

Effect of platelet derived growth factor on heterotopic bone formation in rats

Olof Risto¹, Ola Wahlström¹, Avni Abdiu² and Thomas Walz²

Platelet derived growth factor (PDGF) and induction of newly woven bone growth were studied in rats. PDGF (20 ng/mL) was administered continuously for 2 weeks via microosmotic pumps to 6-mm-long pieces of demineralized rat femur inserted into muscle pouches. Each rat had a control

piece of demineralized bone inserted into the contralateral gluteal muscle. The samples were collected after 4 weeks, and wet and ash weight were recorded. Fourteen rats were evaluated. There were no differences as regards wet weights. PDGF increased the ash weights.

¹Departments of Orthopedics and ²Cell Biology, University Hospital, S-581 85 Linköping, Sweden

Platelet derived growth factor (PDGF) is the most important growth factor in serum for connective-tissue cells. It is a protein with biological activity in a dimer structure (8); and receptors have been demonstrated on fibroblasts, smooth muscle, and glial cells (9). PDGF is released from platelets during clotting processes, and acts as a chemoattractant, recruiting cells into the wound (6, 13). The proliferation of these cells, which results in increased connective tissue outgrowth, is also believed to be stimulated by PDGF (7).

Cellular processes, such as migration and proliferation of immature connective-tissue cells, are stimulated by PDGF, and are also observed in the early phases of experimentally induced heterotopic bone formation (HBF) (4, 7, 12, 13, 14).

We have studied the effects of PDGF on the mineral contents of experimentally induced new bone formation in rats.

Material and methods

We used the classical model for inducing new bone formation as described by Urist (14). Rat femurs were demineralized in 6 M HCl for 12 h at room temperature. The demineralization was only checked by palpating the preparations. Delloye et al. (5) found demineralization in 6 M HCl for only 4 h at room temperature equally effective for inducing new bone as demineralization at 40 °C for longer periods at room temperature.

Next, we inserted 6-mm-long pieces of demineralized rat diaphyses into the gluteal muscular pouches on each side of the rats. PDGF (200 mL, 20

ng/mL) was continuously administered by microosmotic pumps (Altazet 2002, Alza Corp., Paulo Alto, California) to one of the bone pieces during a 2-week period. A piece of catheter was fixed to the other bone piece (control piece) in one series of 22 cases; and in another series of 10 cases, saline was administered to the control piece in the same way as PDGF (Figure 1).

For anesthesia, we used xylazin (Rompun® Vet., Bayer) and ketamin (Ketalar®, Parke-Davies/Warner-Lambert) intraperitoneally, with mean doses of 4 and 8 mg, respectively. Cefuroxim (Zinacef®, Glaxo) was administered intraperitoneally, with a mean dose of 30 mg as a prophylactic against infection.

The rats were kept in cages and received water and a standard laboratory diet *ad libitum*. After 4 weeks, the rats were killed with overdoses of xylazin, and the implants were collected to measure wet and total-ash weights (750 °C, 8 h; Figure 2).

In accordance with our pilot studies, we considered production of new bone, represented by at least 2 mg of ash, to be a criterion for the functioning of the model.

To obtain samples for histologic examinations, another 4 rats, treated in the same way, were killed after 1, 2, 3, and 4 weeks. These samples were demineralized, embedded in paraffin, and stained with hematoxylin and eosin. Further, these samples were analyzed blindly with light microscopy.

We used the paired Student's *t*-test and Wilcoxon's signed-rank test for statistical studies.

Seven rats were excluded because of their poor postoperative condition and early death, wound ruptures with disruption of the catheter-muscle pouch connection, and improper preparation. A

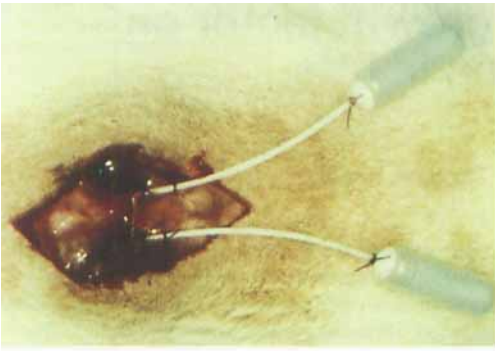


Figure 1. The pumps and catheters, which are fixed to the muscle pouches just before subcutaneous insertion of the pumps.

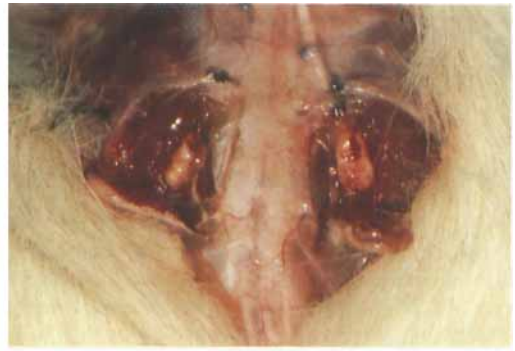


Figure 2. The pieces of newly formed bone during the collection procedure.

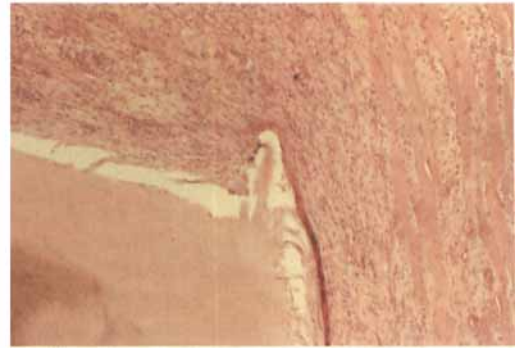
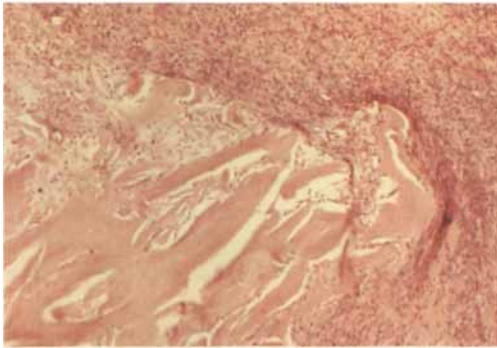


Figure 3. Photomicrographs showing the histologic appearance in samples collected after 1 week. Observe the increased density of fibroblasts surrounding the decalcified bone in the PDGF-treated sample (left). H&E, $\times 60$.

nonfunctioning model, for no definite reasons, with less than 2 mg of ash on one or both sides was found in another 11 rats, and therefore these rats were also excluded.

The remaining 14 rats, with a mean weight of 324 g, all showed similar growth during the 4 weeks (mean weight gain 111 g). Of these 14 rats, only 3 rats from the series with saline controls remained.

Results

There were no differences as regards wet weight. The mean ash weight on the PDGF-treated side was 7.8 ± 4.4 mg and on the control side in both series 5.2 ± 1.7 mg. Using standard weights, i.e., weights proportionate to the square root of the number of rats in each series, we obtained a difference between ash weights of PDGF-treated samples and their controls at the 5 percent level of significance (11) in

both single- and double-tailed tests, and when using the Student's paired *t*-test as the basic test ($t = 2.21$, $df = 12$, $P < 0.05$) and when considering Wilcoxon's signed-rank test as the primary test ($z = 2.31$, $P < 0.05$). If the results are augmented by including the 11 rats that were discarded because of very small observed ash-weight values, the difference was no longer significant. These values are quite variable and marginally favorable to the treatment side. The net effect was a not quite significant difference at the 5 percent level when using a single-tailed test ($t = 1.60$, $df = 23$, $P > 0.10$) ($z = 1.624$, $P > 0.10$).

The histologic examination showed considerably more connective-tissue proliferation in the PDGF-treated preparations after 1 week (Figure 3). This difference was not so apparent after 2 weeks. After 3 weeks, the inflammatory reaction was still present to some extent on the control side, and the bone piece was resorbed on the PDGF-treated side.

Discussion

We believe that the experimental design—with untreated controls in each animal, which eliminates the hazards of different potential for bone formation in different animals (1)—gives our results validity even with the relatively high exclusion rate.

Whole-blood serum contains about 20 ng/mL PDGF, and plasma contains less than 0.2 ng/mL (2). Thus, the free, active fraction of PDGF in the circulation is very low, and most of the substance is bound in platelets and to plasma proteins (3). PDGF stimulates cultured fibroblasts at very low concentrations ($ED_{50} \approx 0.3$ ng/mL; 3). Our choice of PDGF concentration in the pumps (20 ng/mL), accordingly, seems quite adequate. Injected PDGF has a half-life of less than 2 minutes (2), and it is likely that the newly released PDGF has local effects rather than systemic ones. The binding of PDGF to the receptors and nonspecific binding to exposed tissue by virtue of its general adsorptive tendency would also cause PDGF to be retained at the release site. Analogous to this, we believe that all the PDGF released from the pumps in our study has acted locally. The importance of an experimental design with slow release, which protects the active substance from rapid inactivation under physiologic conditions, has been pointed out by Aspenberg and Lohmander (1).

According to Urist et al. (15), there is a direct correlation between rate of increase in ash weight and deposition of calcified, newly woven bone; and induced bone formation can be quantitated simply by determining the ash weight. In the same study, the yield of new bone tapered off after about 30 days. Their study was done on rabbits, but it is very unlikely that the yield of new bone would taper off later in rats. Therefore, we believe that we have measured maximum yield of new bone.

PDGF has recently been shown to stimulate new bone formation in adult rats (10). We believe that our results support the theory that PDGF stimulates new bone formation, but that the mechanism is probably complex. Histologic examinations have indicated that one mechanism may be increased connective tissue outgrowth (7, 13). Probably several growth factors are involved in the multifactorial process of new bone formation, because fibroblast growth factor FGF also contributes to the process (1).

Acknowledgments

The authors would like to thank Professor Ulf Brunk, pathologist at Linköping University Hospital, for performing the histologic examinations.

References

1. Aspenberg P, Lohmander L S. Fibroblast growth factor stimulates bone formation. Bone induction studied in rats. *Acta Orthop Scand* 1989; 60 (4): 473-6.
2. Bowen Pope D F, Malpass T W, Foster D M, Ross R. Platelet derived growth factor in vivo: levels, activity, and rate of clearance. *Blood* 1984; 64 (2): 458-69.
3. Bowen Pope D F, Ross R. Platelet derived growth factor. II. Specific binding to cultured cells. *J Biol Chem* 1982; 257 (9): 5161-71.
4. Buring K. On the origin of cells in heterotopic bone formation. *Clin Orthop* 1975; 110: 293-301.
5. Delloye C, Hebrant A, Munting E, Piret L, Coutelier L. The osteoinductive capacity of differently HCl decalcified bone alloimplants. *Acta Orthop Scand* 1985; 56 (4): 318-22.
6. Grotendorst G R, Pencer D, Martin G R, Sodek J. Molecular mediators of tissue repair. In: *Soft and Hard Tissue Repair*. (Eds. Hunt T K, Heppenstall R B, Pines E, Rovsee D). Praeger, New York 1984: 20-40.
7. Grotendorst G R, Martin G R, Pencev D, Sodek J, Harvey A K. Stimulation of granulation tissue formation by platelet derived growth factor in normal and diabetic rats. *J Clin Invest* 1985; 76 (6): 2323-9.
8. Heldin C H, Westermark B, Wasteson A. Platelet derived growth factor: purification and partial characterization. *Proc Natl Acad Sci U S A* 1979; 76 (8): 3722-6.
9. Heldin C H, Westermark B, Wasteson A. Specific receptors for platelet derived growth factor on cells derived from connective tissue and glia. *Proc Natl Acad Sci U S A* 1981; 78 (6): 3664-8.
10. Howes R, Bowness J M, Grotendorst G R, Martin G R, Reddi A H. Platelet derived growth factor enhances demineralized bone matrix induced cartilage and bone formation. *Calcif Tissue Int* 1988; 42 (1): 34-8.
11. Lehmann E L. *Nonparametrics, statistical methods based on ranks*. Holden Day Inc, San Francisco 1975: 132.
12. Reddi A H. Regulation of local differentiation of cartilage and bone by extracellular matrix: a cascade type mechanism. *Prog Clin Biol Res* 1982; 110 Pt B: 261-8.
13. Seppä H, Grotendorst G, Seppä S, Schiffmann E, Martin G R. Platelet derived growth factor is chemotactic for fibroblasts. *J Cell Biol* 1982; 92 (2): 584-8.
14. Urist M R. Bone: formation by autoinduction. *Science* 1965; 150 (698): 893-9.
15. Urist M R, Jurist J M Jr, Dubuc F L, Strates B S. Quantitation of new bone formation in intramuscular implants of bone matrix in rabbits. *Clin Orthop* 1970; 68: 279-93.