Histological evaluation of repair of the rotator cuff in a primate model

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The establishment of a suitable animal model of repair of the rotator cuff is difficult since the presence of a true rotator cuff anatomically appears to be restricted almost exclusively to advanced primates. Our observational study describes the healing process after repair of the cuff in a primate model. Lesions were prepared and repaired in eight ‘middle-aged’ baboons. Two each were killed at four, eight, 12 and 15 weeks post-operatively. The bone-tendon repair zones were assessed macroscopically and histologically.

Healing of the baboon supraspinatus involved a sequence of stages resulting in the re-establishment of the bone-tendon junction. It was not uniform and occurred more rapidly at the sites of suture fixation than between them. Four weeks after repair the bone-tendon healing was immature. Whereas macroscopically the repair appeared to be healed at eight weeks, the Sharpey fibres holding the repair together did not appear in any considerable number before 12 weeks. By 15 weeks, the bone-tendon junction was almost, but not quite mature.

Our results support the use of a post-operative rehabilitation programme in man which protects the surgical repair for at least 12 to 15 weeks in order to allow maturation of tendon-to-bone healing.

It has been almost a century since Codman first described surgical repair of the rotator cuff.1,2 The operation was rarely performed, however, until Neer’s3 description of impingement of the cuff in 1972. Since then, with improved understanding of the pathomechanics of tears of the rotator cuff and refinements in surgical technique, repair has been undertaken with increasing frequency. Despite the popularity of operative treatment, there is limited scientific evidence to assist surgeons in developing guidelines for treatment.4 In particular, regimes for post-operative rehabilitation are mostly empirical, relying on clinical experience and a small number of animal studies.5-12

Most tears of the rotator cuff are thought to occur at the bone-tendon junction and repair involves reattachment of the tendon to bone at that site. While tendon-to-tendon repair has been studied in many species and tendon-to-bone repair to a less extent, the repair process of a flat tendon such as the rotator cuff is less well known.7,8,13-18 The search for a suitable animal model for the study of the pathology of the rotator cuff has been frustrating. The use of tendons which are round or oval in cross-section, with fibres aligned longitudinally in bundles, is not directly relevant. While repair of the tendons of supraspinatus and infraspinatus has been studied in many different species, the tendons in these animals do not exert multidirectional forces or form a true rotator cuff.7,16,19-21 The microstructure of the human rotator cuff is one of multiple orthogonally aligned layers, reflecting the multidirectional mechanical stresses applied by the contributing tendons.22 This complex arrangement is peculiar to the rotator cuff, which appears to be unique to those animals which use their arms overhead for at least part of the time and, as such, is found almost solely in advanced primates.23 Ethical problems associated with primate research have generally prevented the undertaking of systematic experimental study of the pathology of the rotator cuff in such animals.

Our aim was to document the histological pattern, progression and rate of healing in an experimental repair of a primate rotator cuff shown to be very similar to the human shoulder.23

Materials and Methods

Eight adult female baboons, all aged over 15 years and part of a colony maintained for ethical primate medical research, were scheduled for euthanasia. Their mature age was

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thought to be particularly relevant to the investigation of the pathology of the rotator cuff which in man is found increasingly from middle age onwards. Repair of the rotator cuff in man is most commonly performed in patients aged between 50 and 65 years. Since the average life span of the baboon is approximately 25 years, the age of these baboons correlated with the human age range for pathology of the rotator cuff. The ethics committee of the University of New South Wales and the Central Sydney Area Health Service approved the use of these baboons for this study (ACE 94/96). Considerable attention was given to their peri- and post-operative welfare. An initial pilot study involving two baboons was performed to establish the validity and practicability of the proposed study. These two baboons were monitored daily by video recording at their afternoon feeding session for the first three weeks post-operatively, then again at eight weeks post-operatively, just before they were killed, to assess the rate of return of function in the operated arms.

The baboons were operated on in pairs and were killed at four, eight, 12 and 16 weeks after repair. These times were determined as being the most appropriate to study the repair process, since most patients undergoing repair begin active-assisted or active exercises at between four to eight weeks post-operatively and are usually progressed to early strengthening or active-resisted exercise at 10 to 16 weeks. For logistical reasons, the 16-week study group had to be killed at 15 weeks but the results were not expected to be altered by the loss of only one week. A standard operative protocol was used as described below and acromioplasty was not performed as part of the operation. Formal post-operative immobilisation was not used since immobilisation of the baboon shoulder in a sling or splint was not feasible and post-operative plaster casts would not be the case.

Before the baboons were killed the range of movement of the shoulders was examined under general anaesthesia to exclude any ‘capsulitis’. For the pilot study, plain radiographs (antero-posterior in external rotation) were taken of both shoulders to determine the presence of obvious osteoporosis or deformity. None was observed. Both shoulders were exposed surgically and the proximal humeral shaft, together with the attached muscles and tendons of the rotator cuff, were removed. On examination, particular attention was directed to any macroscopic adhesions or inflammation involving the subdeltoid bursa. After this and before the removal of the specimens, saline arthrography was performed on the shoulders to assess the integrity of the repair process. Serial longitudinal sections were then prepared through the bone-tendon junction of the repaired tendons of supraspinatus.

**Histological examination.** The specimens were fixed in two changes of freshly prepared 4% neutral paraformaldehyde for 24 hours, and decalcified in three changes of 0.5 molar ethylenediaminetetra-acetic acid for three weeks. They were then processed through graded alcohols for 5.5 hours, cleared in two changes of chloroform overnight and embedded in paraffin. Sections cut at 4 μm on a rotary microtome were mounted on glass slides and dried overnight at 45°C. They were stained with haematoxylin and eosin and a modified version of the Picro-Sirius Red staining method of Junqueira, Bignolas and Brentani.

Histological examination was performed by a qualified orthopaedic histopathologist (CRH). Each specimen was examined completely and particular attention was paid to four areas: 1) the bone-tendon repair site where the end of the cut tendon had been apposed to the decorticated greater tuberosity; 2) the area of tendon immediately adjacent to the bone-tendon repair; 3) the tissues adjacent to...
the operation site, including the periosteum and synovium; and 4) the area adjacent to the bone-tendon repair from which hyaline cartilage had been removed at operation in the hope of increasing the repair response and possibly the area over which the tendon healed to bone.

Results
After their operations, the baboons did not move the operated shoulder voluntarily for several days, but held the limb closely by their side. They then gradually began to use the operated arm, mainly from the elbow distally, for feeding and grooming, but avoided climbing for approximately three weeks. At four weeks, their behaviour was grossly normal. They were, however, seen to use the non-operated arm preferentially when only one arm was required. By eight weeks no discernible difference in use was detected between the operated and non-operated arms.

Anatomical and histological observations of the normal baboon rotator cuff. Macroscopic examination of the contralateral non-operated shoulder confirmed the similarity to the human shoulder. There was a definite rotator cuff, with the tendons of supraspinatus and infraspinatus blending well medial to their insertion to the greater tuberosity (Fig. 1). As in man, the tendon of teres minor was much shorter than those of the spinati, but it blended with the posterior margin of the tendon of infraspinatus, thereby completing the rotator cuff. Histological examination confirmed the multilayered structure of the tendon with blending of the individual tendons into a true rotator cuff. The most lateral portion, immediately adjacent to the bone-tendon junction, contained longitudinal fibres with some transverse fibres present in layers, particularly medially, where the tendons blended. Sharpey fibres were observed anchoring the tendon to bone and were aligned at right angles to a distinct tidemark (Fig. 2). At the site of blending, there appeared to be many longitudinal blood vessels, especially on the bursal aspect of the cuff. At the most lateral portion of the tendon, most of the transverse fibres appeared to be much closer to the articular aspect.

Macroscopic and histological examination after surgery. At the time of killing, the repair site of one of the four-week specimens was disrupted. The two four-week baboons had fought on one occasion through the bars of their adjacent cages a week earlier and this may have been relevant. Macroscopically, there were adhesions between the deep layer of the subdeltoid bursa and the bone-tendon repair of both operated shoulders. This was not seen in either of the contralateral shoulders. Saline arthrography showed no leakage in the baboon with the intact repair and in both
non-operated shoulders. There was, however, leakage of saline when injected under pressure from the shoulder of the baboon with the failed repair. The disruption of the repair did not appear to have occurred at the suture line, but some millimetres medial to it. The superficial surface of supraspinatus had become detached anteriorly and had retracted approximately 3 mm. The resultant space was filled with friable granulation tissue. The deep surface appeared to be grossly intact, but leaked on saline arthrography. Both of the four-week specimens were considerably more friable than those of the pilot study which had been processed at eight weeks post-operatively.

In the intact four-week specimen, it appeared that the repair process was more mature anteriorly, than in its mid-zone. Histological examination showed a considerable amount of fibrin clot at the bone-tendon junction with some early new bone extending from the greater tuberosity and some randomly orientated collagen fibres from the tendon side of the repair invading the fibrin clot (Fig. 3a). There was a definite layer of fibrovascular tissue overlying the thin area of the fibrin clot at the bone-tendon junction. The periosteum immediately adjacent to the bone-tendon repair was hypercellular and hypervascular, suggesting that it was actively involved in the repair process.

The tendon just medial to the bone-tendon repair, extending from approximately 1 mm medial to the repair for a further 2 mm medially, contained an area of undifferentiated mesenchymal tissue between existing tendon layers. This area appeared to be somewhat oedematous and was maximal in the mid-zone of the repaired tendon and less apparent at its anterior and posterior margins. Necrosis was observed within this area, with associated haemorrhage in the tendon rather than in the bone-tendon junction, suggesting that the blood supply to this area of tendon had been interrupted by surgery (Figs 3c and 3d).

In the area immediately adjacent to the repair, from which the articular cartilage had been removed at the time of surgery, a thin layer of fibrous tissue appeared to have been laid down. There was no apparent involvement of most of that area in the repair process. This area was not attached to the adjacent tendon, but there was a small area of vascular proliferative tissue, which appeared to be synovial, at the 'commissure' (Fig. 3b).

Histological examination of the torn specimen was difficult to interpret. The impression was that the disruption might have occurred not exactly at the bone-tendon repair but perhaps a millimetre or so medial to it, leaving a very small amount of tendinous material and fibrin clot attached at the repair. It was not possible to tell whether this disruption occurred through the area of necrosis noted in the tendon of the intact four-week specimen.

At eight weeks, the bone-tendon repair appeared macroscopically to be well healed. The overlying bursa was adherent to it in both cases and blood vessels could be seen macroscopically (Fig. 4). Saline arthrography confirmed the integrity of both repairs. There were obvious histological differences between the anterior and mid-sections of the repair in both cases. Anteriorly, there were foci of thin collagen fibres extending from the new bone deposited at the repair into the tendon (Fig. 5a). There was more new bone present than in the four-week specimens and the collagen fibres had effectively established a new bone-tendon junction in the anterior portion of the repair zone. In the mid-section of the repair in both baboons, the histological remodelling was considerably less advanced, with residual fibrin-containing areas and randomly aligned collagen fibres which seldom crossed the bone-tendon repair (Fig. 5b).
In the periosteum alongside the repair, the tissue had become very organised and layered. In both eight-week specimens, there was a suggestion that the collagen fibres immediately over the repair, which were in continuity with the periosteum, were already starting to align themselves at right angles to the longitudinal axis of the tendon. The cartilage removal area showed areas of formation of new bone, with overlying new fibrous tissue, but again appeared to be taking no part in the process of bone-tendon healing.

Although the pattern of healing was similar in both eight-week baboons, with the anterior portion of the repair site considerably more mature than the mid-portion, the repair process of the first baboon was, in all areas, considerably more mature than that of the second baboon. In this baboon, there were fewer fibres crossing the bone-tendon junction anteriorly and in the mid-zone the bone-tendon demarcation was more obvious. This emphasised the variability in the repair process, even in healthy baboons apparently treated identically.

By 12 weeks, the anterior portion of the bone-tendon repair appeared to be relatively mature both macroscopically and histologically (Fig. 6). There was an almost continuous plate of new bone in continuity with pre-existing trabecular bone. Collagen fibres were seen to stream from the new bone into the tendon, forming new Sharpey

Photomicrographs of specimens at four weeks. a) a surgical suture is seen in the bone (*). Small amounts of new woven bone are already present in the bone-tendon junction (BTJ) (left side). Healing appears to be more advanced adjacent to the suture (haematoxylin and eosin (BDH, Poole, United Kingdom). b) articular cartilage has been removed from the bone surface adjacent to the bone-tendon repair site (cartilage removal area, CRA). A very thin layer of granulation tissue has formed in this region, but there appears to be no healing between the tendon and the CRA. Tissue consisting of a proliferation of synovial cells with a dense collagen core (bottom left) lies on the bone, but has not healed to it (open arrows) (haematoxylin and eosin). c) a section of the BTJ showing an area of necrosis at the bottom right (**), with associated haemorrhage. There is some early healing of the BTJ on the left (haematoxylin and eosin). d) section of the BTJ in (c) at higher power showing early healing (top left) and areas of necrosis (bottom right) with some haemorrhage (arrows) (haematoxylin and eosin).
fibres. In a few foci, they appeared to extend from the original bone across the bone-tendon repair and into the tendon. With regard to cell numbers and fibre size, the size and shape of the collagen fibre bundles in this area appeared to be similar to those in the anterior sections of the control specimens.

In the mid-portion of bone-tendon repair, collagen fibres again cross from bone to tendon, but this interface appeared to be less mature, with the collagen fibres being finer and less numerous than in the anterior portion or control specimens. A tidemark between the calcified and uncalcified tissue was evident anteriorly but present discontinuously in the mid-zone.

In both 12-week specimens, the repair process appeared to be more mature in the regions immediately adjacent to the surgical sutures than in those between sutures. Blood vessels, usually arterioles, were more prominent in the repaired bone-tendon junctions than in the non-operated control specimens. Small clusters of haemosiderin-laden macrophages were found in all parts of the bone-tendon repair, but not in the control specimens. There was no evidence of the cartilage removal area being involved in the bone-tendon repair process.

The histological appearance of the 15-week specimens (Fig. 7) was similar to that at 12 weeks. The anterior and posterior sections of the bone-tendon repair were more mature than the mid-section, with some coarse collagen fibres crossing the junction in the anterior and posterior zones, but not in the mid-zone. Again, there were a few areas in which coarse collagenous bundles appeared to run from pre-existing mature lamellar bone across the new bone-tendon junction into the tendon. Thus, Sharpey fibres of varying degrees of maturity crossed the latter in an aligned, stress-bearing orientation. A tidemark had formed anteriorly and posteriorly, with a few foci of an apparent new tidemark in the mid-zone in which the other areas had developed a fibrocartilaginous interface without a new tidemark. Although the repair did not appear significantly more mature than at 12 weeks, there were no active osteolytic areas at the bone-tendon repair in the 15-week specimens.

As with the 12-week specimens, one baboon was considerably more advanced than the other. Its tendon had numerous, thick collagenous bundles, with strong crimping. The
bundles appeared to be arranged directionally and there was some interlacing and crossing. The periosteum alongside the repair was very thick and well-organised and perhaps somewhat less cellular than in the 12-week specimens. In one of the 15-week baboons, there was a small area involving the cartilage removal area and the adjacent tendon in which there were some collagen fibres apparently joining this area at its most lateral extent with the adjacent tendon.

Discussion

The baboon is the best animal model for the study of repair of the rotator cuff because of the similarity of the shoulder to that of man. To our knowledge, the only reported study to date of repairs of the rotator cuff in a primate model is that of Paulos et al\textsuperscript{29} who recognised the difference between the anatomy and function of primate and quadrupedal shoulders. They used a primate model, the cynomolgus monkey, but it should be noted that monkeys have a very rudimentary rotator cuff with little cross-connection between the contributing tendons. However, few histological details were given in their study. Other studies on experimental healing of the rotator cuff in animal models may be less relevant to man, because of the relatively small size of the animals. It is generally accepted that small animals heal more quickly and there is a lack of true rotator cuff.

It has been well established that tendons attach to bone by four sequential zones, namely, pure tendon, uncalcified...
cartilage, calcified fibrocartilage and bone. We have shown in the baboon that repair of the tendon of the rotator cuff after surgical detachment heals by the re-establishment of Sharpey’s fibres across the bone-tendon junction with the formation of a tidemark. Healing was observed to occur more rapidly anteriorly and posteriorly than in the mid-portion of the repair. We also observed that healing occurred more quickly at the most firmly held points of the repair, namely, the sutures. This suggested that firm apposition of the tendon to bone at many fixation points promoted improved healing. However, the surgical removal of hyaline cartilage adjacent to the site of the bone-tendon repair and not incorporated in the repair appeared to contribute little to the healing process. This suggests that in order to maximise the area of the tendon-bone healing while re-establishing the so-called ‘footprint’, points of fixation should ideally be located at the most medial aspect of the prepared bone-bed.

It has been suggested that the bursa plays an active and important role in the healing process. We found the overlying subdeltoid bursa to be adherent to the repair site in all cases and it was obviously vascular. While this observation did not necessarily prove that the bursa contributed to healing, it would appear to be likely that it was involved. The prominent arterioles noted at 12 weeks also suggested a role for neovascularisation in the repair process. The subacromial bursa has been shown to be an important source of cells during the proliferative phase of bone-tendon healing in repair of the rotator cuff. Upregulated bursal expression of a number of important extracellular matrix molecules, including collagen type I, has been demonstrated during healing and remodelling of tears. Some authors have recommended excision of the bursa at the time of repair because of the increased expression of pro-inflammatory cytokines and metalloproteinases in the bursa in patients with tears. Further studies may clarify the precise mechanisms leading to the cellular proliferation in early bone-tendon healing and the relative contributions of different tissues to this process. Meanwhile, we believe that it is important to maintain as many potential sources as possible of cells capable of contributing to the healing process during repair. Therefore, for open and arthroscopic repairs, we currently perform only sufficient bursectomy to allow adequate exposure for repair.

Our study is purely observational and has limitations. Variability in the healing rates was noted. Because of the small number of specimens, statistically meaningful quantitative histological assessment could not be performed. Similarly, while it would have been useful to have performed biomechanical testing on the specimens, we were restricted by the small numbers. Our study evaluated the surgical repair of an acute injury, which we recognise is not the common clinical problem. The age of the baboons in our study, between 15 and 20 years, classified them as being of ‘middle age’. Although this correlated with typical patients undergoing repairs, the baboons in our study were healthy and, aside from age-related changes, their tendons and bones were essentially normal. The consistent underlying histopathological feature associated with failure of the cuff in man is that of degeneration within the tendon. Importantly, Longo et al showed that these tendon changes were not only localised at the site of rupture, but were generalised throughout the macroscopically-intact tendon. Larger tears are associated with more severe degeneration and this has been shown to have a negative effect on successful healing after surgical repair. We therefore suggest that this animal model represented only a best-case scenario for repair. For reasons mentioned earlier, formal post-operative immobilisation was not used and this may have also influenced the healing process.

Whereas the early transition to active movement in this primate model did not appear to be detrimental to successful repair, one of the eight repairs failed within four weeks. Active use of the limb was essentially only from the elbow distally for the first three weeks, possibly equivalent to passive movement after cuff surgery in man. Further work is required to investigate the role of immobilisation and the introduction of early active versus passive movement after cuff repair.

Our study has shown that repair of the tendon of supraspinatus to bone in the baboon involves a sequence of events resulting in an almost, but not quite mature, bone-tendon junction with a tidemark and thick Sharpey fibres by 15 weeks after surgery. While the site of bone-tendon repair seemed to be healed macroscopically by eight weeks, the Sharpey fibres holding the bone and tendon together did...
not appear in any considerable number before 12 weeks. This suggests that excessive tension on the repair site should be avoided for at least 12 weeks and possibly longer.

Since we can assume, for the reasons outlined above, that healing of the rotator cuff in man would occur no more rapidly than that observed in this primate model, our results provide evidence supporting the recommended time periods for each phase of a typical post-operative rehabilitation programme after surgery on a rotator cuff. Post-operative physiotherapy should be restricted to passive exercises during the initial four and probably six weeks and active-resisted or strengthening exercises should be avoided for at least 12 weeks post-operatively.

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