Release of prostaglandins from bone and muscle after femoral osteotomy in rats

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In rats after nonstabilized femoral osteotomies, the changes in the release of prostaglandins (PGs) during bone healing (from bone and surrounding muscle tissue) were determined for PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and thromboxane B_2. A unilateral osteotomy, with contralateral soft-tissue dissection, was performed. After 4 or 10 days, the rats were killed and soft tissue and femoral bone were incubated, and the release of PGs was measured with specific radioimmunoassays. The release of PGs from rat femurs without previous surgery and from the sham-operated on side did not differ after 180 minutes' incubation. The release of PGE_2, 6-keto-$PGF_{1\alpha}$, and thromboxane-B_2 from the osteotomy site was increased for bone on Day 4 and for muscle on Day 10 when compared with the controls. The release of $PGF_{2\alpha}$ from bone and muscle was about the same on both days, but increased as compared with the controls on Day 10 for bone. On Day 10, the other PGs for muscle and bone tissue were decreased as compared with Day 4. The most pronounced release of PGs occurred during the early healing phase after osteotomy; as early as 10 days after surgery, most of the PGs were not increased when compared with the sham-operated on side.

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The initial reaction to a fracture or osteotomy is characterized by an inflammatory response. Histologically, polymorphonuclear leukocytes, lymphocytes, histiocytes, and monocytes, as well as macrophages, are seen (Simmons 1985). Most of these cells are known to release prostaglandins (PGs) and leukotrienes (Vane 1976, Schroer 1985). The platelets in the fracture hematoma release thromboxane A_2 and a growth factor, which is mitogenetic for fibroblasts and can be stimulated by arachidonic acid (Linder et al. 1979, Key et al. 1983, Canalis 1985). This inflammatory reaction is present during the first few days (Days 0 to 5, depending on the species) after bone injury.

The second stage is that of callus formation. Motion in the fracture site and PGs (mainly PGE_2) stimulate differentiation of the osteoprogenitor cells and fibrocartilaginous callus growth (Cohen 1956, Jee et al. 1987, Shih and Norrdin 1986). Bone remodeling does not commence until the late phase of callus formation (Frost 1973).

PGs are released during fracture healing. After pin stabilization of rabbit fractures, an increased release of PGs of the E and F series was found in bone and muscle when compared with controls (Dekel et al. 1981). PGs stimulate bone formation by preosteoblast cell differentiation, osteoblast proliferation, and collagen synthesis (Jee et al. 1987, Chyun and Raisz 1984, Rodan and Rodan 1985, Shih and Norrdin 1986).

Histologic evidence of the importance of PGs in bone remodeling was presented by Ro et al. (1978) when they demonstrated inhibition of fracture healing by indomethacin. In other studies, ibuprofen and acetylsalicylic acid (potent inhibitors of PGs) inhibited fracture healing and remodeling of the haversian system (Huusko et al. 1975, Sudmann 1975, Ro et al. 1978, Sudmann and Bang 1979, Norrdin et al. 1990).

In the present study, release of PGs from muscle and bone was measured during the early healing phase after unstable osteotomies. The primary goal of our study was to gain a better understanding of the pharmacodynamics of PGs in bone remodeling.

Materials and methods

Thirty-two male, adult, 3-month-old Sprague-Dawley rats weighing 400–500 g were used. The animals were kept in standard cages and fed and watered ad libitum.

Osteotomy

For the operative procedures, 24 animals were anes-

thetized with ether. The femur was exposed by a longitudinal medial incision. With a slow-speed, 1-mm dental burr, a mid-diaphyseal oblique femoral osteotomy was performed. During this procedure, the wound was constantly irrigated with sterile saline to reduce cell necrosis. The soft-tissue dissection was repeated contralaterally, but the femur was not osteotomized. After surgery, the animals were returned to their cages and allowed free motion without internal or external immobilization of the fracture.

Tissue sampling

From 8 control rats, 300–400 mg bone samples, free of soft tissue, were removed from the femur and incubated.

The other 24 animals were randomized into two test groups and killed after 4 or 10 days. Control radiographs were obtained from each animal. From the muscle surrounding the fracture site, tissue pieces of up to 100 mg were obtained. The whole femur was then removed and cut with a diamond disc (Brassler, Germany) 10 mm distal and proximal to the osteotomy site. The weight of the bone tissue was 500–600 mg.

It was macroscopically evident on Day 10 that all the animals manifested callus formation. On Day 4, there was no firm connecting tissue between the bone fragments. The muscle and bone tissue samples were then washed in iced (4 °C) Tyrode's solution and immediately incubated (Tyrode's solution: NaCl 9 percent, KCl 11.5 percent, MgCl 10.5 percent, $NaHC0_3$ 6.5 percent, NaH_2P0_3 2.9 percent, $CaC1_2$ 8.1 percent, and glucose 5 percent. This solution was degassed for 30 min with 95 percent CO_2 and 5 percent O_2).

Release of prostaglandin (PGs)

Rinsed samples of nonoperated on and operated on bone (500–600 mg) and muscle (100 mg) were placed in 3 mL of Tyrode's solution and incubated in a water bath at +37 °C under continuous degassing with 95 percent CO_2 and 5 percent O_2. The incubation time for bone was 180 min and 90 min for muscle. After 30 min and at the end of the incubation period, samples were obtained and immediately deep-frozen (–20 °C). Previous studies, based on repeated measurements over a 6-month period, have shown that this method does not affect the levels of PGs in Tyrode's solution. The release of PGE_2, 6-keto-$PGF_{1\alpha}$, thromboxane B_2, and $PGF_{2\alpha}$ was measured by radioimmunoassay (RIA; Peskar et al. 1979).

Totally, 0.02 to 0.1 mL of the incubated Tyrode solution was added, without prior processing, to the assay to a total volume of 2 mL. The assay consisted of 0.5 mL radioactive tracer (7,000–9,000 dpm = 117–150 Bq) and 0.5 mL of specific antibodies. The samples were kept for 20–24 hours at 4 °C. Charcoal was added to the sample to bind the nonbound radioactive PGs. After centrifugation, the sample volume was added to 7 mL scintigel and the H^3 activity was measured. The amounts of PGs were calculated by comparison with standard curves, where known amounts of PGs were added to radioactive PGs.

The detection limit of the radioimmunoassay was 32 ng/mL for PGE_2, 68 ng/mL for 6-keto$PGF_{1\alpha}$, 28 ng/mL for $PGF_{2\alpha}$, and 42 ng/mL for thromboxane B_2.

Histologic, as well as microbiologic, studies were performed on the tissue samples to exclude fractures with acute infection and to define the incubated tissue.

Data were analyzed according to the paired and unpaired Student's *t*-test (significance level $P < 0.05$). The mean and standard deviation of the PGs and thromboxane levels were calculated.

Results

All the animals survived the surgical procedure, and none showed clinical, histologic, or microbiologic signs of infection when killed. The histologic examination of the samples collected on Day 4 demonstrated only a nonspecific inflammatory response on the osteotomy side, whereas on Day 10, the osteotomy was bridged by fibrocartilagenous callus.

Release of PGs from bone without prior surgery

The release of PGs was measured in 8 rats. The PGE_2 release at 30 min was 5.9 ± 0.51 ng/g and at 180 min 21 ± 4.1 ng/g bone weight. The 6-keto-$PGF_{1\alpha}$ release at 30 min was 59 ± 5.3 and at 180 min 124 ± 8.2 ng/g bone weight. The values for thromboxane B_2 at 30 min were 12 ± 1.8 ng/g and at 180 min 21.1 ± 4.2 ng/g bone weight. $PGF_{2\alpha}$ release at 30 min was 7.2 ± 0.92 ng/g and at 180 min 24 ± 5.2 ng/g bone weight.

Release of PGs from bone on Day 4

The amounts of PGE_2, 6-keto-$PGF_{1\alpha}$, and thromboxane B_2 measured after 30 or 180 min of incubation were increased from osteotomized femora compared with contralateral controls. After 180-min incubation, the PGE_2 concentration in the osteotomy group was 10 times higher than that in the control group. The 6-keto-$PGF_{1\alpha}$ release was increased 4-fold, $PGF_{2\alpha}$ 3.5-fold,

Table 1. Mean (M) and standard deviation (SD) of the prostaglandins (PGs) and thromboxane released from rat bone and muscle 4 or 10 days after femoral osteotomies and the sham procedure. Each group included 12 animals and values were measured after 180 min (bone) and 90 min (muscle). The release of PGs was increased when compared with the controls for PGE_2, 6-keto-$PGF_{1\alpha}$, and thromboxane B_2 on Day 4 and $PGF_{2\alpha}$ on Day 10. The release of PGE_2, 6-keto-$PGF_{1\alpha}$, and thromboxane B_2 from muscle after 10 days was increased when compared with the controls

		Bone				Muscle			
		Day 4		Day 10		Day 4		Day 10	
		Control	Osteotomy	Control	Osteotomy	Control	Osteotomy	Control	Osteotomy
PGE_2	M	33	341	18	60	58	439	14	78
	SD	*6.1*	*76*	*2.6*	*21*	*17*	*269*	*5.8*	*23*
6-keto-$PGF_{1\alpha}$	M	102	402	90	107	116	488	32.0	152
	SD	*7.8*	*50*	*11*	*47*	*23*	*292*	*10*	*46*
$PGF_{2\alpha}$	M	21	73	27	69	45	144	21	136
	SD	*4.7*	*21*	*5.7*	*16*	*7.3*	*57*	*6.9*	*56*
Thrombo xane B_2	M	27	78	22	44	39	238	12.4	133
	SD	*3.3*	*5.9*	*5.6*	*11*	*7.3*	*116*	*4.9*	*41*

and thromboxane B_2 3-fold (Table I) as compared with the controls. The highest amount for the control and osteotomy sides was measured for 6-keto-$PGF_{1\alpha}$, (102 and 402 ng/g bone weight). $PGF_{2\alpha}$ showed the lowest concentration (21 and 73 ng/g bone weight; Table 1).

Release of PGs from bone on Day 10

The amounts of PGs for PGE_2, 6-keto-$PGF_{1\alpha}$, and thromboxane B_2 were not increased when compared with the controls. $PGF_{2\alpha}$ release was increased (69 ng/g bone weight) when compared with the controls (27 mg/g bone weight). Upon comparing the release of PGs from osteotomies on Days 4 and 10, a 2–5-fold decrease of PGE_2, 6-keto-$PGF_{1\alpha}$, and thromboxane B_2 occurred. Only $PGF_{2\alpha}$ exhibited a similar release (21/27 and 73/69 ng/g bone weight). The control groups for Days 4 and 10 showed a similar bone release of PGs (Table 1).

Release of PGs from muscle on Days 4 and 10

The amounts of PGs in the muscle osteotomy groups on Day 4 were not increased when compared with the control groups. On Day 10, increased levels in the muscle osteotomy group compared with the controls were measured for PGE_2, 6-keto-$PGF_{1\alpha}$, and thromboxane B_2. Upon comparing the 4- and 10-day results, a decreased release of PGs was seen on Day 10 except for $PGF_{2\alpha}$, where the release pattern for both days was nearly constant. These results correlate with those of the bone groups. Controls on Day 4 were elevated when compared with Day 10 (Table 1).

Discussion

Bone repair has a defined time frame for each stage, but varies in different species. In rats, it is five times faster then in man and the defined phases are shorter. Our 2 examination days were chosen to include the inflammatory and callus phase of bone formation. The amount of callus has been shown to increase with instability. Therefore, the osteotomies were not stabilized, and the animals were allowed to move freely without any internal or external fixation (Mindell et al. 1971). The release of PGs in the sham-operated on femora did not differ from intact, nonoperated on femora. Therefore, the increase in the release of PGs after osteotomy must be attributed to the bony lesion.

During callus formation, bone resorption and osteogenesis take place. The callus formation stage corresponds to Day 10 in our study. At this phase, the levels of the PGs (except $PGF2_{\alpha}$) and thromboxane B_2 were decreased when compared with the inflammatory stage. Raised levels of PGs after fracture were first detected by Dekel et al. (1981) in rabbits. In their study, they chose Days 1, 3, 7, 14, and 28 after the operation, and found that the highest bone release of PGE was on Day 3 and the highest release of PGF was on Day 7, whereas the release from muscle was highest on Day 14 for both PGE and PGF. They observed no definitive decrease in PGs from the inflammatory to the callus stage. This might be due to group antibodies against the E and F series. In our study, antibodies against specific prostaglandins with a cross-reaction of less than 10 percent were used.

Therefore, it was possible to measure specific prostaglandin release on Days 4 and 10. As regards different PGs, the decrease in PGE_2 was most

pronounced from Days 4 to 10. Upon reviewing the literature, we found that PGE_2 has a stimulatory effect in high doses on the division of prechondroblasts (Falconer et al. 1980) and on the formation of fracture callus (Juhn et al. 1989). Further, PGE_2 and PGE_1 increase endosteal bone growth in infants and bone formation in dogs after rib fractures (Ueda et al. 1980, Ueno et al. 1985, Shih and Norrdin 1986, High 1987, Norrdin and Shih 1988). PGE_2 might therefore be important in the initiation of bone healing. The decrease in PGE_2 from Days 4 to 10, however, is probably also due to the decreased inflammation. It has been shown that the degree of inflammation is correlated with PGE_2 release (Corbett et al. 1979, Dekel and Francis 1981).

The elevated $PGF_{2\alpha}$ release on Day 10 is consistent with the results of Wientroub et al. (1983). They found, using an endochondral bone-formation system, high $PGF_{2\alpha}$ levels during chondrogenesis and chondrolysis. Thus, $PGF_{2\alpha}$ seems to influence the callus stage of bone healing. Moreover, 6-keto-$PGF_{1\alpha}$ showed the highest amounts of PGs during chondrogenesis and chondrolysis. Presumably, this is partly related to revascularization, which is highest on Day 4 (Wray and Lynch 1959). Besides endothelial cells, osteoblasts are known to release PGI_2 in cell cultures (Rodan and Rodan 1985, Schroer 1985). Accordingly, the release of 6-keto-$PGF_{1\alpha}$ might be partly due to osteoblasts. An increase in blood flow is also related to PGI_2, and plays a role in bone healing. Keller et al. (1987) showed a reduction in blood flow during treatment with indomethacin. This is most likely due to the inhibition of PGs. The release of PGI_2 might therefore contribute to bone formation.

The importance of thromboxane B_2 in bone formation is unknown. So far, it has only been reported that thromboxane B_2 does not stimulate bone resorption (Raisz and Martin 1983). In our present study, thromboxane B_2 showed the same release profile as PGE_2 and 6-keto-$PGF_{1\alpha}$. Peaks of thromboxane B_2 were detected during the proliferation of mesenchymal cells (Wientroub et al. 1983). The thromboxane B_2 release on Days 4 and 10 can be partly attributed to platelets and mesenchymal cells on the fracture side.

The amounts of PGs released from muscle tissue around the fracture sites were higher than released from the control side. The release of PGs and thromboxane decreased from Days 4 to 10 with the exception of $PGF_{2\alpha}$. The release of $PGF_{2\alpha}$ was the same on Days 4 and 10. One explanation for the high level of release of PGs is the inflammatory reaction around the fracture site and the presence of monophagocytic cells. Monocytes, macrophages, and polymorphonuclear leukocytes synthesize PGs (Corbett et al. 1979). The proliferation of mesenchymal cells from muscle surrounding fractures might also contribute to the elevated levels, especially of thromboxane B_2 (Tonna and Cronkite 1961, Brookes 1971). The decreased release of PGs in the control and osteotomy groups from Days 4 to 10 is related to a less pronounced inflammatory response on Day 10.

Early release of PGs effects bone healing. Therapy with nonsteroidal anti-inflammatory drugs influenced bone healing and prevented heterotopic ossification after hip surgery (Törnkvist et al. 1985, Sodemann et al. 1988). In our model, using a whole-tissue preparation, we were able to demonstrate the release pattern of specific PGs and thromboxane B_2 in the early stage of bone healing (Days 4 and 10).

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