EXPERIMENTAL STRESS FRACTURES OF THE TIBIA

BIOLOGICAL AND MECHANICAL AETIOLOGY IN RABBITS

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We have shown that stress fractures can be induced in the tibial diaphysis of an animal model by the repeated application of non-traumatic impulsive loads. The right hind limbs of 31 rabbits were loaded for three to nine weeks and changes in the bone were monitored by radiography and bone scintigraphy. The presence of stress fractures was confirmed histologically in some cases.

Most animals sustained a stress fracture within six weeks and there was a positive correspondence between scintigraphic change and radiological evidence. Microscopic damage was evident at the sites of positive bone scans. The progression, location, and time of onset of stress fractures in this animal model were similar to those in clinical reports, making the model a useful one for the study of the aetiology of stress fractures.

Stress fractures have been defined as partial or complete fractures in apparently normal bone (Hartley 1943) with no specific history of trauma (Hopson and Perry 1977; McBryde 1985). In recent years the basis for the diagnosis of a stress fracture has shifted from radiology to bone scintigraphy. Although this provides early detection of potential stress fractures, there are problems in the interpretation of a positive bone scan. Not all periosteal reactions are indicative of a stress fracture.

Clinical decisions on treatment are based on radiology and scintigraphy, yet the relationships between these, the histological changes and clinical symptoms have not been determined. Only when these relationships are clear will scintigraphy be useful to distinguish between true stress fractures and stress-induced periosteal reactions to other causes (Johnell et al 1982).

Study of the pathophysiology of stress fractures has been delayed because of the difficulty in finding a suitable experimental model. Human studies are difficult to control, and bone biopsies from an area suspected of a stress fracture are difficult to obtain. Few clinical studies have used biopsy to confirm the diagnosis, though one exception is Johnson et al (1963). An animal model is needed to define the parameters that indicate true stress fractures; once these have been defined in an animal model, they can be studied clinically.

This paper reports that stress fractures can be produced in rabbit tibiae within three to six weeks by repeated application of non-traumatic impulsive loads. The scintigraphic and radiological changes are repeatable, are similar to the progressive changes in the development of human stress fractures, and are associated with histological micro-damage to bone.

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MATERIALS AND METHODS

The right hind limbs of 31 skeletally mature 4 kg female New Zealand white rabbits were subjected to repetitive impulsive loading (1.5 × body-weight, 1 Hz, 40 min/day, 5 days/week, 25 msec rise time) using a device we described earlier (Radin et al 1973). During the loading period, the hind limb was splinted in methylmethacrylate to prevent movements of the ankle that might attenuate the force (Fig. 1). The left hind limb was also placed in a splint, but not loaded, and used as an internal control. Skeletal maturity was verified radiographically. All animals were acclimatised to the apparatus for at least

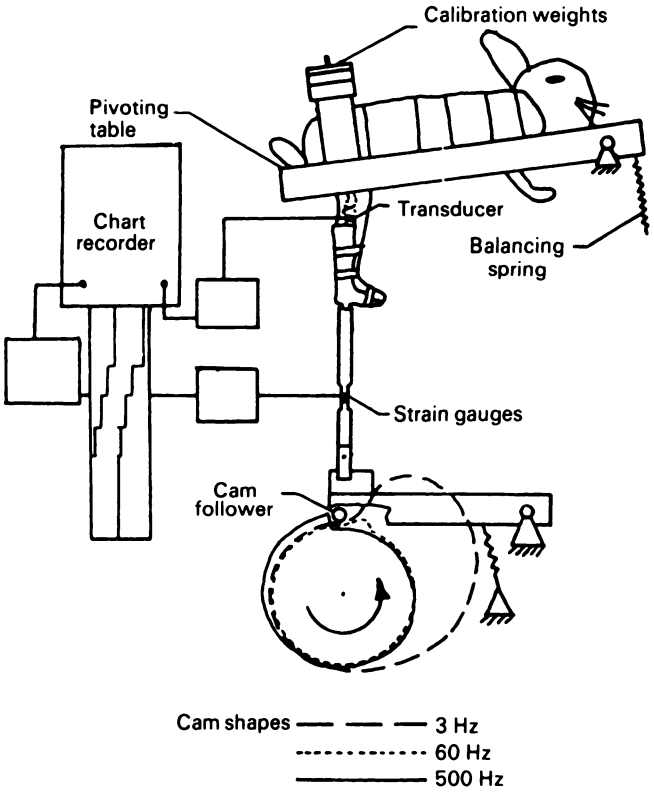

Fig. 1

The apparatus used to apply cyclical loading to the rabbit hind limb. Reproduced with permission from Paul et al (1978).

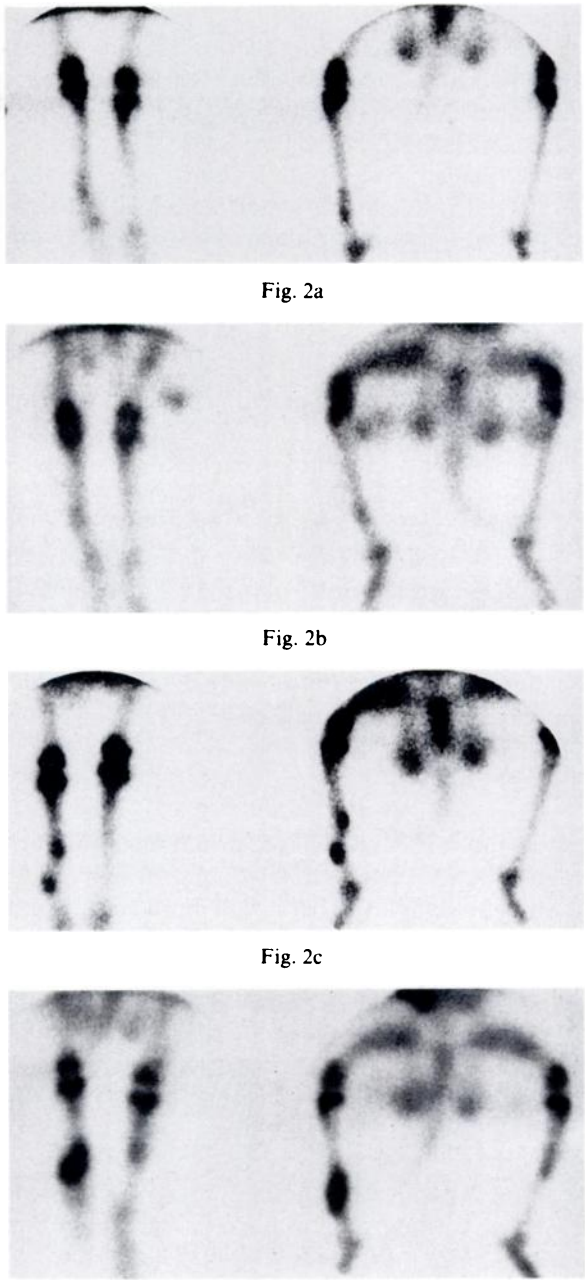

Fig. 2a

Fig. 2b

Fig. 2c

Fig. 2d

Scintigraphic images of rabbit hind limbs to show the grading system used to evaluate uptake of ^{99m}Tc: a) grade 1, b) grade 2, c) grade 3, and d) grade 4. Focal changes around the knee and ankle were excluded from the evaluation.

two weeks before the start of loading, and received a standard diet and water *ad libitum* during the experiment.

Before loading a baseline bone scan was obtained, by intravenous injection of 3 mCi ^{99m}Tc HDP (oxidronate). Three hours after this injection, the animals were anaesthetised, and the hind limbs fixed in a specially designed splint to maintain a standard position during the examination. Five-minute bone scans were made of the hind limbs in anteroposterior (AP) and lateral planes using a Picker Dyna-Mo camera. A converging collimator and NaI crystal were used to image the activity to an ADAC system IV computer. Pretreatment radiographs of both hind limbs were also taken. Repeat bone scans and radiographs were made of all surviving animals at one, three, six and nine weeks, and subgroups of the sample were killed at three weeks (n = 7), four weeks (n = 1), five weeks (n = 1), six weeks (n = 19) or nine weeks (n = 3). Radiographs and bone scans were also made of the hind limbs of three non-loaded control rabbits. After killing, AP and lateral radiographs were taken of the hind limbs both in situ and after dissection.

Scintigraphic activity in the tibial diaphysis was evaluated by a four point grading system (Roub et al 1979; Zwas et al 1980) which has been further modified for assessing change in human bone (Milgrom et al 1985a; Zwas, Elkanovitch and Frank 1987). The grading system as illustrated in Figure 2 is:

Grade 1: A poorly defined area of slightly increased uptake is present, as compared to the contralateral side;
Grade 2: The uptake is greater than that in grade 1, but there still are no well-marginated borders;
Grade 3: A focal area of increased uptake can be observed. The lesion is well marginated, but both cortices are not involved;
Grade 4: The lesion is focal and well marginated and both cortices are involved.

Following the convention set by others (Orava and

Hulkko 1984; Milgrom et al 1985c), grade 3 or 4 lesions were considered diagnostic of a stress fracture. Increased ^{99m}Tc uptake was generally observed at the knee and around the ankle, but only increased uptake in the diaphysis was considered to be positive.

The loaded and control limbs were evaluated separately. Those animals which were loaded for three weeks or longer had multiple bone scans and radiographs. Each set of these was evaluated without knowledge of the other assessments for that animal so that progressive changes, should they occur, could be assessed without bias.

Following the loading period, the rabbits were killed and both tibiae removed. Each tibia was sectioned into seven pieces using a Buehler Isomet low-speed saw. The pieces were embedded in methylmethacrylate, and each block was ground to the periosteal surface of the embedded bone. This surface was polished. Sections that came from an area of the tibial diaphysis associated with a positive bone scan were viewed at 110 × using reflected light. This procedure was used because embedding and polishing whole sections of bone reduces the likelihood of producing artefactual cracking of histological sections.

RESULTS

Figure 3 shows the frequency distribution of the various grades of lesion in the tibial diaphysis at each time period. Bone scans taken before loading were all negative (grade O). After one week of loading, nearly 50% of the animals showed some evidence of a positive bone scan (grade 1 or 2), but only 4% showed more severe lesions. By three weeks, 48% of the animals demonstrated grade 3 or 4 lesions, while after six weeks 68% of the surviving animals had such a lesion. Fewer than 10% of the animals showed no evidence of change after six weeks of loading.

Some healing occurred between six and nine weeks of loading. All nine-week animals presented with grade 1 lesions, even though all of them had had more severe lesions at three or six weeks (Fig. 4). The bone scans for several of these clearly demonstrated evidence of healing (Fig. 5).

The incidence of grade 3 or 4 stress fractures increased markedly between one and three weeks of loading, and peaked at six weeks (Fig. 3). The percentage of animals with no change at all steadily declined to zero by nine weeks.

No significant scintigraphic changes were observed in non-loaded control animals (Fig. 4), or in the control limbs of loaded animals. The profiles shown in Figure 4 demonstrate that the bony lesions in the treated animals became progressively more severe with continued loading.

Fisher exact probability tests (Siegel 1956) showed a highly significant ($p < 0.001$) association between positive scintigraphic change and radiological evidence of periosteal stress-induced callus formation. There was

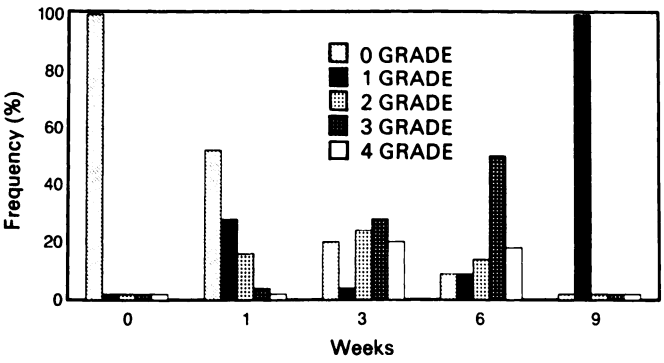

Fig. 3

Histogram showing the grade of scintigraphic lesions after each time period. The severity of the lesions increased up to six weeks; the absence of severe lesions after nine weeks may indicate some spontaneous healing.

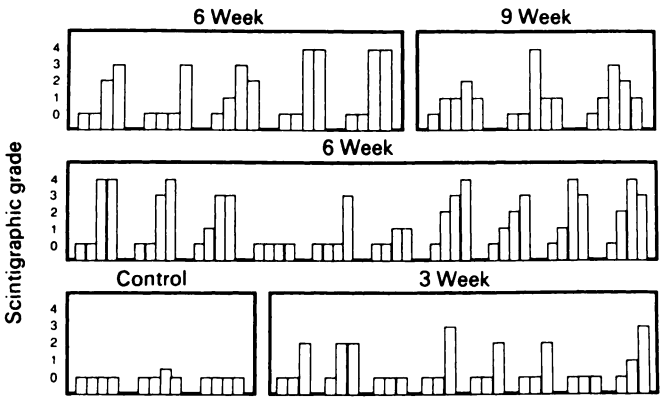

Fig. 4

Individual profiles showing the progression of the scintigraphic grade in all rabbits that demonstrated increased ^{99m}Tc uptake. The four-week and five-week results are included with the three-week animals. Progression is seen in nearly all animals.

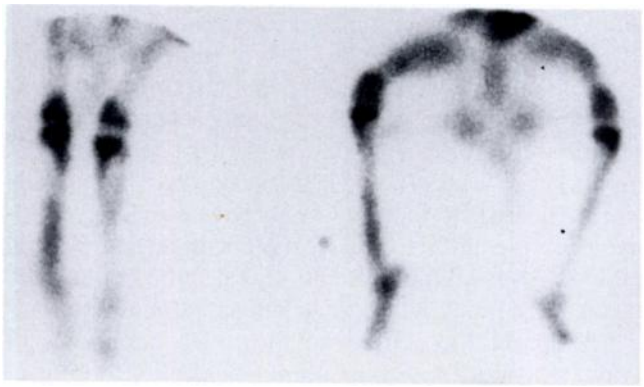

Fig. 5

Scintigraphic image of a rabbit hind limb which showed evidence of spontaneous healing. The edges of the lesion are no longer clearly demarcated and the uptake of ^{99m}Tc is diffuse.

complete correspondence between radiological and scintigraphic changes at the time of sacrifice (Fig. 6). In no case was scintigraphic change observed without radiological evidence of periosteal reaction. When scintigraphic change was absent, radiological change was also absent.

The only radiographic evidence of a fracture was the formation of callus. No definite fractures were observed on radiographs, but microscopic damage was evident at sites of positive bone scan in the tibial diaphysis (Fig. 7). Damage generally consisted of small cracks with a mean length of 346 μm. These were often, but not always, in anatomical association with periosteal callus.

DISCUSSION

To be useful, an animal model for stress fracture should demonstrate several characteristics of the clinical situation. First, bone changes should be progressive; the lesion should become increasingly more severe with continued loading, and regression to an earlier grade should be rare. The individual profiles in Figure 4 demonstrate that progression occurred in every animal. Regression to a previous grade was seen, but only in the later time periods.

Regression was associated with less clearly demarcated edges to the lesion and a diffuse uptake of ^{99m}Tc; we interpret this as evidence of healing. This indicates that lesions may resolve even with continued loading, contrary to current opinion which prescribes rest as the treatment of choice for stress fractures (Orava 1980). All our animals showed progressively more severe changes up to six weeks, so the model does demonstrate progression.

Secondly, positive scintigraphic findings should correlate well with radiological evidence of a stress-induced periosteal reaction (Matin 1983; Milgrom et al 1985a; Uhthoff and Jaworski 1986). Scintigraphic changes predate radiographic changes by two to 12 weeks (Savoca 1971; Fordham and Ramachandran 1974; Geslien et al 1976; Marty et al 1976; Prather et al 1977; Wilcox, Moniot and Green 1977; Roub et al 1979; Mills, Marymont and Murphy 1980; Norfray et al 1980), so the

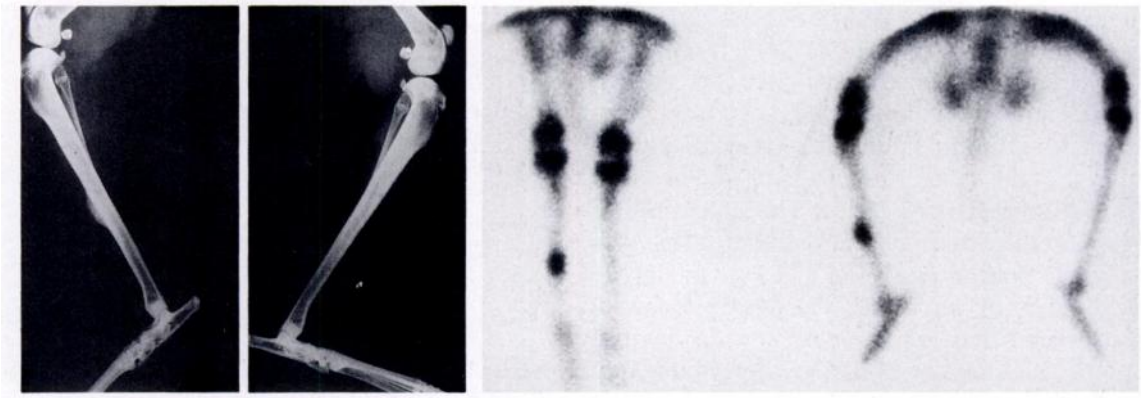

Fig. 6

Radiographs and scintigraphic images showing the corresponding change produced by a stress fracture.

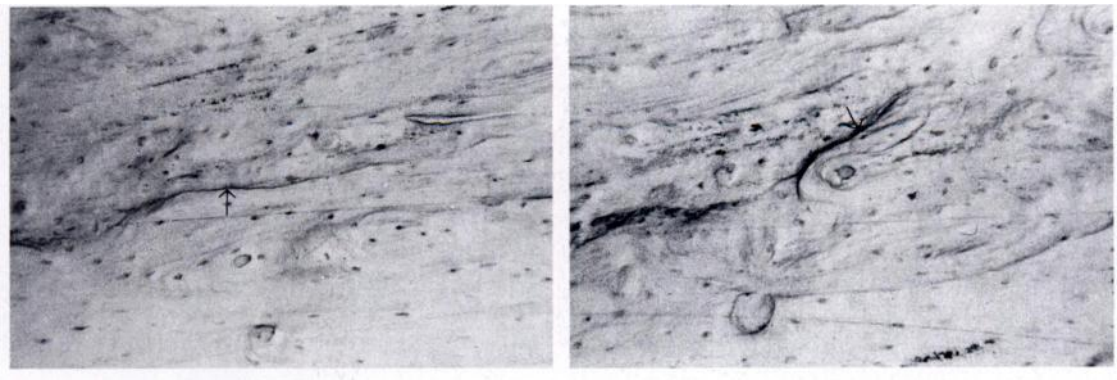

Fig. 7a

Fig. 7b

Many micro-cracks were observed in the tibiae with positive bone scans: photomicrographs from separate animals show micro-cracks (arrows) found in the areas in which ^{99m}Tc uptake was increased (Magnification × 30).

time at which each is first observed may be different. Radiographic confirmation of stress fractures diagnosed by scintigraphy depends upon the duration of symptoms and the location of the lesion (Greaney et al 1983; Milgrom et al 1985a). In our series scintigraphic change was associated with radiological change in every case.

Thirdly, an animal model should demonstrate penetrance. Not all people develop stress fractures under similar loading conditions. If all the animals had developed evidence of stress fracture under our loading regime, then the regime could be considered too severe; the skeletal changes would not accurately represent the physiological adaptation of the system. We found grade 3 or 4 lesions in 48% of the rabbits by three weeks and in 68% by six weeks, comparing well with the reported incidence of stress fracture in physically-active humans (Meurman and Elfving 1980; Rosen, Micheli and Treves 1982; Milgrom et al 1985b).

Fourthly, the location of the stress fractures should be similar. The tibia is reported to be the most common site (Armstrong and Tucker 1964; Orava, Puranen and Ala-Ketola 1978; Belkin 1980; Clement et al 1981; Taunton, Clement and Webber 1981; Sullivan et al 1984; Orava and Hulkko 1984; McBryde 1985; Matheson et al 1987; Hulkko and Orava 1987), lesions in this bone constituting between 20% (McBryde 1975; Walter and Wolf 1977) and 72% (Giladi et al 1985; Zwas et al 1987) of all stress fractures. In our rabbit model, stress fractures develop reliably in the middle and distal tibial diaphysis; 89% were in the midshaft of the tibia, and 74% involved the anterior or anteromedial cortex. The similarities in location support the clinical relevance of the model.

Finally, the time of onset after a change in activity should be similar. In our model, most stress fractures occurred between three and six weeks after the start of loading, and 72% of the stress fractures appeared within the first three weeks. Greaney et al (1983) reported that in US Marines 64% of stress fractures occurred in the first two weeks of training, while Milgrom et al (1985b) found that in Israeli Army recruits, 33% occurred during the first two weeks of basic training with over 50% of the tibial stress fractures within the first four weeks. These timings compare well with those in our model.

Li et al (1985) have also reported a rabbit model for stress fractures in the tibia. They used an electric cage in which rabbits were induced to jump and run. Radiographs showed progressive periosteal bone reactions, like those associated with stress fractures, during the 60-day experimental period. They were able to control the intensity, duration and interval of the electric currents, but not the actual load experienced by the animals, nor were these loads monitored throughout the experiment, and only two control animals were used. While this work provides useful information about the histological changes, the model does not have much flexibility. In our model it is possible to alter specific aspects of the loading regime and to monitor them closely.

Our rabbit model can be used to study the development of stress fractures and permits correlation between the histological, radiological and quantitative scintigraphic changes in a single animal. We hope to be able to define the specific levels of increased activity which will carry a significant risk for a stress fracture, and study appropriate treatment and rehabilitation programmes. The model will also facilitate collection of the basic information required to relate scintigraphic change to the actual skeletal pathology after repetitive stress injury and in this way lead to the earlier diagnosis of potential stress fractures, before the occurrence of clinical symptoms.

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