Extracorporeal irradiated autogenous osteochondral graft

A HISTOLOGICAL STUDY

We examined osteochondral autografts, obtained at a mean of 19.5 months (3 to 48) following extracorporeal irradiation and re-implantation to replace bone defects after removal of tumours. The specimens were obtained from six patients (mean age 13.3 years (10 to 18)) and consisted of articular cartilage (five), subchondral bone (five), external callus (one) and tendon (one). The tumour cells in the grafts were eradicated by a single radiation dose of 60 Gy. In three cartilage specimens, viable chondrocytes were detected. The survival of chondrocytes was confirmed with S-100 protein staining. Three specimens from the subchondral region and a tendon displayed features of regeneration. Callus was seen at the junction between host and irradiated bone.

The development of adjuvant chemotherapy, radiation therapy and wide local resection has improved the prognosis of patients with bone and soft-tissue sarcomas. Extracorporeal irradiation of tissue followed by re-implantation can be used to replace bone defects following removal of tumours. This technique enables biological reconstruction with a precise fit, helps to restore the function of joints and avoids long-term complications of endoprosthetic replacements such as loosening and breakage. It avoids rejection, transmission of disease and the need for allograft. The results of irradiated bone graft have been encouraging. However, the recent literature indicates that mechanical complications associated with irradiated osteochondral grafts, such as collapse of subchondral bone and joint degeneration, remain common.

The integrity of bone and articular cartilage is crucial to long-term success following re-implantation of irradiated tissue. Several animal studies have documented the histological features of irradiated grafts. Rabbit articular cartilage tolerates irradiation without undergoing serious degenerative changes. The survival of articular cartilage after irradiation suggests that this technique can be advantageous for limb-salvage surgery involving a joint. However, there has been no study of the histological features of irradiated grafts in humans.

The objective of our study was to examine the histological features in six patients receiving irradiated osteochondral grafts, obtained from three to 48 months after re-implantation, in order to provide insight into the mechanism of regeneration or degeneration of the joint.

Patients and Methods

Between 1994 and 2002, 23 extracorporeally-irradiated autografts were used to reconstruct skeletal defects created in the treatment of musculoskeletal tumours. In this series, 14 bone and nine osteochondral grafts were implanted with a mean post-operative follow-up of 47.4 months (3 to 120). Five examples of nonunion at the site of the osteotomy and two wound infections occurred. Of the nine osteochondral grafts, six were revised because of implant-related complications or local tumour recurrence (Table I). One patient developed a recurrence which occurred outside the irradiated graft and was treated by amputation (case 1). One patient underwent surgery as a result of contracture of the knee joint (case 2). Removal of fixation devices was performed in three patients because of irritation of the surrounding tissues (cases 3, 4 and 6). In case 5, collapse of the implanted tibia caused severe instability of the knee joint which was treated by resection and total knee replacement.

In cases 1 and 5, the whole joint was obtained. Samples from the remaining four cases involving osteochondral graft were obtained by biopsy during revision surgery. In total, six irradiated and two non-irradiated specimens derived from these six patients underwent histological analysis. Clinical
The mean age at the time of retrieval was 13.3 years (10 to 18). All six patients had received pre- and/or post-operative chemotherapy. The mean follow-up for the six patients was 61.3 (6 to 100) months after surgery, the mean size of the graft was 18 cm (7 to 28) in length and the mean duration in situ was 19.5 months (3 to 48).

Extracorporeal irradiation. The tumours were resected with wide margins and removed from the patients en bloc. The autograft, including its sterile drape in a plastic cover, was surrounded by additional water containing antibiotics. All autografts were given a single dose of 60 Gy. The grafts were returned to the operating theatre in less than one hour and were re-implanted using fixation devices.

Immunohistochemistry. Immunostaining was performed using a standard avidin-biotin complex method. Polyclonal antibodies to S-100 protein and vimentin were obtained from DakoCytomation Co, Ltd (Kyoto, Japan). The primary antibodies were applied at a dilution of 1:1000. Following incubation with biotinylated secondary antibody, avidin-enzyme complex was applied. Antibody binding was visualised by incubating the slides in 3',3-diaminobenzidine solution containing hydrogen peroxide. The percentage of S-100 protein immunoreactive cells was evaluated semi-quantitatively by counting at least 1000 chondrocytes within the articular cartilage.

Results

Joint cartilage. Five cartilage specimens were examined (cases 1 to 5; Table II). The thickness of the irradiated articular cartilage displayed little or no narrowing in all five specimens in comparison with the non-irradiated cartilage (Figs 1a and b). Surface irregularities varied in severity with fibrillation of the superficial surface of the cartilage and fragmentation. The specimen obtained four years after surgery (case 5) exhibited the most severe degenerative changes. Intriguingly, in this specimen, the appearance of some normal chondrocytes was retained; moreover, nuclei of these chondrocytes were well stained with haematoxylin despite the degenerative change in the cartilage matrix (Figs 1c and d). The specimens from cases 1 and 2 were also populated with viable-appearing chondrocytes lying within a chondroid matrix. The cartilage obtained from cases 3 and 4 showed empty lacunae without chondrocyte composition.

Positive staining was found in cases 1, 2 and 5 (Figs 1e and f). The mean of positive cells in the cartilage layers from cases 1, 2 and 5 was 3%, 65% and 79%, respectively. The specimen from case 1 showed fewest positive cells, which were distributed mostly in the superficial layer. In the other two positive cases (cases 2 and 5) obtained at longer intervals, chondrocytes positive for

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Table I. Details of the six patients whose specimens were analysed

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yrs)</th>
<th>Gender</th>
<th>Diagnosis*</th>
<th>Graft site</th>
<th>Graft size (cm)</th>
<th>Reason for revision</th>
<th>Site of histological examination†</th>
<th>Duration in situ (mths)</th>
<th>Total follow-up‡ (mths)</th>
<th>Collapse of the grafts</th>
<th>Outcome§</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>M</td>
<td>OS</td>
<td>Femur</td>
<td>22</td>
<td>Recurrence</td>
<td>Callus, CA, SB</td>
<td>3</td>
<td>6</td>
<td>–</td>
<td>DOD</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>F</td>
<td>OS</td>
<td>Femur</td>
<td>28</td>
<td>Contracture of the knee</td>
<td>CA, SB</td>
<td>6</td>
<td>41</td>
<td>+</td>
<td>CDF</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>F</td>
<td>OS</td>
<td>Humerus</td>
<td>18</td>
<td>Irritation by fixation devices</td>
<td>CA, SB</td>
<td>13</td>
<td>75</td>
<td>–</td>
<td>CDF</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>F</td>
<td>OS</td>
<td>Tibia</td>
<td>14</td>
<td>Irritation by fixation devices</td>
<td>CA, SB</td>
<td>34</td>
<td>92</td>
<td>+</td>
<td>CDF</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>F</td>
<td>OS</td>
<td>Tibia</td>
<td>19</td>
<td>Collapse of subchondral bone</td>
<td>CA, SB</td>
<td>48</td>
<td>100</td>
<td>+</td>
<td>NED</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>M</td>
<td>DFSP</td>
<td>Patella</td>
<td>7</td>
<td>Irritation by fixation devices</td>
<td>Tendon</td>
<td>13</td>
<td>54</td>
<td>–</td>
<td>CDF</td>
</tr>
</tbody>
</table>

* DFSP, dermatofibrosarcoma; OS, osteosarcoma
† CA, cartilage; SB, subchondral bone
‡ from initial surgery to the latest follow-up
§ CDF, continuous disease-free; DOD, died of disease; NED, no evidence of disease

Table II. Summary of the histological features of the irradiated joint

<table>
<thead>
<tr>
<th>Case</th>
<th>Duration in situ (mths)</th>
<th>Cartilage</th>
<th>Subchondral bone</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Surviving chondrocytes</td>
<td>S-100 positive cells (%)</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>+</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>–</td>
<td>NA*</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>–</td>
<td>NA*</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>+</td>
<td>79</td>
</tr>
</tbody>
</table>

* NA, not analysed
Histology of non-irradiated (left column) and irradiated (right column) articular cartilage. A non-irradiated specimen was obtained from the femur and an irradiated specimen was obtained from the tibia of case 5, who had undergone resection of the proximal tibia and re-insertion of an extracorporeally-irradiated osteochondral graft. Low-powered microphotographs of a) non-irradiated and b) irradiated articular cartilage (x40). The irradiated cartilage exhibited more severe surface irregularity and fibrous degeneration of the cartilage matrix in comparison to non-irradiated cartilage. High-powered microphotographs of c) non-irradiated and d) irradiated articular cartilage (x400). The appearance of normal chondrocytes was retained; moreover, the nuclei of chondrocytes were well stained with haematoxylin in both types of cartilage. Immunohistochemical staining for S-100 patients in the e) non-irradiated and f) irradiated articular cartilage (x400). S-100-positive cells were evident in both types of cartilage.
S-100 protein were seen in all layers, although the superficial and deep layers stained most intensely. The remaining two specimens (cases 3 and 4) that lacked viable chondrocytes were negative for S-100 protein.

Subchondral bone. Five specimens of subchondral bone were examined (cases 1 to 5; Table II). In the specimens of cases 4 and 5, which were obtained nearly three and four years, respectively, after surgery, surrounding osteoblasts and osteocytes embedded in trabeculae of the subchondral bone were evident and the marrow was filled with fibrovascular tissue which could represent bone regeneration. Case 3, obtained 13 months after surgery, did not show osteoblasts, although newly-formed vessels were present among trabeculae of subchondral bone.

Cases 1 and 2, obtained six months or less after irradiation, exhibited features of osteonecrosis, in which trabeculae lacked both surrounding osteoblasts and reparative vascular formation.

The cortical junction. The site of the cortical junction was examined as a single specimen. A lateral radiograph taken three months after surgery revealed that external callus bridged the posterior aspect (Fig. 2a). There was a radiolucent line between the irradiated and the host bone which was confirmed on histological examination (Fig. 2b). The bridging external callus extended equally on the surface of both the host and the irradiated bone (Figs 2c and d) and was well incorporated with the cortex of irradiated bone. The gap between the cortex of the host bone and the irradiated bone was filled with fibrovascular tissue (Fig. 2e). Blood vessels extended into the Haversian canals in the cortex of the irradiated bone, the canals being surrounded by newly-recruited osteoblasts (Fig. 2f).

Discussion

In our study, we confirmed that the tumour cells had been devitalised using morphological and immunological analysis of samples which were irradiated with a dose of 60 Gy. One case developed local recurrence of tumour in the soft tissue outside the irradiated bone, however, the tumour cells were completely eradicated from the irradiated bone. Our study shows the viability of chondrocytes in the irradiated cartilage. Several animal investigations support this finding. We attempted to stain specimens with antibodies, including type II collagen, the major protein.
synthesised by chondrocytes and Ki67, a marker of proliferative activity,\textsuperscript{22,23} to investigate the biological and proliferative activity of chondrocytes. However, we were unable to stain even the control cartilage (the non-irradiated cartilage in the same subject), employing these antibodies, with the exception of the S-100 protein. The antigenicity of collagen type II and Ki67 was eliminated probably due to hard decalcification.\textsuperscript{24} S-100 protein is present in chondrocytes\textsuperscript{16-19} and its expression is correlated with the metabolic activity of the cartilage matrix, i.e., collagen and proteoglycans.\textsuperscript{19,20,25} Distribution of S-100 protein-positive chondrocytes in the irradiated cartilage was similar to that of the normal cartilage, the superficial and deep layers stained most intensely.\textsuperscript{18} The S-100 protein-positive chondrocytes, therefore, are not only viable but should also possess, at least in part, biological activity similar to that of chondrocytes in the normal cartilage. In addition to cartilage, the irradiated tendon was also populated with fibroblast-like cells. The mechanical strength of this tendon was unknown; however, the histological appearance was indicative of regeneration of the irradiated tendon. The existence of these chondrocytes and regenerated tendon might be advantageous in reconstruction around the joint.

In the subchondral region, two cases (cases 4 and 5) obtained more than three years post-operatively exhibited the features of bone regeneration. However, the two cases (cases 1 and 2) obtained six months or less after implantation lacked reparative processes. The subchondral bone region is most distant from the osteotomy site. In allografting, new bone formation begins at the junction between the host and graft bone; moreover, it creeps towards the subchondral bone.\textsuperscript{26} Enneking and Campanacci\textsuperscript{27} showed that the total extent of repair remained approximately 30% in the majority of allografts retrieved two years after implantation; however, a specimen retrieved at eight years showed 70% repair. As shown in case 1, the new bone is well incorporated with the cortex of irradiated bone at the osteotomy site. However, regeneration of the irradiated subchondral area, which is distant from the osteotomy site, will occur at the last stage of regeneration. In rabbits, irradiated subchondral bone in the femur showed osteonecrosis for a specific period of time; however, a tendency to recover 15 months after grafting was also observed.\textsuperscript{13} In humans, osteonecrosis may not recover for extended periods, resulting in mechanical failure and degenerative change. Subchondral collapse was not obvious in case 1, probably because of the relatively short duration in situ. In case 3, the graft was used to reconstruct a non-weight-bearing joint, which could account for the absence of joint collapse.

If chondrocytes can survive following irradiation at 60 Gy, is it safe to employ this method for cartilaginous tumours? We have treated three patients presenting with
chondrosarcoma using this method. These patients were not included in this histological study since they did not develop complications requiring revision surgery. They are free of recurrence after a mean of 79.3 months (58 to 120). There have been no reported cases of chondrosarcoma that have developed local recurrence following extracorporeal irradiation in other series. Additionally, the direct effect of irradiation on chondrocytes is different in DNA and matrix synthesis.8,9,28,29 Cultured chondrocytes continued to produce glycosaminoglycan, whereas DNA synthesis and cell proliferation were inhibited following a single dose of 10 Gy.28 Synthesis of collagen fibres was also reported to be more radioresistant compared with DNA synthesis.29 Our results indicate that matrix synthesis in grafted articular cartilage is preserved at a dose of 60 Gy, whereas cell division of the cells of cartilaginous tumours was inhibited.

The collapse of the irradiated joint is inevitable, especially for weight-bearing joints. We recommend the use of irradiated osteochondral graft only in non-weight-bearing joints or for supplementation of biological reconstructions (e.g. vascularised bone grafts). Further investigation is needed to clarify the nature of the chondrocytes in irradiated grafts.

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References