Effect of local prostaglandin E\textsubscript{2} on periosteum and muscle in rabbits

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We assessed the target tissue for the stimulatory effect of prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) on bone formation previously observed during fracture healing. PGE\textsubscript{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\textsubscript{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\textsubscript{2} infusion compared to solvent infusion. In the muscle, PGE\textsubscript{2} infusion caused the formation of connective tissue with small amounts of woven bone. Thus, the major effects of PGE\textsubscript{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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Submitted 91-11-12. Accepted 92-04-17

Prostaglandin E\textsubscript{1} (PGE\textsubscript{1}) and prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) stimulate bone formation (Chyun and Raisz 1984) and resorption (Dietrich et al. 1975) in vitro. Parenteral PGE\textsubscript{1} or PGE\textsubscript{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, Host et al. 1988, Jørgensen et al. 1988) and long-term subcutaneous PGE\textsubscript{2} administration increases the metaphyseal bone mass in rats (Jee et al. 1985). Oral PGE\textsubscript{2} treatment in dogs increases regional remodeling after rib fracture (Shih and Norridin 1986). Previously we have demonstrated that local PGE\textsubscript{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\textsubscript{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\textsuperscript{®} 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\textsubscript{2} in the solvent (pumping rate: 0.8 mg/h per kg body weight). We chose a dose of PGE\textsubscript{2} known to stimulate callus formation after a plated osteotomy and a solvent solution, in which PGE\textsubscript{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 lidocaine) 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.
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.

**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 PGE2-infused leg with the solvent-infused leg. A P-value < 0.05 was considered to be significant.

**Results**

**Infusion in the periosteum**

All PGE2-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 PGE2-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 PGE2 infusion, compared with the control legs. In the
Figure 2. Histological section of formed callus after 6 weeks of local infusion in periosteum.

PGE₂. Periosteal callus with disconnected bone trabeculae of primitive woven bone and connective tissue, x40.

Solvent solution. Periosteal callus on the cortical bone surface. Toluidine blue, x100.

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

PGE₂ Solvent solution

Figure 4. 6 weeks after infusion into muscle tibialis anterior. PGE₂ (left) and solvent solution (right).

Figure 5. Histology after 6 weeks of PGE₂ infusion.

Depletion of muscle fibers by connective tissue. x40

Metaplastic bone formation in the connective tissue. x100
neighboring cortical bone, PGE2 caused an increase in
the diameter of Haversian canals and the number of
tetracyclin-labeled Haversian canals.

Histology of the PGE2-infused legs revealed a
primitive periosteal woven bone with thin, partly dis-
integrated trabeculae in a richly vascularized connec-
tive 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 forma-
tion with negligible amounts of connective tissue (Fig-
ure 2). On microradiography the bone appeared regu-
lar (Figure 3).

Infusion in the muscle
The mean weight of the PGE2-infused muscles was
10.1 ± 0.8 g, whereas that of the control legs was 7.7 ±
0.7 g (P < 0.05). After PGE2 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 PGE2-infused
legs, but none were evident in the control legs (Figure
4). After PGE2 infusion the muscles were normal only
in the most superficial parts. In the center of the mus-
cles 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 connec-
tive tissue were located at the catheter tip in the mus-
cle (Figure 5).

Discussion
The present study demonstrates that PGE2 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 periostem may dif-
ferentiate into bone forming cells after osteogenic
induction (IOPC; inducible osteoprogenitor cells)

The observed effects of PGE2 infusion at the peri-
stem in the present study may be caused by PGE2
directly or indirectly via release of cytokines or growth
factors (Hulth 1989). In vitro PGE2 stimulates replica-
tion 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, PGE2 may stimulate bone
formation in osteoblastic cells (Chyun and Raisz 1984,
Nefussi and Baron 1985, Raisz and Fall 1990). How-
ever, PGE2 is a known stimulator of inflammatory
reactions (Vane 1971, Bomalaski et al. 1983), which
may cause release of different growth factors. Finally,
PGE2 in vitro stimulates insulin-like growth factor I
synthesis in osteoblast-enriched cultures (McCarthy et

We chose a dose of PGE2, known to stimulate callus
formation after a plated osteotomy in rabbits (Keller et
al. 1992b) and a solvent, in which PGE2 is stable (Kel-
ler et al. 1992a). In the present study, periosteal bone
formation after PGE2 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,
PGE2 increased the total amount of mineral, indicating
that PGE2 induces a true bone stimulating effect and
not a normal bone formation response with concomi-
tant 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 PGE2 infu-
sion in the muscle may cause osteogenic induction.
However, it is obvious that the main effect is the stim-
ulation 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 PGE2 has a direct activating
effect on the remodeling process. Local application of
PGE2 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 PGE2
stimulated the RAP. However, the increase in remodel-
ing may be initiated by the non-specific tissue reaction
as well.

Our present study shows that PGE2 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). PGE2 may prove useful for bone stimulation in
combination with specific growth factors.
Acknowledgements

This work was supported by grants from the University of Aarhus, the Danish Medical Research Council and the Upjohn Company, Denmark. Technical assistance with the bone density measurements from J. Marquersen, pharmacist, Department of Clinical Physiology and Nuclear Medicine, Århus Kommunehospital is gratefully acknowledged. Furthermore, we thank Poul Erik Nielsen, photographer, for help with the photos.

References


