Osteogenic capacity of vascularised periosteum: experimental study using rib periosteum in rabbits

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Summary—The osteogenic capacity of vascularised periosteum was investigated in rabbits using island rib periosteum nourished by intercostal vessels as an experimental model. Twenty-three adult white female rabbits were used. Excellent bone formation was observed after 2 postoperative weeks in each case. In the early stages of bone formation, extensive chondral ossification was observed. Newly formed bone became mature after 4 to 8 postoperative weeks, on histological examination.

There has been great controversy about the osteogenic capacity of periosteum since Ollier (1867) first reported on it. This was mainly because stable results with free periosteal grafts could not be obtained in experimental studies. Clinically, Skoog (1967) introduced the use of the maxillary periosteal flap in primary repair of the alveolar cleft. Ritsilä et al. (1972) used free periosteal grafts from the tibia in repair of the primary palate.

Finley et al. (1978) reported on excellent osteogenic capacity of revascularised periosteal grafts in tibial defects of dogs. It was suggested that vascularisation was very important in bone formation from periosteum. Wildenberg et al. (1984) reported that periosteal grafts taken from different donor sites showed different osteogenic capacity.

In this experimental study, the osteogenic capacity of vascularised rib periosteum was investigated using rabbits, and the process of bone regeneration was investigated by histological examination and bone scintigrams using Tc-99m Methylene Diphosphonate (MDP).

Materials and method
Twenty-three adult white female rabbits weighing 2800 to 3500 g were used. The rabbits were sedated intravenously with sodium pentobarbitone and anaesthetised through inhalation of a mixture of halothane, nitrous oxide and oxygen. The left chest was shaved and prepared with povidone-iodine and the operation carried out under strict aseptic conditions.

A skin incision was made along the 7th rib on the left side of the chest. The latissimus dorsi muscle was cut in order to expose the rib and the intercostal muscles were divided for about 6 cm along both sides of the 7th rib. Incisions were made through the parietal pleura, care being taken not to injure the lung. Next, an island of rib about 5 cm long, nourished by intercostal vessels, was elevated. The pedicle was carefully dissected for about 1 cm. Bleeding from the distal stump of rib was observed. Thereafter, the rib was removed subperiosteally and the island musculoperiosteal flap, which was composed of the intercostal muscles and periosteum, was completed (Fig. 1). The periosteum was tubed. The musculoperiosteal flap was replaced

Figure 1—Diagram of an elevated island rib musculoperiosteal flap pedicled on intercostal vessels (P = periosteum, R = rib, V = vessels of pedicle, L = lung).
in situ and covered with latissimus dorsi muscle. The skin incision was closed.

As a control, the pedicle was ligated before the flap was replaced in three rabbits.

Six rabbits died from respiratory failure due to pneumothorax just after operation. Three of these were caused by injury to the lung and the other three by technical errors in closing the wound. Rabbits which died or whose wounds were infected were replaced.

Five rabbits were examined postoperatively at each of 2, 4, 8 and 16 weeks, and the three control rabbits were examined at 8 weeks. X-ray examinations were carried out and the specimens of regenerated tissue were decalcified and embedded in paraffin. Sections 10 μm thick were prepared and stained with haematoxylin and eosin and examined under a light microscope.

Bone scintigraphy with Tc-99m MDP was performed just after sacrifice in three rabbits at each of 2 and 8 weeks and in the three control rabbits and quantitative analysis carried out. Positive uptake of Tc-99m MDP was taken to reflect bone viability. 2–4 mCi of Tc-99m MDP were administered intravenously via an ear vein to each rabbit. Approximately 4 hours after the injection the rabbit was sacrificed. The left chest wall was obtained en bloc. After X-ray examination, bone scintigraphy was performed using a gamma camera (G.E. Maxicamera 400 AT). A 128 × 128 digital image was obtained at 500 000 counts. The uptake of Tc-99m MDP in the regenerated bone was expressed as the count ratio to that in the 9th rib, which was not touched at the operation.

Results
The major data are summarised in Table 1. The incidence of bone regeneration was 100% at each of the postoperative weeks studied. The patency of all the pedicles was confirmed at sacrifice.

At only 2 weeks postoperatively, roentgenograms showed evidence of excellent bone regeneration. The volume of the regenerated bone was about the same as, or slightly larger than, that of the original 7th rib on X-ray films (Fig. 2). Histological examination showed woven fine cancellous bone with large areas of cartilage. The regenerated bone was derived from cartilage in some areas and fibrous tissue in other areas (Fig. 3). Bone scintigrams revealed much larger uptake of Tc-99m MDP in the regenerated bone than in the 9th rib (8.25 ± 2.138; ratio: mean ± SD) (Fig. 4). At 4 postoperative weeks, all cases showed the same evidence of excellent bone regeneration.

Histological examination showed woven fine cancellous bone and fine-fibred compact bone with small areas of cartilage. At 8 postoperative weeks, the volume of the regenerated bone was about the same as that of the original 7th rib (Fig. 5). Histological examination showed fine-fibred compact bone having a central marrow cavity (Fig. 6) very similar to the original ribs. Bone scintigram revealed slightly larger uptake of Tc-99m MDP in the regenerated bone (1.74 ± 0.267).

In the control cases whose pedicles were ligated, no bone formation was seen on X-rays and bone scintigram showed no uptake of Tc-99m MDP (Fig. 7). At 16 postoperative weeks, both the X-ray and histological findings were the same as those at 8 weeks.

Discussion
Finley et al. (1978) reported the rapid formation of new bone, with bony union, after the transplanta-

| Table 1 | Incidence of bone regeneration and uptake of Tc-99m MDP in the regenerated bone. The uptake is expressed as the ratio to that in the 9th rib. |
|---|---|---|---|---|
| **Postoperative Weeks** | 2 | 4 | 8 | 16 |
| **Experimental Group** | | | | |
| Island rib periosteum (vascularised) | | | | |
| Percentage of bone regeneration (%) | 100 | 100 | 100 | 100 |
| Ratio in bone scintigram (mean ± SD) | 8.25 ± 2.138 | 1.74 | 0.267 |
| Control (pedicle ligated) | | | | |
| Percentage of bone regeneration (%) | 0 | | | |
| Ratio in bone scintigram | 0 | | | |
Figure 2—X-ray after 2 postoperative weeks. Arrows indicate the regenerated bone which is larger in diameter than the original rib. Figure 3—Histology after 2 postoperative weeks. Note regenerated woven bone and cartilage (H.E. x 100). Figure 4—Bone scintigram after 2 postoperative weeks. An intense uptake of Tc-99m MDP is shown (arrow). Figure 5—X-ray after 8 postoperative weeks. Arrows indicate the regenerated bone which is about the same in diameter as the original rib. Figure 6—Histology after 8 postoperative weeks. Fine-fibred compact bone having a central marrow cavity is observed (H.E. x 40). Figure 7—Control case whose pedicle was ligated. Bone scintigram after 8 postoperative weeks. No uptake of Tc-99m MDP is observed (arrows).
tion of vascularised rib periosteal grafts to defects in the tibias of dogs. Puckett et al. (1979), however, reported the uniform failure of revascularised rib periosteum to form bone in the fibula of dogs. They emphasised the influence of weight-bearing or stress on bone formation from grafted periosteum. Wildenberg et al. (1984) reported that tibial periosteum had greater osteogenic capacity than rib periosteum. We (Takato et al., 1986) also reported the excellent osteogenic capacity of vascularised tibial periosteum in rabbits. We concluded that the musculoperiosteal flap, which had ample blood circulation, had a greater osteogenic capacity than the fascioperiosteal flap. We believe that osteogenic capacity mainly depends on the volume of periosteum and blood circulation.

In this experimental study, we investigated the osteogenic capacity of the vascularised rib musculoperiosteal flap in rabbits. This island flap, pedicled with intercostal vessels, was replaced in situ and, under this ideal condition, excellent bone formation was obtained in every case as early as 2 postoperative weeks. At this early stage chondral ossification seemed to play a principal role in bone regeneration. The histological findings were similar to those at the epiphyseal line of a long bone, where active osteogenesis takes place. Bone scintigram revealed an uptake of Tc-99m MDP in the regenerated bone about eight times larger than in the untouched rib. (In our experimental study using vascularised tibial musculoperiosteal flaps, the uptake of Tc-99m MDP in the regenerated bone was 20 times as large as that in the untouched tibia at 2 weeks postoperatively (Takato et al., 1986)).

Berggren et al. (1982) reported that even a small amount of osteoblastic activity in free periosteal grafts produced positive bone scintiscans in their experimental study using dogs, and unsuccessfully revascularised and dead bone showed negative or subnormal uptake in the early postoperative period. In clinical cases, unsuccessfully revascularised bone never shows a positive uptake of Tc-99m MDP (Takato et al., 1988). We therefore believe that bone scintigrams using Tc-99m MDP accurately reflect osteogenic activity. The larger uptake in the tibial musculoperiosteal flap than in this model confirms the suggestion by Wildenberg et al. (1984) that tibial periosteum has greater osteogenic capacity than rib periosteum.

At 8 postoperative weeks the volume and histological findings of the regenerated bone were about the same as those of the original rib and the uptake of Tc-99m MDP had subsided considerably. Osteogenesis was probably completed by this time and, as the regenerated bone was slightly smaller in volume than at 2 weeks, remodelling had presumably started.

Although good results using rib periosteum have not previously been reported, the excellent osteogenic capacity of a vascularised rib musculoperiosteal flap was confirmed in this study. We have previously reported the development of bone after vascularised perichondrial grafting (Takato et al., 1987). A good blood circulation seems to have a strong influence on the development of bone.

As regards osteogenesis of periosteum, mechanical stress may not be a prerequisite for new bone formation. Although this experimental model did not undergo such a stress, a large amount of bone was obtained. Thereafter, the regenerated bone is remodelled according to the surrounding conditions and we think that weight-bearing or stress may have a strong influence on such remodelling. If there is no mechanical stress, the regenerated bone may be resorbed. In this rib model, perhaps the regenerated bone was stimulated by the respiratory muscles; not much was resorbed and it was remodelled to resemble the original rib.

In conclusion, it seems that a vascularised periosteal graft has promising possibilities provided that it is to be used as a musculoperiosteal graft under the stimulus of weight-bearing or stress.

References


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