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Traumatic heterotopic bone formation in the quadriceps muscle

No progression by continuous passive motion in rabbits

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One hind limb in each of 20 New Zealand White rabbits was immobilized for 3 weeks together with daily forcible manipulation to induce heterotopic bone formation in the quadriceps muscle. The rabbits were then divided equally into a control group and a group treated with continuous passive motion (CPM). The effect of CPM on the development of heterotopic bone formation was assessed by radiographs of the femur and by histology. Treatment with CPM did not lead to increased heterotopic bone formation, as compared to the control group.

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Traumatic heterotopic bone formation in soft tissues of an extremity may impair mobility of the adjacent joint (Huss and Puhl 1980, Carlson and Klassen 1984, Nalley et al. 1985). Passive manipulation of joints is commonly employed in a variety of circumstances to improve this range of motion, but has itself been implicated in the development of myositis ossificans (Hait et al. 1970, Ivey 1985). Initial physiotherapy, massage and stretching of a joint should therefore also be avoided after trauma (Nalley et al. 1985). On the other hand, temporary application of Continuous Passive Motion (CPM) is recommended as additional treatment after joint surgery, especially for elbow and knee fractures (Salter 1993). This is consistent with the stimulating effect of CPM on the repair of articular and periarticular tissues demonstrated in previous experimental studies (Salter et al. 1980, O'Driscoll and Salter 1986, Kim et al. 1991, Moran et al. 1992).

Michelsson and Riska (1979) and Michelsson et al. (1980, 1983) described a rabbit model that reliably induces heterotopic bone similar to myositis ossificans in humans. In this model, immobilization of a hind limb in extension, combined with daily forcible mobilization of the knee for about 5 minutes, leads to heterotopic bone formation in the quadriceps muscle within 2 weeks. We used this model to determine whether treatment with CPM might adversely stimulate the development and progression of heterotopic bone formation after muscle injury.

Animals and methods

Using the animal model described by Michelsson et al. (1980), each right hind limb of 20 adolescent New Zealand White rabbits (weight 2.0–2.5 kg) was immobilized during 3 weeks by means of a fiberglass cast, with the knee in maximum extension and the hip free. Once a day, the cast was removed (6 days a week) and the knee was forcefully manipulated for 5 minutes through a maximum passive range of motion. After the daily manipulation, the cast was reapplied. The animals could move in their cages with the cast in place. 6 rabbits were excluded because of epiphyseal fractures of the femur caused by the manipulation, which is a recognized complication (Moed et al. 1994). An additional 6 rabbits with closed epiphyseal plates were admitted to the study. The casting and manipulations were continued for 3 weeks.

Thereafter the rabbits were divided into 2 groups of 10 animals each, based on the grade of ossification present (Table 1). The first group was remobilized on a CPM apparatus for 2 weeks, whereas the control group was allowed to move freely in their cages, without a cast in place for the same period. The CPM apparatus provided continuous passive flexion and extension of the previously immobilized knee joint over an arc of 70 degrees (from 40 to 110 degrees), every 45 seconds, 24 hours a day (Salter et al. 1980).

Lateral soft-tissue radiographs were obtained from the manipulated hind limb of each rabbit after weeks 1, 2, 3, and 5 to evaluate the progression of the ossifications. Radiographs were taken in a standard posi-

Table 1. Number of rabbits in each radiographic group (grade of ossification) against time

	Immobilization period				Remobilization	
	All n 20				Control n 10	CPM n 10
Week no.	0	1	2	3	5	
Grade 0	20	20	2	0 (/2)	0	0
I	0	0	7	6 (/2)	3	3
II	0	0	9	8 (/2)	4	4
III	0	0	2	6 (/2)	3	3

tion, under general anesthesia. Unlabeled radiographs were evaluated and graded for heterotopic ossification independently by two of us (JVS, HK) using the method of Scott et al. (1987).

After killing at 5 weeks, the femora with attached quadriceps muscle were excised and prepared for histological evaluation with hematoxylin and eosin and alcian blue (O'Driscoll and Salter 1986). An average of 10 transverse sections of the specimen (5 mm thick) were cut to include the heterotopic ossification. The sections were spaced at regular 500 μm intervals so that multiple areas of the lesion could be assessed and inadequate sampling diminished.

Results

After 2 weeks of immobilization and manipulation, heterotopic ossifications appeared on lateral radiographs immediately anterior to the femur in the quadriceps muscle in 18 of the 20 rabbits. At the end of the immobilization period of 3 weeks, all rabbits showed some grade of ossification, with 6 rabbits in grade I (mild), 8 in grade II and 6 in grade III (Table 1). After a remobilization period of 2 weeks, no radiographic differences were found in the progression of heterotopic bone between the groups. Each rabbit had maintained the same grade of ossification as before remobilization, only now the ectopic bone appeared to be more organized and had become attached to the anterior side of the femur, whereas earlier the lesion had been slightly separated from the femur. In case of a grade I ossification, the femur was eventually covered by only a thin layer of new bone, whereas in grade III this bone extended as a convexity over the anterior side of the femur (Figure 1).

Histology

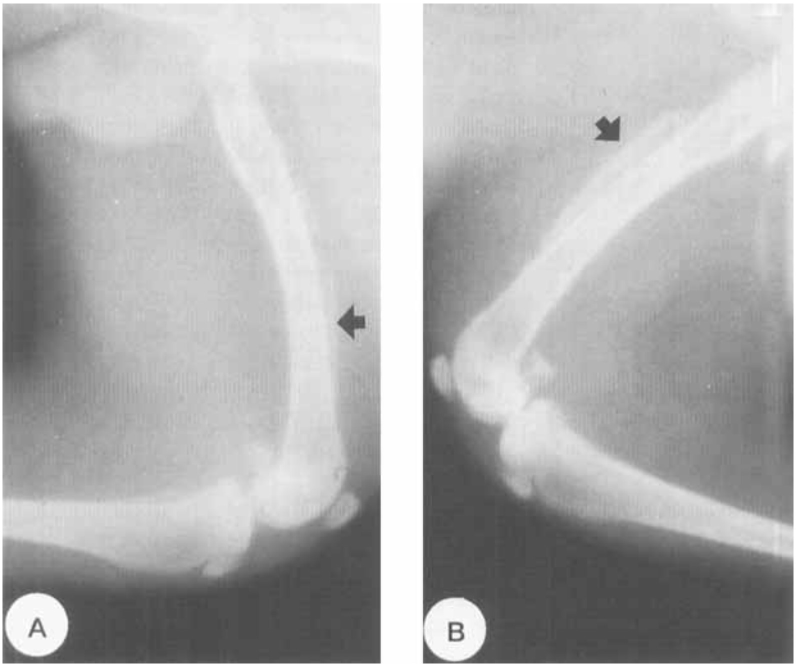
Transverse sections showed the various grades of heterotopic ossification in accordance with the radiographs. The vastus intermedius area had changed into lamellar bony tissue covering the anterior part of the femoral cortex 5 weeks after the start of the experiment. In the outer zone of the ossification, mature calcified, almost lamellar, bone had been formed, while less well organized osteoid-like tissue was found in the middle zone and, centrally, proliferation of granulation and fibrous covering the femoral cortex took place. Calcifying woven bone seemed to be formed directly from the periosteum, which appeared to have been elevated from the femur with subsequent lifting of the vastus intermedius muscle. Occasionally, cartilage was seen in the outer zone, representing enchondral ossification (Figure 2).

Discussion

Several authors have used the Michelsson model to study the development and prevention of heterotopic ossification in animals (Michelsson and Riska 1979, Michelsson et al. 1980, 1983, Aho et al. 1988, Moed et al. 1994). This model has consistently led to bone formation in the extensor muscles of the rabbit. Repetitive mobilization of an initially immobilized joint seems to induce heterotopic bone formation and the periosteum is also believed to participate in this process to some extent, because of muscle attachments. The heterotopic ossification is morphologically and radiographically similar to myositis ossificans in humans (Michelsson et al. 1983). Therefore, this model provides a good basis for determining whether treatment with CPM of a muscle-injured extremity may provoke or increase ectopic bone formation.

In our study, treatment with CPM did not lead to an increased heterotopic bone formation in a muscle-injured limb. We performed CPM for 2 weeks only and the follow-up was no longer than 5 weeks. However, Michelsson et al. (1980) observed marked progression of heterotopic bone formation from the third to the fifth week when manipulation was continued and, if passive manipulation with CPM had a similar stimulating effect on bone formation, at least some progression could have been expected. CPM is of value in stimulating the repair of various articular and periarticular tissues (Salter 1993) and is often used after articular fractures. The role of passive exercising of immobilized joints in humans is still controversial. In some clinical studies, passive mobilization of joints was of no benefit and, indeed, sometimes harmful (Michelsson et al. 1983). Our findings indicate that application of CPM does not stimulate the development or progression of heterotopic bone formation in muscle-injured rabbits, which supports our assump-

Figure 1. Radiographic evaluation of ectopic bone formation.



First signs of ossification after 2 weeks, starting with a convex line parallel to the anterior side of the femur (arrow), representing the ossifying elevated periosteum.

Severe ossification (arrows, Grade III; B) at 5 weeks after treatment with CPM

tion that CPM can safely be applied to a muscle-injured extremity.

The ossifications in the quadriceps muscle in both the control group and the CPM-treated group had an appearance like that described in earlier studies using the Michelsson model. All lesions appeared first on radiographs as a convex radio-dense line in the extensor muscles parallel to the anterior side of the femur (Figure1). This line could represent the ossifying periosteum elevated from the femur because of repeated manipulation of the limb with resultant traction on the quadriceps origo. An elevated periosteum is known to induce a strong osteoblastic reaction and in humans it is also regarded as one of the etiological factors involved in the development of heterotopic bone (Ivey 1985). Histology showed that the ossification had indeed coalesced with the anterior side of the femur and maturation of bony-tissue was clearly visible from the periphery to the center. This maturation pattern, with mature lamellar bone on the outside and active granulation tissue in the center, can be interpreted as the "zonal phenomenon" observed in human myositis ossificans.

Acknowledgement

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Figure 2.

A. Histological findings showing HE-stained transverse section of ectopic bone formation in a rabbit hind limb at 5 weeks, showing the heterotopic ossification. Maturation of bony tissue from the periphery (P) to the center (C), ×60.

B. Detail of mature ectopic bone, ×150.

C and D. Middle of ectopic bone after 5 weeks, showing first signs of remodeling of woven bone, ×150.

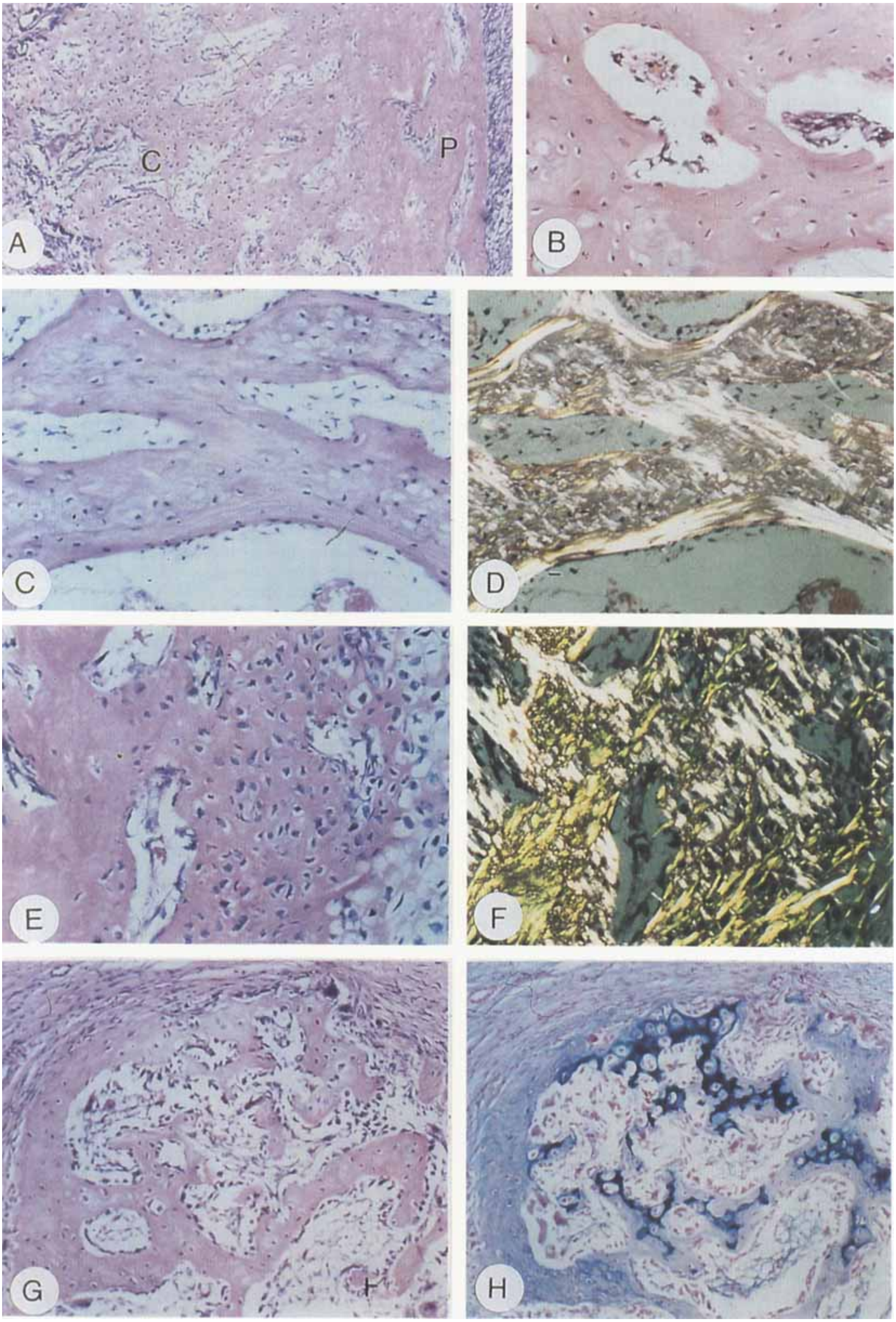
D. Same location as C but with polarized light.

E and F. Center of ossification showing woven bone.

F. Same location as shown in E but with polarized light, ×150.

G and H. Local areas at the outside of the ectopic bone of partly cartilaginous tissue.

H. Same location as shown in G, but with alcian blue staining, ×90.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P
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