976-50 Effect of Selective PDGF-Receptor Versus Non-Selective Protein Tyrosine Kinase Blockers on Aortic Smooth Muscle Cells (SMC's) and Endothelial Cells Proliferation

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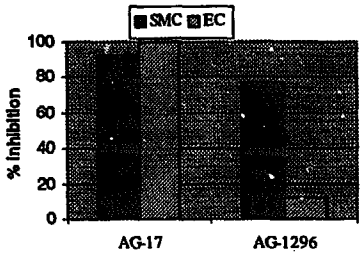
Protein tyrosine kinases (PTK) activity is involved in multiple steps of signal transduction of SMC's growth factors. It is essential for normal cell proliferation, and greatly amplified in proliferative disorders. Thus, blocking the activity of tyrosine kinases may provide a unique and useful strategy for the treatment of syndromes involving accelerated proliferation of vascular SMC's. We evaluated the inhibitory effect of AG-1296, a selective blocker of PDGF-receptor kinase and PDGF-dependent DNA synthesis, and compared it to the inhibitory effect of AG-17, a non-selective PTK blocker, on porcine aortic SMC's and endothelial cells (EC) proliferation. 10 μM of AG-17 or AG-1296 dissolved in 0.1% DMSO were added to the culture medium twice: on day 1 and 3. DMSO at equal concentration was added to control cultures. Cells were counted on day 8. AG-17 caused marked inhibition of both SMC's (92%) and EC (100%) proliferation. AG-1296 caused selective inhibition of SMC's proliferation (77%) with only a minor (12%) inhibition of EC growth.



Conclusions: both PTK blockers tested were very effective inhibitors of SMC's proliferation. While AG-17 markedly inhibited SMC's and EC, AG-1296 selectively inhibited SMC's. Selective inhibition of vascular SMC's proliferation while preserving the EC makes AG-1296 a likely candidate to treat syndromes of injury-induced arterial SMC's proliferation and prevent neointimal formation.

976-51 Which Proto-Oncogene Is More Suitable as a Target for Antisense Inhibition of Human Vascular Smooth Muscle Cell Proliferation?

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Immediate-early proto-oncogenes (IEPs) are considered as putative targets for gene therapy to prevent restenosis, since their expression may be necessary and permissive for cell proliferation. However, oligonucleotides (ODNs) targeting the mRNAs of these IEPs display different inhibitory properties when used with vascular smooth muscle cells (VSMCs). The purpose of this study was to evaluate the comparative efficiency and sequence-specificity of ODNs targeting *c-myc, c-myb, c-jun* and *c-fos* in human VSMC culture systems. Phosphorothioate ODNs (15 to 18 mer, antisense (AS) and sense (S)) targeting these mRNAs were added to synchronized human VSMC cultures at concentrations ranging from 1.25 to 10 μM, concomitant with serum stimulation. Percent growth inhibitory effect was assessed by measurement of [^{3}H]-thymidine incorporation in ODN-treated cells as compared to that in controls (untreated). Sequence-specific effect of the ODNs was measured by using S controls. Results were obtained from up to 21 wells per target per dose, in up to 7 different sets of experiments. Growth inhibition followed a dose-response curve. Maximal effect of antisense ODNs was seen at 10 μM for each IEP and was averaging 70% inhibition. No significant antiproliferative effect was observed with S ODNs at up to 10 μM for *c-jun*, whereas uses of S sequences of *c-fos* at 10 μM and of S *c-myc* and *c-myb* at as low as 5 μM showed an inhibitory effect, although significantly lower than that seen with the corresponding AS ODN. The use of 1.25 μM AS ODNs targeting *c-jun* resulted in a significantly higher inhibition of proliferation (42.8%) than that observed either with *c-myc, c-myb,* or *c-fos* ($p < 0.05$). Conclusions: 1 – *C-fos* and *c-jun* mRNAs are two new suitable targets for AS ODN inhibition of cell proliferation. 2 – Inhibitory effect of AS ODNs targeting *c-fos* and *c-jun* appears to be more sequence-specific than that observed with ODNs targeting *c-myc* and *c-myb*. 3 – At lower doses, AS ODNs targeting *c-jun* are the most potent inhibitors of human VSMC proliferation.

976-52 Improved Efficiency of Percutaneous Adenoviral-Mediated Arterial Gene Transfer by Pre-Treatment With Elastase

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Endothelium and the internal elastic lamina (IEL) are the main barriers to adenovirus mediated gene transfer to the media. Controlled digestion of the IEL by elastase may enhance the efficiency of medial transduction, a critical step for many arterial gene therapy strategies. A pilot experiment showed that the safest elastase regimen avoiding light microscopic damage to the IEL and the media was 2.10^{-7}UI over 5 min. To test whether this dose enhances arterial gene transfer in vivo, an adenoviral vector carrying the nlslacZ gene (AdRSV-βgal) was delivered in both iliac arteries in 10 rabbits after endothelial abrasion, using a double balloon catheter (Mansfield) (5.10^9 pfu during 30 min). In each animal, each iliac artery was randomly assigned to pre-treatment with either elastase or a control infusion of saline. Three days later, transduction was assessed by X-gal staining and cell count (144 sections/artery). Transduction was observed in 10/10 elastase treated vessels vs 5/10 controls ($p < 0.04$). Transduction was confined to the most superficial cell layers of media and was significantly (3-fold) higher than control (7.6 ± 8 vs 2.3 ± 3 cell/section, $p = 0.003$). No dilatation or aneurysm were observed. Therefore, elastase pre-treatment effectively augments transduction efficiency during adenoviral-mediated percutaneous arterial gene transfer, while necessitating neither prolonged vessel occlusion nor increased viral concentrations.

976-53 Local Administered Argatroban Inhibits Intimal Thickening Induced by Balloon Injury in Rabbit Model

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Restenosis after PTCA is one of the major problems in the coronary intervension. The purpose of this study was to evaluate the inhibitory effects of locally delivered argatroban(Arg), a competitive inhibitor of thrombin-induced platelet activation, on intimal thickening in a rabbit carotid injury model. Hydrogel-coated balloon was immersed three times in the Arg solution(1 mg/ml) for 60 seconds. The acute effect of Arg in situ was estimated by scanning electron microscopy, and the contents were determined by chemical determination of Arg using HPLC. The long-term effect of Arg was observed histologically and immunocytochemically. 2 hours after inflation, Arg-treated artery showed a smaller amount of platelet adhesion, compared with saline-treated controls(C). The concentration of Arg at 0, 5, 15 minutes after inflation were 8.4, 5.6, 5.1 μM/g of wet weight of artery, respectively. Intima-media area ratio after 20 days were 0.33 ± 0.12 ($n = 6$) in Arg group and 1.33 ± 0.43 ($n = 6$) in C ($p < 0.001$). Neointimal cells were positively stained against HHF-35 and negatively against HAM-56. These results support the hypothesis that blood coagulation plays an important role in the restenosis after PTCA. Arg, locally administered using hydrogel-coated balloon, inhibits intimal smooth muscle cell migration and proliferation in a rabbit cartotid-injured model.

977 Hypertrophic Cardiomyopathy Mechanisms Causing Exercise Intolerance

Tuesday, March 26, 1996, 3:00 p.m.–5:00 p.m.
Orange County Convention Center, Hall E
Presentation Hour: 3:00 p.m.–4:00 p.m.

977-116 Exercise-Induced Alterations in Diastolic Properties of Regional Myocardium in Hypertrophic Cardiomyopathy

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We examined changes in regional diastolic function during exercise in hypertrophic cardiomyopathy (HCM). Micromanometer left ventricular (LV) pressure and LV short axis M-mode echocardiogram were simultaneously obtained at rest and during supine bicycle exercise (25 W) in 9 patients with HCM and asymmetric septal hypertrophy (the HCM group) and 6 control patients (the control group). The regional diastolic myocardial stiffness constant (KDM) was calculated by fitting the diastolic mean wall stress and the natural logarithm of reciprocal of wall thickness data from minimal stress to regional end-diastole in exponential form. While there was no shift in the LV