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PLATELET-DERIVED GROWTH FACTOR A-CHAIN GENE ACTIVATION AND GROWTH FACTOR PRODUCTION BY HUMAN AORTIC SMOOTH MUSCLE CELLS.
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The many contributory factors leading to the development of cardiovascular disease are currently thought to induce a common pathological change involving smooth muscle cells, which migrate from the vessel wall, proliferate, accumulate at the sites of endothelial cell damage, and then secrete connective tissue proteins and lipids which contribute to the plaque which results in the occlusion of the vessel. According to the recently modified hypothesis of Ross (1), a key event in the development of atheroma may be the abnormal release of a number of growth modulatory polypeptides, including platelet-derived growth factor (PDGF), which can potentially originate from platelets, endothelial cells, monocytes or macrophages, and smooth muscle cells themselves.

We have isolated smooth muscle cell lines from 25 samples of human aorta, using digestion with collagenase and elastase. With DNA synthesis and Northern blot techniques, we examined them for both the production of PDGF-like proteins, and for the possible activation of the PDGF A-chain and B-chain genes. Several lines secreted a growth factor and were still viable after culture for 57 days in serum-free medium. Parallel experiments using Northern blot analysis revealed the activation of the PDGF A-chain gene in all lines examined with no detectable B-chain gene transcripts.

These data raise the possibility that vascular damage may activate the gene encoding the A-chain of PDGF in adjacent smooth muscle cells. Such cells might then become capable of autonomous growth, in an analogous manner to cells transformed by Simian Sarcoma Virus, whose *sis* oncogene encodes the B-chain of PDGF.

1. Ross, R. N. Engl. J. Med. 1986; 314:488-500.

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SELECTIVE EXPRESSION OF PLATELET-DERIVED GROWTH FACTOR B-CHAIN mRNA BY HUMAN ENDOTHELIAL CELLS AND BY HUMAN PERIPHERAL BLOOD MONOCYTES, BUT NOT BY HUMAN SMOOTH MUSCLE CELLS.
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Vascular injury may occur by a variety of mechanisms. Episodes of local hypoxia or conditions leading to local generation of thrombin may influence local cells to release growth regulatory molecules such as platelet-derived growth factor (PDGF) in the surrounding connective tissue. The roles of the cells and of PDGF in these processes are not entirely understood, and this prompted us to investigate effects of hypoxia (5% O_2) on cultured human saphenous vein endothelial cells and human thoracic aorta smooth muscle cells. Freshly isolated human peripheral blood monocytes were exposed to 3.0 U/ml α-thrombin. PDGF-A and PDGF-B mRNAs were analyzed by Northern blots, and their levels were assessed by dot blots utilizing ^{32}P nick-translated cDNA probes. Selective expression of PDGF-B mRNA occurred in endothelial cells during hypoxia and in monocytes exposed to thrombin. Genes coding for PDGF-A and PDGF-B are expressed constitutively in endothelium, and after 48 hr of hypoxia a nine-fold increase of PDGF-B mRNA is detected (9 pg mRNA/ug total RNA). No detectable levels of mRNA encoding PDGF-A and PDGF-B were observed in freshly isolated monocytes; however, a 4-hr exposure to α-thrombin resulted in a selective and transitory increase in PDGF-B mRNA, amounting to 1 pg mRNA/ug total RNA. No PDGF-B mRNA was detected after 20 hr. Hypoxic conditions did not trigger any selective expression of PDGF-B mRNA in smooth muscle, including arterial smooth muscle derived from 1-day- and 3-month-old individuals, or from adult venous smooth muscle. However, constitutive expression of PDGF-A mRNA was observed in each of these, amounting to 0.4 pg mRNA/ug total RNA in the 1-day- and 3-month-old cells, and 0.2 pg mRNA/ug total RNA in the venous smooth muscle. Our data show that endothelium and monocytes selectively express PDGF-B mRNA in vitro in response to conditions mimicking those encountered during vascular injury in some in-vivo situations. The data imply that both endothelial cells and monocyte/macrophages may be sources for mitogens that induce intimal hyperplasia and eventually plaque formation.

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A SPECIFIC THROMBOXANE A2 ANTAGONIST, GR32191, REDUCES PLATELET DEPOSITION IN PTFE GRAFTS.
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Platelet inhibitory therapy improves patency in arterial grafts but when aspirin is given over 20% of patients discontinue therapy. We evaluated a specific Thromboxane A2 antagonist (GR32191 - Glaxo Group Research) on graft platelet uptake and pseudo-intimal hyperplasia in a canine model.

Thirty greyhounds were randomised to orally administered placebo, GR32191 25mg, or aspirin 150mg (ASA) plus dipyridamole 50mg (DPM) 12 hourly, commencing 48 hours prior to implantation of a 6cm length of 6mm PTFE in the femoral artery. Autologous ^{111}In-platelets were infused on the fifth postoperative day and platelet uptake measured by probe and ratemeter with the daily rise in graft radioactivity over reference expressed as Thrombogenicity Index (TI). Drugs were continued to 8 weeks when ^{111}In-platelets were again infused and graft uptake measured on the excised graft and expressed as a ratio to blood. Percentage luminal stenosis was measured by grid microscopy.

	Placebo	GR32191	ASA+DPM
Graft TI	0.14± 0.07	0.014±0.012	0.088± 0.029
Luminal stenosis %	57.7 ±10.4	16.0 ±7.6*	50.5 ±13.5
Graft platelet uptake	21.1 ± 4.8	5.8 ±2.4*	16.8 ± 5.5

(All results mean ± sem, *$p<0.01$ Mann Whitney U-test)

GR32191 significantly reduced luminal stenosis and graft platelet uptake compared to placebo and although TI appeared lower with both platelet inhibitory regimens this did not achieve statistical significance ($p<0.1>0.05$). Thromboxane A2 antagonists reduce thrombus formation on artificial surfaces and being specific may have less undesirable effects.

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THE EFFECTS OF GR32191, A NEW THROMBOXANE RECEPTOR BLOCKING DRUG, ON PLATELETS AND VASCULAR SMOOTH MUSCLE IN VITRO.
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The effect of a new thromboxane receptor blocking drug GR32191 ([1R-[1α(Z),2β,3β,5α]]-(+)-7-[5-[[(1,1'-biphenyl)-4-yl]methoxy]-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptenoic acid, hydrochloride) has been examined upon platelets and vascular smooth muscle. In human platelet-rich plasma (PRP), aggregation to thromboxane (Tx) A_2, PGH_2, arachidonic acid, collagen and U-46619 was antagonised by GR32191 (IC_{50} range 2-36 nM). Primary aggregation (PRP treated with aspirin 10 μM) to ADP, 5-HT and adrenaline were unaffected by concentrations of GR32191 up to 10 μM. In human PRP, U-46619-induced aggregation and 5-HT release were antagonised by GR32191 (10-100 nM). In contrast, in the absence of aspirin, ADP-induced 5-HT release, but not aggregation, was antagonised by the compound implicating a role for TxA_2 in the release process. In human PRP GR32191 (up to 30 μM) did not itself induce aggregation or, in the presence of EGTA (4 mM), induce detectable shape change. Up to 10 μM GR32191 was without effect upon the inhibitory activity of PGI_2 or PGD_2 and at 1 μM had no significant inhibitory activity upon fatty acid cyclooxygenase, thromboxane synthase, prostacyclin synthase, 12-lipoxygenase or phosphodiesterase. The effect of GR32191 was quantified further in human platelets suspended in whole blood or physiological salt solution. Aggregation to U-46619 was antagonised by GR32191 with a pA_2 (slope of the Schild regression) of 8.2 (1.3) in whole blood and 8.8 (1.3) in resuspended platelets. The compound competitively and specifically antagonised the contractions of strips of human isolated pulmonary blood vessels and rat and guinea-pig aortic strips produced by U-46619 with pA_2 (slope) values of 8.2 (1.1), 7.9 (0.9) and 8.7 (0.9) respectively. In contrast contractions induced by KCl and 5-HT (rat) or KCl and histamine (guinea-pig) were unaffected by concentrations of GR32191 up to 30 μM. Thus GR32191 is a potent and specific thromboxane receptor blocking drug on platelets and vascular smooth muscle in vitro. It is orally active and long lasting in man (Thomas, M et al., this meeting).