant from the German Research Foundation (DFG: AzIIB10 Re570/1-1 BA5161).

References

Amiel D, Akeson W H, Harwood F L, Frank C B. Stress deprivation effect on metabolic turnover of the medial collateral ligament collagen. A comparison between nine and 12 week immobilization. Clin Orthop 1983; 172: 265–70.

Amiel D, Ishizue K K, Harwood F L, Kitabayashi L, Akeson W H. Injury of the anterior cruciate ligament: the role of collagenase in ligament degeneration. J Orthop Res 1989; 7 (4): 486–93.

Amiel D, Woo S L, Harwood F L, Akeson W H. The effect of immobilization on collagen turnover in connective tissue: a biochemical biomechanical correlation. Acta Orthop Scand 1982; 53 (3): 325–32.

Boynton M D, Cannistra L M, Walsh W R, Sasken H F, Russel M S, Akelman E. Early effects of local fibronectin application on ligament healing. Trans Orthop Res Soc 1992; 17: 77.

Braun A, BruwerW, Schumacher G, Heine W D. Die fibrinolytische Aktivität im traumatisierten Kniegelenk und ihre Bedeutung bei der Fibrinklebung osteochondraler Frakturen. Hefte Unfallheilkd 1980; 148: 814–7.

Frank C, MacFarlane B, Edwards P, Rangayyan R, Liu Z Q, Walsh S, Bray R. A quantitative analysis of matrix alignment in ligament scars: a comparison of movement versus immobilization in an immature rabbit model. J Orthop Res 1991; 9 (2): 219–27.

Kohn D. Arthroscopy in acute injuries of anterior cruciate-deficient knees: fresh and old intraarticular lesions. Arthroscopy 1986; 2 (2): 98–102.

Sommerlath K, Lysholm J, Gillquist J. The long-term course after treatment of acute anterior cruciate ligament ruptures. A 9- to 16-year follow-up. Am J Sports Med 1991; 19 (2): 156–62.

Warren R F. Primary repair of the anterior cruciate ligament. Clin Orthop 1983; 172: 65–70.

Wruhs O, Vecsei V, Herts H, Czerwenka R. Ergebnisse der Sehnenklebung im Experiment und in der Klinik. Hefte Unfallheilkd 1980; 148: 818–22.