CHANGES IN BONE AFTER HIGH-DOSE IRRADIATION

BIOMECHANICS AND HISTOMORPHOLOGY

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We studied the effects of high-dose irradiation on the mechanical properties and morphology of cortical bone in rabbits for 52 weeks after a single dose of 50 Gy of electron-beam to the tibia.

After four weeks, the bending strength of the irradiated bone was unchanged, but at 12 weeks, the strength had decreased significantly. At 24 weeks after irradiation mean strength was less than half of controls but by 52 weeks there was a tendency toward recovery. Similar, synchronous changes of damage and recovery were seen in cortical porosity, haematopoietic cells in the bone marrow and endosteal new bone formation.

Radiation therapy plays an indispensable role in the current treatment of musculoskeletal tumours. Radiosensitive primary sarcomas such as Ewing's sarcoma and lymphoma are often treated successfully by radiation in combination with chemotherapy and surgery. Palliative radiation is an established treatment for metastatic bone lesions. Osteosarcoma, fibrosarcoma and chondrosarcoma have been difficult to treat by conventional methods of external irradiation, but newly developed techniques have allowed successful local control of these tumours (Abe et al 1975; Uyttendaele et al 1988). We have used intraoperative irradiation therapy at a single dose of 50 Gy for 33 patients with osteosarcoma since 1978. There was no recurrence of tumour in any of these cases; the main problems were fractures in the irradiation field, seen in 10 cases (Nagashima et al 1983; Yamamura et al 1989). The increase in the indications and use of radiation therapy have produced more associated morbidity, including bone damage and osteonecrosis. Radiographic changes and pathological fractures have been extensively reported, as have histological changes in irradiated skeletal tissue in both humans and experimental animals. However, the process by which bone atrophy and mechanical weakness develop remains obscure, and little is known about possible recovery of bone injured by irradiation.

We aimed to reproduce radiation-induced atrophy of cortical bone in an animal model and to use this to quantify and confirm the development of mechanical weakness.

MATERIALS AND METHODS

We used 14-week-old Japanese white rabbits weighing about 3 kg. They were housed in cages, fed commercial food pellets (CR-2, Clea, Japan) with free access to water. Each of 20 rabbits was given 50 Gy of electron-beam irradiation to the right proximal tibia in a single dose. Five animals were killed at each of 4, 12, 24 and 52 weeks after irradiation.

Irradiation. Each rabbit was anaesthetised with intravenous pentobarbital sodium (30 mg/kg body-weight), and placed supine with the right knee flexed to about 90°. A longitudinal 7 cm incision over the right knee allowed the skin to be retracted away from the irradiation field. The proximal 3 cm of tibia and the knee were irradiated at a dose of 50 Gy using 14-MeV electron-beam from a betatron. The skin was then closed with interrupted nylon sutures.

Sham operation. The left unoperated tibiae were used as controls. To monitor the effect of the skin incision and retraction, the operation was performed on the right knee of another group of five animals, but no irradiation was given. These tibiae served as sham-controls.
**Fluorescent analysis.** Bone was labelled by the intravenous injection of tetracycline, 25 mg/kg body-weight (Hostacrylicin; Hoechst, Japan) for three consecutive days at two weeks before sacrifice. An intraperitoneal injection of 20 mg/kg body-weight Calcein solution (Nacalai Tesque Corp., Japan) was given three and two days before the rabbits were killed with an overdose of pentobarbital.

**Biomechanical tests.** Both tibiae were harvested from each rabbit and stripped of soft tissues. Radiographs were taken with fine-resolution films (Fuji, Japan).

From each tibia a rectangle of cortical bone 14 × 5 mm was cut from the flat portion of the medial cortex (Fig. 1) using a diamond band-saw (Exakt, Germany). These specimens were tested to failure at room temperature with a three-point bending load, using an Autograph machine (DSS-2000, Shimadzu, Japan) with a cross-head speed of 1 mm/min, a maximum load of 2 kg and a span of 10 mm. The longitudinal axis of the cross-head was placed parallel to the haversian systems. The load was delivered onto the endosteal side of the specimen at right angle to the surface.

The bending strength (S) was calculated from the equation:

\[
S (\text{MPa}) = \frac{3pl}{2wt^2} \times 9.8
\]

where \( p \) is the load necessary to cause fracture, \( l \) is the distance between the two supporting knife edges (span), and \( w \) and \( t \) are the width and thickness respectively of the specimen. The bone was kept wet with Ringer lactate solution during the whole process to avoid any adverse effect of drying.

**Morphometry and histology.** After mechanical testing, the specimens were fixed in 10% phosphate-buffered formalin solution for four days, as were 10 mm long pieces of diaphysis cut from a non-irradiated area of each tibia. The specimens were dehydrated in ethanol, embedded in polyester resin, and 80 μm slices prepared for contact microradiography. The slices were ground to a thickness of 40 μm and stained with Giemsa solution for histological and fluorescence studies.

The rate of endosteal new bone formation (mineral apposition rate) and the cortical bone porosity were examined morphometrically. Endosteal new bone formation was measured as the interval between tetracycline- and Calcein-labelled bands divided by the period between the times of labelling, examining three different points in each cross-section under ultraviolet light.

Porosity of the cortical bone was measured by image analysis of the contact microradiograms using a micro-

![Fig. 1](image1.jpg)

**Fig. 1**

Site and orientation of the specimen used for mechanical testing.

![Fig. 2](image2.jpg)

**Fig. 2**

Radiograph showing local bone porosity at 12 weeks, seen only in the irradiated proximal area of the tibia on the left.
scope (× 100) and a minicomputer system, digitiser and plotter (Model-G/A, Mutoh Industries, Japan). Three standardised fields in the intracortical areas of each cross-section of the bending test specimens were examined. The number of resorption cavities were also counted. In the non-irradiated part of the diaphyses, four fields, the intracortical areas of the medial, lateral, anterior and posterior sides, were analysed. The porosity of the cortical bone was determined by the area of haversian canals and resorption spaces as a percentage of total cortical bone area in the field.

The remaining portion of metaphysis was decalcified for histological processing, embedded in paraffin, cut in 5 μm sections and stained with haematoxylin and eosin. Osteocytes and bone marrow cellularity were examined at × 400, and 200 osteocyte lacunae were examined from each specimen to determine the presence or absence of an osteocyte nucleus. The numbers of bone marrow cells were counted in five fields of the marrow space.

Statistics. The mean and standard deviation for each parameter was calculated from five results for each time period. Differences were examined by paired and unpaired Student’s t-tests. A confidence level of 95% (p < 0.05) was set as a measure of significance.

RESULTS

All the wounds healed within 10 days, and no loss of hair, skin erosion or ulcer was seen. Radiographs showed porotic changes in the irradiated area at 12, 24 and 52 weeks after irradiation (see Fig. 2).

Mechanical changes. Weakness of cortical bone on the irradiated side was first found at 12 weeks after irradiation indicating an increase in endosteal bone resorption. There was a significant increase in intracortical bone porosity at 12 weeks; this progressed to reach its maximum at 24 weeks (p < 0.05). The porosity had decreased a little at 52 weeks, but still remained significantly higher (p < 0.05) than that of the contralateral side (Fig. 3). There were no changes in the porosity of the non-irradiated parts of the diaphyses on either side throughout the study. The number of resorption cavities on the irradiated side was greatest at 12 weeks and decreased gradually thereafter. At 24 weeks the number was significantly larger than on the contralateral side (p < 0.05).

On the irradiated side new bone formation was significantly suppressed at four weeks (p < 0.05) and almost completely suppressed at 12 and 24 weeks (p < 0.01, p < 0.05). At 52 weeks after irradiation, there was an increased rate of new bone formation over that at 24 weeks.

Histopathology. There was a marked decrease in the haematopoietic elements in the irradiated bone marrow at all of the time periods (Fig. 4, Table II), and especially at 12 and 24 weeks. At 52 weeks there was some repopulation of haematopoietic elements in the degener-

Table I. Mechanical strength and morphometric variables (mean ± SD, using five rabbits for each time period)

<table>
<thead>
<tr>
<th></th>
<th>Sham control at 4 weeks</th>
<th>Irradiated</th>
<th>Weeks after irradiation</th>
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<tr>
<td></td>
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<tr>
<td>Bending strength (MPa)</td>
<td></td>
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<tr>
<td></td>
<td>97 ± 8</td>
<td>Yes</td>
<td>98 ± 30</td>
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<tr>
<td></td>
<td>94 ± 6</td>
<td>No</td>
<td>96 ± 29</td>
</tr>
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<td>Bone porosity (per cent)</td>
<td></td>
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<td></td>
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<tr>
<td>Metaphysis</td>
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<td></td>
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<td></td>
<td>2.7 ± 0.5</td>
<td>Yes</td>
<td>3.0 ± 1.9</td>
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<td></td>
<td>2.2 ± 1.3</td>
<td>No</td>
<td>2.9 ± 1.8</td>
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<tr>
<td>Diaphysis</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>1.2 ± 0.2</td>
<td>Yes</td>
<td>1.4 ± 0.6</td>
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<td></td>
<td>1.3 ± 0.2</td>
<td>No</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Number of resorption cavities (per mm²)</td>
<td>0.3 ± 0.2</td>
<td>Yes</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.3 ± 0.3</td>
<td>No</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>Endosteal bone-formation (µm/day)</td>
<td>4.4 ± 0.8</td>
<td>Yes</td>
<td>2.7 ± 0.9*</td>
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<tr>
<td></td>
<td>4.3 ± 0.7</td>
<td>No</td>
<td>4.3 ± 0.4</td>
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*p < 0.05
†p < 0.01
‡the diaphysis on the irradiated side is outside the exposure field

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Fig. 3

Microradiographs to show morphological changes in cortical bone. The periosteal surface is on the right side of each specimen. A, control; B, four weeks after irradiation, bone resorption is seen on the endosteal surface; C, 12 weeks, intracortical absorption is obvious; D, 24 weeks, resorption foci are seen in the outer cortical area; E, 52 weeks, the inner side of the cortical bone has been remodelled (original magnifications ×400).

Table II. Osteocyte count and bone marrow cellularity (mean ± SD, using five rabbits for each time period)

<table>
<thead>
<tr>
<th></th>
<th>Sham control at 4 weeks</th>
<th>Irradiated</th>
<th>Weeks after irradiation</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
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<tr>
<td>Osteocyte nuclei (per cent of lacunae)</td>
<td>73 ± 4</td>
<td>Yes</td>
<td>66 ± 6</td>
</tr>
<tr>
<td></td>
<td>72 ± 6</td>
<td>No</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>Bone marrow cells (×10²/mm²)</td>
<td>71 ± 14</td>
<td>Yes</td>
<td>27 ± 5*</td>
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<tr>
<td></td>
<td>78 ± 10</td>
<td>No</td>
<td>81 ± 7</td>
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* p < 0.001

DISCUSSION

Much attention has been paid to radiation injury of bone but there have been few quantitative analyses of the mechanical properties of irradiated compact bone. The changes in irradiated bone in vivo have been thought to be mainly due to a delayed biological process. These damages must be distinguished from the primary physicochemical reaction which causes immediate structural weakness after very heavy irradiation in vitro (Pelker, Friedlaender and Markham 1983).

The effects of in vivo irradiation on the mechanical properties of cortical bone were first quantified in a rat model by Maeda et al (1988). In their 18-week study, however, rat femora showed no significant mechanical...
weakness after a single dose of 35 Gy. In contrast, the time-related changes found by us correspond well with those reported in clinical cases (Howland et al 1975). Considerable bone loss is reported to be induced by irradiation over 40 Gy (Cutright and Brady 1971), which produces inevitable skin damage. Our method of retracting the skin enabled us to give rabbits a single dose of 50 Gy of electron-beam radiation with no skin damage. Our animal model reproduced the mechanical weakness found in irradiated cortical bone. However, our study differed from that of Maeda et al (1988): they used rats, the dose was lower, the irradiated volume was smaller, and the mechanical test was different. These differences may account for their negative findings.

Maeda et al (1988) reported that the osteocyte lacunae of rats became empty after a single 35 Gy dose of irradiation. We found apparently intact osteocytes with only occasional shrunken nuclei or empty lacunae. Osteocytes are highly differentiated, post-mitotic cells and, as such, are relatively radioresistant. Our findings support the observations of Jacobsson et al (1987) which showed by a histochemical method that osteocytes remained viable after a single dose of 40 Gy.

However, bone marrow is a radio-sensitive tissue: irradiation of 10 to 20 Gy is known to reduce marrow cellularity within a week, followed by a return to normal in eight weeks. El-Naggar et al (1980) found that there was an irreversible reduction of haematopoietic cells 12 weeks after fractionated irradiation of 50 Gy. Our specimens showed similar findings after 12 weeks, but, at 52 weeks we found viable haematopoietic elements in the irradiated marrow. It seems that, in rabbits, the bone marrow is capable of a degree of recovery after a single irradiation of 50 Gy.

Many investigators have considered that vascular ischaemia is part of the pathogenesis of radiation-induced bone weakness; they point out the occurrence of delayed ischaemic damage to the local mesenchymal and haematopoietic cells (Fajardo 1982). Our study has clarified some of the consequences of irradiation, such as loss of bending strength, increased porosity, reduced new bone formation and reduced cellularity of the bone marrow (Fig. 5). Two points must be emphasised. One is

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**Graph to show the time relationship of changes in strength and morphometry, expressed as the ratio between control and irradiated bone. The osteocyte and bone marrow cell counts are shown by broken lines.**

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radiation damage. At this stage the healing process is probably similar to the 'repair' seen in bone grafts and after femoral head necrosis. However, the effects of irradiation on bone resorption need further investigation.

Recovery from radiation damage is of direct concern to clinicians, but the encouraging results we obtained in rabbits are not immediately applicable to patients. Howland et al (1975) reported spontaneous healing within one year in several patients with pathological fracture of irradiated ribs. However, such cases are rare; most radiation-injured bones are extremely slow to recover or fail to do so. It may become possible to improve local revascularisation to facilitate bone marrow recovery, but this will be difficult and perhaps prove insufficient. More fundamental knowledge is needed on the deterioration and recovery of irradiated bone.

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REFERENCES


