Effect of local prostaglandin E_2 on fracture callus in rabbits

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We investigated the effect of local infusion with prostaglandin E_2 (PGE_2) in doses of 0.0003 to 4.0 mg/hour per kg body weight for 6 weeks on a plated unilateral osteotomy in rabbits. PGE_2 caused a dose-dependent stimulation of callus formation. Total bone mineral content increased, although the mineral content per volume of the callus was reduced. In another experiment, PGE_2 was infused either in the first half or in the second half of the healing period. No effect of PGE_2 infusion could be observed in the first half of the 6-week healing period, whereas PGE_2 infusion during the second half caused callus stimulation.

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Prostaglandin E_2 (PGE_2) is probably an important regulator of bone formation and resorption (Hulth 1989, Raisz and Fall 1990). Previously, we have demonstrated that local infusion of PGE_2 at the osteotomy site of a plated tibial rabbit osteotomy stimulates callus development (Keller et al. 1992). In the first part of the present study we investigated the callus-stimulatory effect of PGE_2, using the same model, but with varying doses of PGE_2 over a 6-week healing period. In the second part of the study we infused PGE_2 either in the first three-week period of healing or in the last three weeks to establish which phase of bone healing was most sensitive to PGE_2.

Materials and methods

9-month-old New Zealand white rabbits weighing about 4 kg were used, and kept in separate cages. In the first part of the study, 32 rabbits were given a local infusion of PGE_2 (0.0003, 0.006, 0.16, 0.8 µg/h per kg body weight) at the osteotomy area for 6 weeks. A previously reported group (Keller et al. 1992) of 30 rabbits infused with either solvent solution or 4.0 µg/h per kg body weight of PGE_2 served as controls. In the second part of the study 8 rabbits were given local infusion of PGE_2 (4 µg/h per kg body weight) for 3 weeks, after which the pump was removed, and 10 rabbits were given solvent infusion for 3 weeks followed by a 3-week period of PGE_2 infusion (4 µg/h per kg body weight).

All rabbits were killed 6 weeks after osteotomy (Figure 1). Both tibial bones were dissected free from soft tissue, the plate and screws were removed and the fibular bone resected. The tibial bones were examined by radiography. The mineral content and diameter at the osteotomies were determined, and the bone was mechanically tested by three-point bending.

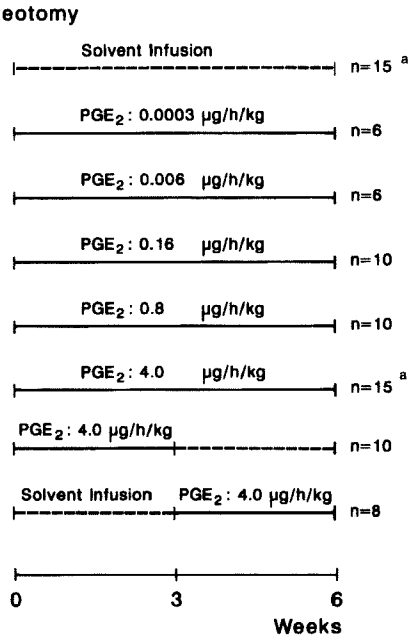

Figure 1. Design of the study.
[a]Data previously published (Keller et al.1992).

Operation

A mid-tibial osteotomy was performed on the right leg and fixed with a four-hole semi-tubular plate (FICS: 49 × 10 mm) and four 2.7-mm screws. A mini-osmotic pump (Alzet® model 2ML4, reservoir volume 2.2 mL) was implanted subcutaneously in the right thigh and a small polyvinyl catheter was tunneled to the osteotomy site for delivery. The catheter was fixed with a suture to the bone and plate to ensure that the tip of the catheter was placed opposite the plate at the osteotomy site (Keller et al. 1992).

Medication

The pumps were filled with either a solvent solution (40 percent ethanol in propylene glycol) or PGE_2 in solvent solution, in which PGE_2 was stable throughout the experiment (Keller et al. 1992). After 3 weeks the rabbits were anesthetized and the reservoir of the pumps replaced with new reservoirs.

Callus area

The cross-sectional area was estimated from the anteroposterior and the lateral diameters of the bone at the osteotomy site, as measured with a sliding calliper. The cross-sectional area was calculated assuming the tibia to be elliptical. Coefficient of variation of a double measurement was 3 percent. In 10 transverse bone sections close to one of the central drill holes, the coefficient of variation was 7 percent between the cross-sectional area, according to diameter and the area obtained by planimetry. Finally, we estimated the cross-sectional callus area as the difference between the cross-sectional area of the osteotomized leg and the intact leg.

The longitudinal callus area was estimated from anteroposterior and lateral radiographs. The callus area was measured by point-counting, using a 5-mm grid of 4.4 magnification. In order to count about 150 points in each sample we made one count of the PGE_2 infused legs and five counts of the controls, revealing a 4 percent coefficient of variation of a double measurement.

Bone mineral content

The plate was removed and the bone scanned by photon absorptiometry at 4-mm increments between the two end-holes (Keller et al. 1987). The mean mineral content of the tibial diaphyseal bone between the peripheral screw holes was calculated as the mean of 8 scans. Coefficient of variation of a double measurement was 3 percent.

The mineral content of the callus at the osteotomy was calculated, assuming the bone to be an elliptical cylinder for 10 mm at the osteotomy site.

Mechanical testing

The tibial shaft was loaded in a three-point bending set-up (Alwetron 250, Lorentsen & Wettre, Stockholm Sweden) at a constant deformation rate of 5 mm/min. The tibia was placed on the posterior surface and a load was applied to the osteotomy site on the anterior edge of the bone. The distance between the supporting points was 40 mm. The intact left tibia was tested similarly (Keller et al. 1987). The ultimate strength and the stiffness were derived from the curves.

3 rabbits were excluded from the study, 1 due to fracture through the screw holes, 1 due to hemiparesis and 1 due to death from unknown cause. The number of participating animals in each group is given in the tables.

Statistics

Differences between the groups were analyzed by the Kruskal-Wallis one-way analysis of variances in combination with the Bonferroni method for multiple comparisons, using the two-tailed Mann-Whitney test. Accuracy was expressed as the coefficient of variation. A P-value < 0.05 was considered significant.

Results

Throughout the experimental period the rabbits in each group had a mean weight gain of –0.1 to 0.4 kg (n.s.).

PGE_2 infusion in doses between 0.006 and 4 μg/h per kg body weight caused a dose-dependent increase in the amount of callus and mineral (Table 1), but a decrease in mineral content per volume of callus. The ultimate strength at three-point bending was not influenced by PGE_2 infusion, but the stiffness was reduced after high-dose PGE_2 infusion.

PGE_2 infusion during the first 3 weeks elicited a callus formation not different from that after solvent infusion (Figure 2), whereas callus formed after PGE_2 infusion from the 4th to the 6th week was of the same amount as callus formed after PGE_2 infusion throughout the entire 6-week period (Figure 3, Table 1).

In the intact legs of the groups, the bone mineral content, the ultimate load and the stiffness did not differ, except for one value (Table 2).

Table 1. The effect of local infusion of different PGE_2 doses on callus formation and mechanical properties of a healing tibial osteotomy in rabbits. Mean *SE*

Experimental group	n	Diaph. mineral content g/cm	Longitudinal callus area mm^2	Cross-section callus area mm^2	Callus mineral content g/cm^3	Ultimate load Newton	Stiffness Newton/mm
Solvent[b]	8	1.37 *0.06*	19 *3*	28 *8*	3.25 *0.89*	271 *29*	334 *45*
PGE_2 (µg/h per kg body weight for 6 weeks)							
0.0003	6	1.24 *0.09*	24 *6*	33 *4*	1.81 *0.18*	282 *31*	355 *24*
0.006	6	1.49 *0.07*	74 *8*[a]	89 *10*[a]	1.16 *0.09*	254 *27*	370 *86*
0.16	10	1.80 *0.11*[a]	176 *15*[a]	142 *13*[a]	0.70 *0.10*[a]	300 *11*	348 *29*
0.8	8	1.84 *0.13*[a]	182 *35*[a]	142 *26*[a]	0.78 *0.07*[a]	279 *35*	312 *47*
4.0[b]	12	2.23 *0.10*[a]	181 *14*[a]	180 *19*[a]	0.88 *0.04*[a]	215 *20*	180 *24*[a]
PGE_2 (4 µg/h per kg body weight)							
0 to 3rd week	10	1.41 *0.06*	82 *8*[a]	52 *8*	1.37 *0.19*	214 *12*	319 *27*
4th to 6th week	7	2.53 *0.23*[a]	261 *24*[a]	213 *35*[a]	0.91 *0.07*[a]	223 *26*	131 *13*[a]
One-way Kruskal-Wallis *P*		< 0.001	< 0.001	< 0.001	< 0.001	0.07	< 0.001

[a] $P < 0.05$ compared with the corresponding leg of the placebo group
[b] Data previously published (Keller et al. 1992)

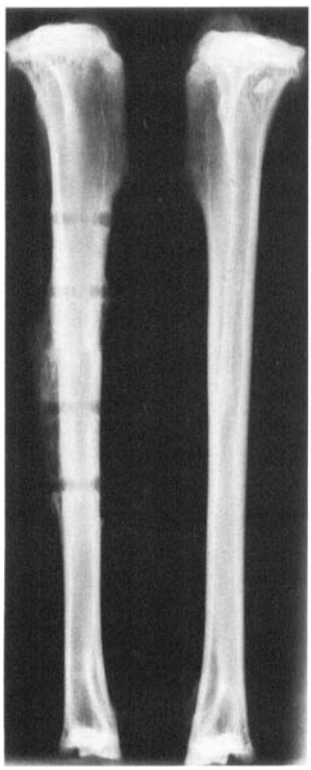

Figure 2. Plated osteotomy after 6 weeks. PGE_2 was infused at the osteotomy site in the first 3 weeks.

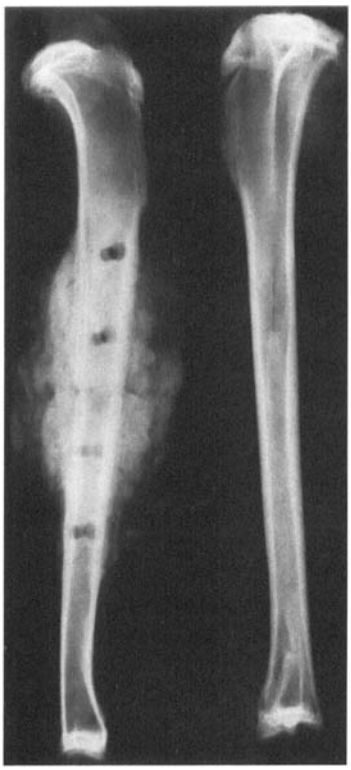

Figure 3. Plated osteotomy after 6 weeks. PGE_2 was infused at the osteotomy site from the 4th to the 6th week after osteotomy.

Discussion

The present study demonstrates that local infusion with PGE_2 at an osteotomy site dose-dependently affects callus formation. This confirms in vitro experiments where PGE_2 in the concentration range of 10^{-8}–10^{-5} M showed a dose-dependent stimulation of chick embryonal osteoblast-like cells (Feyen and Raisz 1987). In fetal rat osteoblast-like cells (Raisz and Fall 1990), the stimulation was found to be dose-dependent only if cortisol was added in physiological concentrations (10^{-8} M), whereas the effect was biphasic in the absence of cortisol with stimulatory effects at 10^{-8} and 10^{-7} M and decreasing effects at higher doses. Since glucocorticoids are usually present in vivo in concentrations required to show a stimulatory effect of PGE_2 in bone organ cultures, it is not surprising that the stimulatory effect dominates when exogenous PGE_2 is administered in vivo (Raisz and Fall 1990).

The callus reduced the mineral content after PGE_2 infusion for 6 weeks, regardless of dose. This finding agrees well with the histological appearance of the callus, as previously reported (Keller et al. 1992), where PGE_2 infu-

Table 2. The effect of local infusion of different PGE_2 doses on the diaphyseal mineral content and the mechanical properties of the corresponding intact leg of a healing tibial osteotomy in rabbits. Mean *SE*

Experimental group	n	Diaph. mineral content g/cm	Ultimate load Newton	Stiffness Newton/mm
Solvent[b]	8	0.97 *0.04*	533 *21*	761 *46*
PGE_2 (µg/h per kg body weight for 6 weeks)				
0.0003	6	0.90 *0.04*	575 *22*	822 *46*
0.006	6	0.84 *0.01*	543 *12*	743 *43*
0.16	10	0.94 *0.02*	564 *21*	783 *29*
0.8	8	0.96 *0.03*	560 *23*	679 *47*
4.0[b]	12	0.95 *0.03*	503 *17*	627 *29*
PGE_2 (4 µg/h per kg body weight)				
0 to 3rd week	10	0.97 *0.04*	500 *22*	710 *41*
4th to 6th week	7	1.00 *0.03*	484 *21*[a]	708 *21*
One-way Kruskal-Wallis *P*		0.05	0.001	0.03

[a] $P < 0.05$ compared with the corresponding leg of the placebo group
[b] Data previously published (Keller et al. 1992)

sion caused a callus formation with thin and partly disintegrated bony trabeculae, compared with thick bony trabeculae forming a continuous network in the controls. The results suggest a mechanism of callus formation after PGE_2 infusion which is different from the normal. Consequently, exogenous PGE_2 stimulates connective tissue proliferation more than bone cell replication. Evidence for this hypothesis was found in the in vitro experiments of van der Plas et al. (van der Plas et al. 1985), who found that PGE_2 had a dose-dependent stimulatory effect on both serum-starved osteoblast-like cells and periosteal fibroblasts. The increase in total amount of mineral after PGE_2 infusion in the present study indicates that it was not a "normal" callus response within an increased mass of fibrous tissue.

As a consequence of the relatively low mineral content in the callus after PGE_2 infusion, it was expected that strength and stiffness would be unchanged or reduced. The results indicate that the PGE_2-stimulated callus had no positive influence on osteotomy healing after 6 weeks. Similarly, no effect was observed on the biomechanics of the control legs.

In the second part of the study we found that PGE_2 infusion during the latter half of a 6-week healing period stimulates callus formation, whereas PGE_2 infusion in the first half has no effect on callus formation. In a pilot investigation (not published) we have shown equal callus formation at 6 weeks after solvent infusion at a plated tibial osteotomy during the first 3 weeks (n 5) or 6 weeks (n 6). This suggests that the endogenous increase in local PGE_2 in the first weeks after fracture (Dekel et al. 1981) stimulates callus formation maximally; the exogenous infused PGE_2 had no further effect. Later in the healing period, endogenous PGE_2 decreases to normal (Dekel et al. 1981), which may explain the effects of exogenous PGE_2. Our results do not agree with the report by Voegely and Chapman (1985) who found increased formation of a near normal callus and increased bending strength 4 and 8 weeks after a nailed tibial osteotomy in rabbits which had been infused with PGE_2 (5 µg/h per kg body weight) for 4 weeks. However, fracture healing under relatively unstable conditions in the latter study is dependent on external callus, which may explain a more pronounced effect of PGE_2 than that observed in our study under stable conditions, where the healing is less dependent on external callus formation (Hulth 1989).

It can be concluded that local infusion of PGE_2 at the osteotomy site of a plated osteotomy has a dose-dependent effect on an immature callus formation, although PGE_2 cannot accelerate strength development in normal fracture repair.

Acknowledgements

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