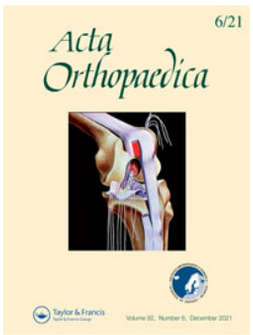

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Effect of local prostaglandin E_2 on periosteum and muscle in rabbits

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We assessed the target tissue for the stimulatory effect of prostaglandin E_2 (PGE_2) on bone formation previously observed during fracture healing. PGE_2 was infused into tibial periosteal tissue in the right leg of 7 rabbits and into the anterior tibial muscle in the right leg of 7 other rabbits for 6 weeks. Solvent solution was infused into the left leg. PGE_2 infusion at the periosteum caused the formation of primitive woven bone with large amounts of connective tissue; solvent infusion caused small amounts of normal periosteal bone formation. In the neighboring cortical bone, remodeling was increased after PGE_2 infusion compared to solvent infusion. In the muscle, PGE_2 infusion caused the formation of connective tissue with small amounts of woven bone. Thus, the major effects of PGE_2 infusion at the site of the periosteum was the formation of primitive woven bone and in muscles the formation of connective tissue.

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Prostaglandin E_1 (PGE_1) and prostaglandin E_2 (PGE_2) stimulate bone formation (Chyun and Raisz 1984) and resorption (Dietrich et al. 1975) in vitro. Parenteral PGE_1 or PGE_2 treatment in infants with persistent ductus arteriosus causes periosteal bone formation (Ueda et al. 1980, Hoevels-Guerich et al. 1984, Poznanski et al. 1985, Williams 1986, Høst et al. 1988, Jørgensen et al. 1988) and long-term subcutaneous PGE_2 administration increases the metaphyseal bone mass in rats (Jee et al. 1985). Oral PGE_2 treatment in dogs increases regional remodeling after rib fracture (Shih and Norridin 1986). Previously we have demonstrated that local PGE_2 infusion at the site of an osteotomy in rabbits causes a profuse irregular callus formation (Keller et al. 1992a). In this rabbit study we investigated the effect of local PGE_2 at the periosteal site in one experiment and in muscle tissue in another experiment.

Material and methods

14 adult (9 month) New Zealand white rabbits, weighing approximately 4 kg kept in separate cages, were randomized into two groups. A mini-osmotic pump (Alzet® model 2ML4, reservoir volume 2.2 mL) was implanted subcutaneously in both thighs and a small polyvinyl catheter was tunneled distally for delivery. The reservoir of pumps implanted in the left leg contained solvent solution (40 percent ethanol in propylene glycol) and those implanted in the right legs contained 2 mg of PGE_2 in the solvent (pumping rate: 0.8 mg/h per kg body weight). We chose a dose of PGE_2 known to stimulate callus formation after a plated osteotomy and a solvent solution, in which PGE_2 was stable throughout the experiment (Keller et al. 1992a). In one group of 7 randomly allocated rabbits, the anterolateral surface of both tibial bones was exposed through an incision in the tibial fascia. The superficial periosteal membrane was removed over a 8 mm wide and 20 mm long area at the tibio-fibular junction. The tip of the catheter was placed at the periosteal defect. In another group of 7 rabbits, the anterior tibial muscles were cut halfway through with a longitudinal anterolateral incision and the tip of the catheter placed in the center of the muscle.

After 3 weeks the reservoirs were replaced with new reservoirs and both tibiae were examined by radiography with anteroposterior and lateral projections. The leg was placed on the film 65 cm from the tube. On Day 32 and Day 39, intravital labeling with oxytetracycline (15 mg/kg body weight in 0.5 mL lidocain) was performed. The rabbits were killed after a total infusion period of 6 weeks. The tibial bones were dissected free from soft tissue and radiographed in two planes again. The bone mineral content of the diaphysis was determined with photon absorptiometry. Transverse bone sections were sawn for microradiography, histology and histomorphometry.

Table 1. Bone mineral content of tibial diaphysis, callus area, cortical porosity, Haversian canal diameter and formative sites after local infusion with PGE_2 in diaphyseal periost. Mean *SE*

Groups	n	Bone mineral content (g/cm)	Callus area mm^2		Cortical porosity (percent)	Haversian canal diam. (µm)	Formative sites per section
			3 weeks	6 weeks			
PGE_2 group	7	1.92 *0.25*[a]	79 *20*[b]	226 *70*[b]	4.2 *0.6*	19.5 *2.1*[a]	12.7 *3.2*[b]
Control group	7	1.19 *0.04*	3 *2*	4 *2*	3.4 *0.4*	13.8 *1.7*	2.1 *0.7*

[a] $P < 0.05$, [b] $P < 0.01$

Infused anterior tibial muscles were removed in toto, weighed on a precision scale and radiographed. Transverse sections were then cut for histology.

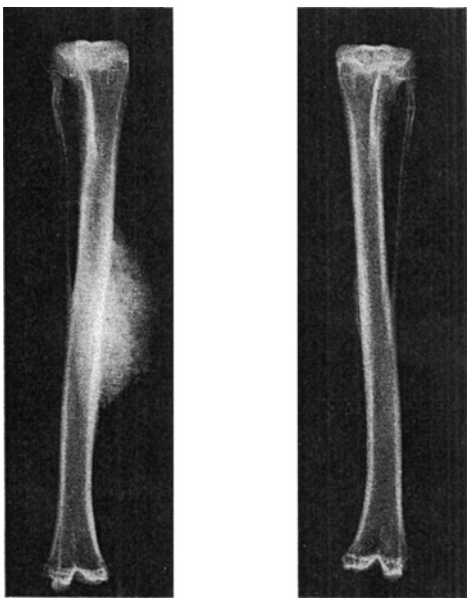
Callus area

After a 4.4 radiographic magnification the callus area was estimated by point-counting, using a 5 mm grid (Keller et al. 1992a). The result was given as the mean of the two radiographic projections. The coefficient of variation of a double measurement was 4 percent.

Bone mineral content

The tibial diaphysis was scanned by photon absorptiometry (Gammatec Osteodensitometer, Model 30 GT, Hareskov, Denmark) every 4 mm, starting 2 cm from the proximal end of the bone and 6 cm distally, which was about the middle 3/5 of the bone (Keller et al. 1987). The mean of the 15 scans was calculated. The coefficient of variation of a double measurement was 1 percent.

Figure 1. 6 weeks after local infusion into periosteum.



PGE_2. Solvent solution.

Histology and histomorphometry

Transverse 100-mm bone sections were sawn from the middle of the tibial bone for microradiography (Faxitron®) and histomorphometry. Porosity was measured using point-counting in 10 randomly selected fields at a 200-fold magnification. Bone formation was measured by counting the number of tetracycline double-labeled canals per section at a 200-fold magnification. Bone resorption was indirectly estimated by the mean diameter of 50 randomly selected Haversian canals at 200-fold magnification. The remaining tibial diaphyseal bone was demineralized in K-EDTA and 10 µm transverse bone sections were cut near the middle of the bone for conventional histology.

Statistics

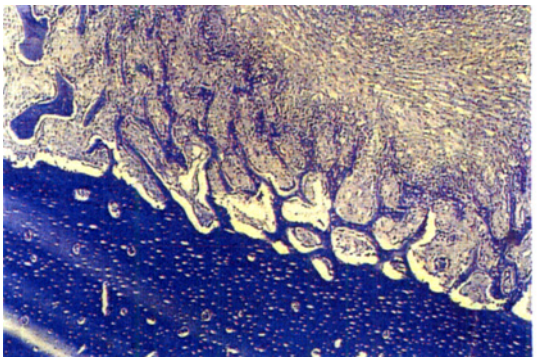
The two-tailed Wilcoxon's test was used to compare the PGE_2-infused leg with the solvent-infused leg. A P-value < 0.05 was considered to be significant.

Results

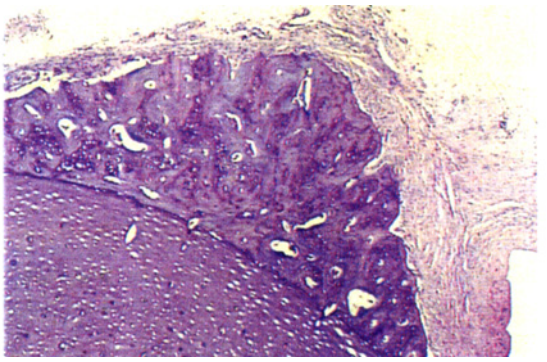
Infusion in the periosteum

All PGE_2-infused legs showed large amounts of periosteal bone formation on radiographs after 3 and 6 weeks, compared with minimal amounts of periosteal bone formation in the control legs ($P < 0.05$; Figure 1). In the PGE_2-infused legs the amount of bone formation increased from 3 to 6 weeks, whereas it was unchanged in the control legs (Table 1). The bone mineral content of the tibial diaphysis increased after PGE_2 infusion, compared with the control legs. In the

Figure 2. Histological section of formed callus after 6 weeks of local infusion in periosteum.

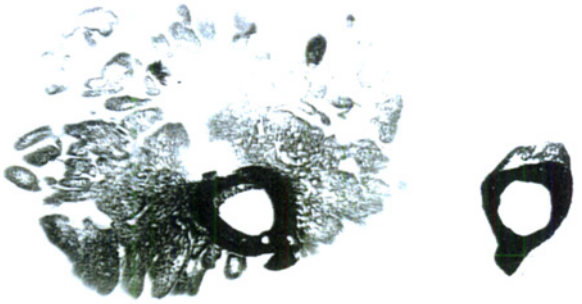


PGE_2. Periosteal callus with disconnected bone trabeculae of primitive woven bone and connective tissue, ×40.



Solvent solution. Periosteal callus on the cortical bone surface. Toluidine blue, ×100.

Figure 3. Microradiography of a transverse bone section after 6 weeks of local infusion in periosteum.



PGE_2

Solvent solution

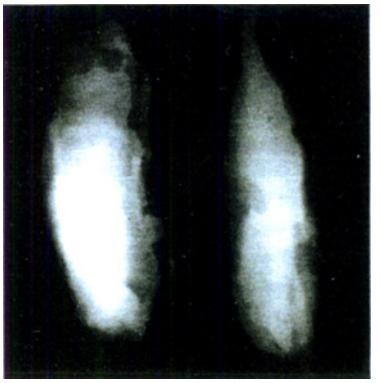
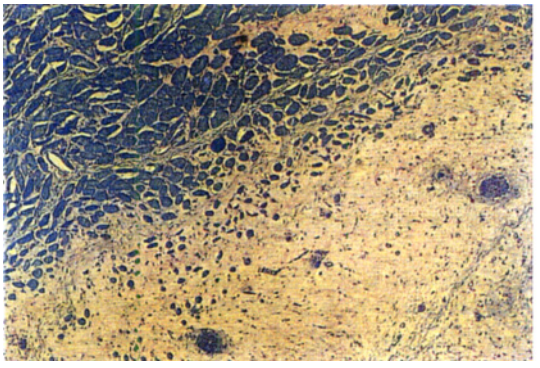
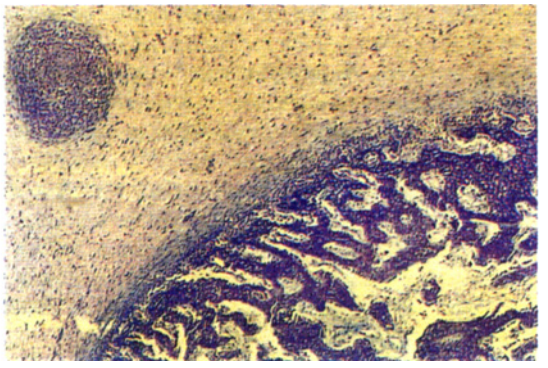


Figure 4. 6 weeks after infusion into musculus tibialis anterior. PGE_2 (left) and solvent solution (right).

Figure 5. Histology after 6 weeks of PGE_2 infusion.



Depletion of muscle fibers by connective tissue. ×40



Metaplastic bone formation in the connective tissue. ×100

neighboring cortical bone, PGE_2 caused an increase in the diameter of Haversian canals and the number of tetracyclin-labeled Haversian canals.

Histology of the PGE_2-infused legs revealed a primitive periosteal woven bone with thin, partly disintegrated trabeculae in a richly vascularized connective tissue (Figure 2). On microradiography the bone formation throughout the area occurred at multiple foci (Figure 3). In the control legs, histology showed only small amounts of normal periosteal bone formation with negligible amounts of connective tissue (Figure 2). On microradiography the bone appeared regular (Figure 3).

Infusion in the muscle

The mean weight of the PGE_2-infused muscles was 10.1 ± 0.8 g, whereas that of the control legs was 7.7 ± 0.7 g ($P < 0.05$). After PGE_2 infusion the muscles were greyish in color in the central parts and small hard islets occurred in the center around the tip of the catheter. The radiographs revealed a few small (mm) mineralized bony islets in 4 of the 7 PGE_2-infused legs, but none were evident in the control legs (Figure 4). After PGE_2 infusion the muscles were normal only in the most superficial parts. In the center of the muscles there was a depletion of muscle fibers replaced by a richly vascularized connective tissue. The bony foci consisted of primary woven trabeculae (Figure 5). In the solvent-infused muscles small amounts of connective tissue were located at the catheter tip in the muscle (Figure 5).

Discussion

The present study demonstrates that PGE_2 infusion at a periosteal defect stimulates bone-forming cells as well as fibroblastic cells. We removed the periosteum anterolaterally to ensure stimulation of the remaining osteoblastic cells on the bone surface (Nijweide et al. 1981). However, the model allows stimulation of the adjacent muscle as well. Primitive mesenchymal cells in the periosteum cambium layer and bone marrow are committed bone-forming cells (DOPC; determined osteoprogenitor cells) (Owen 1970, Friedenstein 1973), whereas less developed primitive mesenchymal cells in muscle, bone marrow or periosteum may differentiate into bone forming cells after osteogenetic induction (IOPC; inducible osteoprogenitor cells) (Owen 1970, Friedenstein 1973).

The observed effects of PGE_2 infusion at the periosteum in the present study may be caused by PGE_2 directly or indirectly via release of cytokines or growth factors (Hulth 1989). In vitro PGE_2 stimulates replication of both osteoblast-like cells and fibroblast-like cells, which may explain the relatively large amounts of connective tissue in the periosteal bone (van der Plas et al. 1985). Moreover, PGE_2 may stimulate bone formation in osteoblastic cells (Chyun and Raisz 1984, Nefussi and Baron 1985, Raisz and Fall 1990). However, PGE_2 is a known stimulator of inflammatory reactions (Vane 1971, Bomalaski et al. 1983), which may cause release of different growth factors. Finally, PGE_2 in vitro stimulates insulin-like growth factor I synthesis in osteoblast-enriched cultures (McCarthy et al. 1991).

We chose a dose of PGE_2, known to stimulate callus formation after a plated osteotomy in rabbits (Keller et al. 1992b) and a solvent, in which PGE_2 is stable (Keller et al. 1992a). In the present study, periosteal bone formation after PGE_2 infusion was similar (structure and amount) to that observed after a plated osteotomy (Keller et al. 1992a). Both in the present experiment and in the previous plated tibial osteotomy experiment, PGE_2 increased the total amount of mineral, indicating that PGE_2 induces a true bone stimulating effect and not a normal bone formation response with concomitant fibrous tissue formation.

In fracture healing, the muscles are probably the source of IOPC (Owen 1970, McKibbin 1978, Hulth 1989). Bone morphogenetic protein as well as different growth factors play important roles in osteogenic induction of these primitive mesenchymal cells (IOPC) (Urist et al. 1983). Our results show that PGE_2 infusion in the muscle may cause osteogenic induction. However, it is obvious that the main effect is the stimulation of connective tissue.

In the last part of the fracture-healing process the bone is reorganized by remodeling (McKibbin 1978). The increase in remodeling activity in the cortical bone is called the regional acceleratory phenomenon (RAP) (Frost 1983). Previous studies (Sudmann and Bang 1979, Keller et al. 1989) have shown that inhibition of the prostaglandin synthesis with indomethacin reduces the RAP, suggesting that PGE_2 has a direct activating effect on the remodeling process. Local application of PGE_2 in fractures has been claimed both to stimulate (Shih and Norridin 1986) and not to stimulate the RAP effect (Keller et al. 1992a). In the present study PGE_2 stimulated the RAP. However, the increase in remodeling may be initiated by the non-specific tissue reaction as well.

Our present study shows that PGE_2 has a general stimulatory effect on cell replication, particularly in bone formation, when infused close to bone or at a fracture site, as previously reported (Keller et al. 1992a). PGE_2 may prove useful for bone stimulation in combination with specific growth factors.

Acknowledgements

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