Early reactions after reimplantation of the tendon of supraspinatus into bone

A STUDY IN RABBITS

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In 14 rabbits we determined the origin of the cells effecting healing of the tendon of supraspinatus inserted into a bony trough. After two weeks both the cellularity of the underlying bone and the thickness of the subacromial bursa were significantly increased in the operated compared with the control shoulders. The cellularity of the stump of the tendon, however, was significantly decreased in the operated shoulders. In this model, both the underlying bone and the subacromial bursa but not the stump of the tendon contributed to the process of repair.

We conclude that the medial stump should be debrided judiciously but that cutting back to bleeding tissue is not necessary during repair of the rotator cuff. Moreover, great care should be taken to preserve the subacromial bursa since it seems to play an important role in the healing process.

J Bone Joint Surg [Br] 2000;82-B:1072-6.

Received 5 March 1999; Accepted after revision 30 September 1999

Since McLaughlin's original description,¹ the standard procedure for surgical repair of a torn rotator cuff has been implantation of the stump of the tendon into a bony trough.^{2,3} Moreover, during surgery on torn rotator-cuff tendons the recommendation has been to cut back the medial stump to bleeding tissue.²⁻⁵ This implies that the cells and vessels necessary for healing arise from the stump of the tendon. Some authors, however, were reluctant to cut back the stump and recommended minimal debridement to

avoid an increase in the size of the defect^{6,7} with implantation of the degenerated stump into a bony trough. They thought that the stump contributed little to the healing process.

There have been several reports of experimental studies on tendon-to-bone healing,⁸⁻¹⁷ but few in regard to the rotator cuff. Miyahara et al¹⁴ studied the late stages of healing of the transection of the supraspinatus tendon in dogs and reported that the repaired tendon showed a gradual reappearance of a normal insertion 24 weeks after surgery. St Pierre et al¹⁶ reported that in goats progressive maturation and reorganisation at the insertion of the infraspinatus tendon were seen at 12 weeks after operation. These authors, however, did not investigate the early stages of healing. Moreover, the tissues contributing to the healing process were not taken into consideration in their study.

We have used a rabbit model in an attempt to identify the most important site(s) of early cell and vascular proliferation leading to an anchorage of tendon into bone.

Materials and Methods

We used 14 mature New Zealand white rabbits with a median body-weight of 3.9 kg (3.6 to 4.2). They were housed individually before and after surgery and had free access to standard food and water.

Operative procedure. The experimental procedure had received approval from the Animal Care Committee of the University of Ottawa.

Under general anaesthesia using halothane or isoflurane and O₂ in a rebreathing circuit, alternate rabbits were allocated to be operated on the right and left shoulders. A longitudinal anterolateral skin incision was made, the omovertebral and deltoid muscles were retracted and the exposed supraspinatus tendon was then transected close to its insertion into the greater tuberosity. Bleeding at the cut surface of the medial stump of the tendon was always observed. The remaining distal fibrocartilaginous stump was resected exposing the greater tuberosity. Using a dental burr, a trough 7 mm long, 2 mm wide and 2 mm deep was prepared in the cancellous bone of the greater tuberosity. Three small drill holes were made from the lateral aspect of the humerus into the bony trough. Two horizontal mattress sutures with non-absorbable 3-0 prolene were placed, each

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0301-620X/00/79986 \$2.00

suture passing through a drill hole then through the tendon and exiting through the second hole. The sutures were then tied to the lateral aspect of the cortex, thus pulling the tendon into the bony trough. The fasciae of the retracted muscles were sutured to cover the operative site and a continuous skin suture was applied. The animals were not immobilised after the operation. An intramuscular injection of 0.5 mg of oxymorphone was given occasionally to reduce pain. The opposite shoulder served as a control.

Preparation of the specimens. All the rabbits were killed two weeks after surgery with an overdose of sodium pentobarbital. Both shoulders, including the deltoid muscle, the rotator-cuff muscles, the proximal one-third of the humerus and the glenoid were removed and fixed in 10% formalin. Specimens were cut parallel to the long axis of the supraspinatus tendon using a low-speed saw (ISOMET; Buehler Ltd, Lake Bluff, Illinois). After decalcification in EDTA, they were embedded in paraffin.

Sections 7 μ m thick were stained with haematoxylin and eosin, Heidenhain's azan and Toluidine Blue. A commercially available staining kit (Biotin-Streptavidin Amplified Detection System; Biogenex, San Ramon, California) was used for immunohistochemical staining. Monoclonal mouse antibodies against type-II collagen (FUJI Chemical Industries Ltd, Toyama, Japan) were used to identify fibrocartilaginous tissue.

Evaluation of histological specimens. The area of interest was divided into four zones: the bone underlying the trough, the small gap between the stump and the bony trough, the stump of the tendon and the bursa. Each zone was evaluated by two observers independently and, in instances of disagreement, consensus was reached by discussion after joint viewing. The corresponding areas of the control shoulders were also evaluated.

Histological features. The proliferation of small vessels, metachromasia in sections stained with Toluidine Blue, the presence of type-II collagen, the continuity of fibres and the localisation of osteoblasts were examined. The presence of each feature was graded as follows: -, not present; $\pm$, weak; +, moderate; ++, strong.

Histomorphometry. To quantify the degree of cell proliferation, cells were counted in the stump of the tendon and in the underlying bone using a computer image-analysing system. A solid-state colour video camera (VK-C350I; Hitachi, Tokyo, Japan) was mounted on a standard Olympus microscope. Using magnification of 133, a video digitiser system of Snappy Video Snapshot (Play Incorporated; Rancho Cordova, California) was used to capture images which were processed on a personal computer with the software of Uthsca Image Tool (version 1.25; University of Texas Health Science Centre, San Antonio, Texas).

Three representative rectangular fields were digitised and analysed. The sites of the digitised images were standardised for each tendon. For the stump of the tendon, these were close to the site of transection at the bursal side and in between and at the articular side and for underlying bone,

close to the surface of the trough. The area of each captured field was always 0.05 mm.² The number of nuclei was counted manually on the monitor. Erythrocytes, either intravascular or extravascular, were not counted. Profiles difficult to diagnose on the monitor were submitted to direct microscopic observation. The cellularity of the subchondral bone of the non-operated shoulders close to the insertion of supraspinatus was determined and compared with that of the underlying bone at the site of the trough; haematopoietic elements were excluded. The cellularity of the tendon proper on the non-operated shoulders was compared with that of the stump of the tendon on the operated side.

The thickness of the bursa was also measured using the computer image-analysing system. In operated shoulders this was measured at the edge of the proximal stump of the tendons and in control shoulders at the site of transition from the tendon proper to non-mineralised fibrocartilage. Care was taken to make the measurements perpendicular to the tendon fibres. Each measurement was taken three times and a mean value used for the analysis.

Statistical analysis. This was performed using the Statview software package (Abacus Concept Inc, Berkeley, California). The paired *t*-test was used to determine the difference in the cell number and in the thickness of bursa between the operated and control shoulders.

Results

Four animals were excluded from the analysis because of wound breakdown at week 1 and suspicion of deep infection (1) and wide dehiscence of the tendon repair (3). This left ten animals in the study.

Histological examination. The findings showed that a small gap (< 1.0 mm) was present between the stump of the tendon and the surface of the bony trough in all specimens. The underlying bone, the gap and the stump were assessed separately as to the presence of vessels, type-II collagen and metachromasia. Moreover, in the underlying bone the presence of osteoblasts and in the gap the continuity of fibres were also semiquantitatively evaluated (Table I).

In the underlying bone, marked proliferation of osteo-

Table I. Histological assessment of each zone (see text)

Underlying bone	
Vessels	++
Osteoblasts	++
Type-II collagen	-
Metachromasia	-
Gap	
Vessels	++
Type-II collagen	+
Metachromasia	-
Continuity of fibres	-
Stump of the tendon	
Vessels	-
Type-II collagen	+
Metachromasia	+

Fig. 1

Photomicrograph showing marked proliferation of osteoblasts lining existing trabeculae and the beginning of the formation of new bone in the zone of underlying bone. Cartilage-like cells cap the surface of the newly-formed bone in the trough (haematoxylin and eosin $\times 8$).

Fig. 2

Photomicrograph showing granulation tissue in the zone between the stump of the tendon and the underlying bone consisting mainly of fibroblasts and small vessels. The continuity between the stump of the tendon and the granulation tissue has been disrupted during processing (haematoxylin and eosin $\times 8$).

Fig. 3

Immunohistochemical staining shows the presence of type-II collagen in the zone of the gap. Differentiation of cells into chondrocytes has already started at this stage ($\times 8$).

blasts and the beginning of the formation of new trabeculae were observed (Fig. 1).

In the area between the stump and the underlying bone, granulation tissue was seen, consisting mainly of fibroblasts and small vessels (Fig. 2). Cartilage-like cells capped the surface of the newly-formed bone in the trough. The continuity between the stump of the tendon and bone had not yet been established. Although no metachromasia of the matrix could be detected, immunohistochemical staining showed the presence of type-II collagen in the gap (Fig. 3).

In the stump no cellular or vascular reaction was observed. The cut surface of the tendon was clearly distinguishable (Fig. 4). Some nuclei of the fibrocartilaginous

Fig. 4

Photomicrograph showing a clear demarcation between the stump of the tendon and granulation tissue. There is no cellular or vascular reaction. The cut surface of the tendon is clearly distinguishable (haematoxylin and eosin $\times 8$).

cells showed cluster formation.

In the subacromial bursa there was a pronounced proliferation of fibroblasts and small vessels resulting in a marked bursal thickening. The cells from the bursal reaction seemed to invade both the stump of the tendon and the gap from the bursal side (Fig. 5).

Histomorphometry. The mean cellularity of the underlying bone (the number of cells per chosen area) was significantly increased in the operated compared with the control shoulders (104.7 ± 4.8 , mean difference = -99.9 , 95% confidence interval (CI) -116.0 to 83.7 , $p < 0.001$, Fig. 6). That of the stump of the tendon, however, was significantly decreased in the operated compared with the

Fig. 5

Photomicrograph showing that in the subacromial bursa there is a marked proliferation of fibroblasts and small vessels resulting in a marked bursal thickening. The bursal reaction reaches into the gap. Articular cartilage is seen in the left upper corner (haematoxylin and eosin $\times 8$).

control shoulders (28.1 v 37.4, mean difference = 9.3, 95% CI 2.2 to 16.3, $p = 0.015$, Fig. 6). The bursa was significantly thicker in the operated than in the control shoulders (0.64 mm v 0.06 mm, mean difference = 0.58 mm, 95% CI 0.20 to 0.96, $p = 0.07$, Fig. 7).

Discussion

Soslowsky et al¹⁸ showed that the anatomy of the rabbit shoulder differed slightly from that of man. Although there is a supraspinatus muscle, a rotator cuff is not present. In addition, there are differences in size and function. The insertion of the supraspinatus tendon is identical in the two species, however, and consists of four zones: the tendon proper, non-mineralised fibrocartilage, mineralised fibrocartilage and bone.¹⁹⁻²¹ Also, type-II collagen is localised only in non-mineralised and mineralised fibrocartilage.^{22,23} We therefore chose the rabbit shoulder as a model for repair of the rotator cuff in man.

Immunohistochemical investigation using monoclonal antibodies against type-II collagen as well as staining with Toluidine Blue allowed changes in the matrix to be assessed. In the gap, type-II collagen was found as well as some evidence for the differentiation of cells into chondrocytes. Re-establishment of the continuity of collagen fibres between the tendon and bone, however, had not occurred after two weeks.

In man, tears of the rotator cuff usually occur at, or close to, its insertion. During surgery, if the distal stump is found to be absent, the proximal stump is implanted into a bony trough. Theoretically, cellular and vascular proliferation leading to healing can start in the tendon, in the bone, or in the bursa. In our former study using biopsy specimens of human rotator-cuff tears, we found active vascular proliferation in the bursa but not in the stump of the tendon.²⁴

Fig. 6

The cellularity in the underlying bone increases significantly in the operated compared with control shoulders, but that of the stump of the tendon is significantly decreased in the operated shoulders (* $p < 0.05$; ** $p < 0.001$; the error bars represent 1 sd).

Fig. 7

The thickness of the bursa is significantly greater in the operated than in the control shoulders (* $p < 0.01$; the error bars represent 1 sd).

Recently, Hamada et al²⁵ showed that the torn supraspinatus tendon had an intrinsic healing capability by measuring in situ hybridisation for α -1 type-I procollagen mRNA. In our study, a marked increase in cells in the cancellous bone underlying the bony trough indicated the intensity of the early proliferation. A marked thickening of the subacromial bursa was also seen. At both sites the proliferation was always accompanied by an increase in vascularity. Cells in the proximal stump, however, showed a significant decrease when compared with control shoulders. These observations provide strong evidence that the stump of the tendon contributes little to the early phases of healing. Although it does have some capability for collagen synthesis, this is not enough for the reformation of the tendon insertion. Clinically, a torn or degenerated tendon may have even less healing potential. Therefore we believe that the proximal stump should be debrided judiciously and that cutting back to bleeding tissue is not necessary. Moreover, great care

should be taken to preserve the subacromial bursa since it seems to play an important role in the healing process.

Our finding that the bone underlying the bony trough constitutes a major source of cells and vessels seems to be supported by a report of Nobuhara, Hata and Komai²⁶ who followed up 189 shoulders operated on for massive tears of the rotator cuff. They observed that 33% of patients who had tendon-to-tendon repair complained of pain after over-use compared with 18% who had undergone the McLaughlin procedure.²⁶ Thus, we believe that the stump of the tendon should be attached to a bleeding surface of cancellous bone during surgical repair.

We wish to thank Ms Clare Booth and Ms Brigitte Eiselt for their technical assistance and Mr David Bachman for his review of the manuscript.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

1. **McLaughlin HL.** Lesions of the musculotendinous cuff of the shoulder. I. The exposure and treatment of tears with retraction. *J Bone Joint Surg [Am]* 1944;26-A:31-51.
2. **Matsen FA III, Arntz CT, Lippitt SB.** Rotator cuff. In: Rockwood CA Jr, Matsen FA III, eds. *The shoulder*. Second ed. Vol. 2. Philadelphia, etc: WB Saunders Company, 1998:755-839.
3. **McLaughlin HL.** Rupture of the rotator cuff. *J Bone Joint Surg [Am]* 1962;44-A:979-83.
4. **DePalma AF.** Ruptures of the musculotendinous cuff. In: *Surgery of the shoulder*. First ed. Philadelphia, etc: J.B. Lippincott Company, 1950:112-37.
5. **Ellman H.** Surgical treatment of rotator cuff rupture. In: Watson MS, ed. *Surgical disorders of the shoulder*. London, etc: Churchill Livingstone, 1991:283-91.
6. **Post M, Silver R, Singh M.** Rotator cuff tear: diagnosis and treatment. *Clin Orthop* 1983;173:78-91.
7. **Bigliani LU, Cordasco FA, McIlveen SJ, Musso ES.** Operative repair of massive rotator cuff tears: long-term results. *J Shoulder Elbow Surg* 1992;1:120-30.
8. **Kernwein G, Fahey J, Garrison M.** The fate of tendon, fascia and elastic connective tissue transplanted into bone. *Ann Surg* 1938;108:285-90.
9. **Kernwein GA.** A study of tendon implantations into bone. *Surg Gynec Obstet* 1942;75:794-6.
10. **Whiston TB, Walmsley R.** Some observations on the reaction of bone and tendon after tunnelling of bone and insertion of tendon. *J Bone Joint Surg [Br]* 1960;42-B:377-86.
11. **Forward AD, Cowan RJ.** Tendon suture to bone: an experimental investigation in rabbits. *J Bone Joint Surg [Am]* 1963;45-A:807-23.
12. **Jones JR, Smibert JG, McCullough CJ, Price AB, Hutton WC.** Tendon implantation into bone: an experimental study. *J Hand Surg [Br]* 1987;12:306-12.
13. **Hausman M, Bain S, Rubin C.** Reluctance of metaphyseal bone to heal to tendon: histologic evidence for poor mechanical strength. *Trans Orthop Res Soc* 1989;14:277.
14. **Miyahara H, Takagishi K, Arita C, et al.** A morphologic and biomechanical study on the healing of the repaired rotator cuff insertion in dogs: a preliminary report. In: Post M, Morrey BF, Hawkins RJ, eds. *Surgery of the shoulder*. St Louis: Mosby-Year Book, 1990:224-7.
15. **Rodeo SA, Arnoczky SP, Torzilli PA, Hidaka C, Warren RF.** Tendon-healing in a bone tunnel: a biomechanical and histological study in the dog. *J Bone Joint Surg [Am]* 1993;75-A:1795-803.
16. **St Pierre P, Olson EJ, Elliott JJ, et al.** Tendon-healing to cortical bone compared with healing to a cancellous trough: a biomechanical and histological evaluation in goats. *J Bone Joint Surg [Am]* 1995;77-A:1858-66.
17. **Liu SH, Panossian V, Al-Shaikh R, et al.** Distribution of collagen types I, II and III during the early phase of tendon to bone healing. *Trans Orthop Res Soc* 1996;21:26-5.
18. **Soslowsky LJ, Carpenter JE, DeBano CM, Banerji I, Moalli MR.** Development and use of an animal model for investigations on rotator cuff disease. *J Shoulder Elbow Surg* 1996;5:383-92.
19. **Benjamin M, Evans EJ, Copp L.** The histology of tendon attachments to bone in man. *J Anat* 1986;149:89-100.
20. **Cooper RR, Misol S.** Tendon and ligament insertion: a light and electron microscopic study. *J Bone Joint Surg [Am]* 1970;52-A:1-20.
21. **Woo S, Maynard J, Butler D, et al.** Ligament, tendon and joint capsule insertions to bone. In: Woo SL-Y, Buckwalter JA, eds. *Injury and repair of the musculoskeletal soft tissues*. Park Ridge, Illinois: The American Academy of Orthopaedic Surgeons, 1988:133-66.
22. **Kumagai J, Sarkar K, Uthoff HK, Okawara Y, Ooshima A.** Immunohistochemical distribution of type I, II and III collagens in the rabbit supraspinatus tendon insertions. *J Anat* 1994;185:279-84.
23. **Kumagai J, Sarkar K, Uthoff HK.** The collagen types in the attachment zone of rotator cuff tendons in the elderly: an immunohistochemical study. *J Rheumatol* 1994;21:2096-100.
24. **Kumagai J, Uthoff HK, Sarkar K, Murnaghan JP.** Collagen type III in rotator cuff tears: an immunohistochemical study. *J Shoulder Elbow Surg* 1992;1:187-92.
25. **Hamada K, Tomonaga A, Gotoh M, Yamakawa H, Fukuda H.** Intrinsic healing capacity and tearing process of torn supraspinatus tendons: in situ hybridization study of $\alpha 1(I)$ procollagen mRNA. *J Orthop Res* 1997;15:24-32.
26. **Nobuhara K, Hata Y, Komai M.** Surgical procedure and results of repair of massive tears of the rotator cuff. *Clin Orthop* 1994;304:54-9.