Poster No. 400

Local I-kappa-bα (Iκbα) Gene Therapy for the Reduction of Neointimal Hyperplasia After PTA or Stent Implantation in a Hypercholesterol Rabbit Model

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PURPOSE: To evaluate feasibility and efficiacy of the inhibition of the activator of smooth muscle cell proliferation NF-κB by local adenoviral mediated gene transfer of IκBα for the reduction of intimal hyperplasia after stent implantation.

MATERIAL AND METHODS: After carotid arterotomy PTA or stent implantation (Symphony stent 3.5mm diam/ 15mm length, Boston Scientific) was performed in the iliac arteries of cholesterol fed New Zealand White rabbits. Local adenovirus mediated transfer of IκBα (3ml of 109 fpu/ml at 6atm) was performed with a local drug delivery system (Multichannel balloon, 3mm diam/ 20mm length, Boston Scientific) after stent implantation (n=8 limbs) or PTA (n=10 limbs). In stented animals two control groups (8 versus 8 limbs, each) received either balloon dilatation alone or balloon dilatation with additional phosphate buffered saline (=PBS) impregnation with the multichannel balloon. In balloon dilated animals controls had balloon dilatation with additional phosphate buffered saline (=PBS) impregnation (n=8).Explantations were performed one and four weeks after gene transfer. Samples were fixed in para-formaldehyd, methacrylat embedded, cut using the sage-grind technique, stained and analyzed histomorphometrically. Efficient transfection was demonstrated by immunehistochemistry with an antibody against IκBα.Results: In IκBα transfected arteries neointimal hyperplasia was markedly reduced 4 weeks after PTA, significantly reduced 4 weeks after stent implantation compared to the control groups (p=0,20 and p=0,02 respectively), consequently patent vessel area was increased.

CONCLUSION: Local delivery of an adenovirus mediated IκBα gene therapy may be an effective way to control intimal hyperplasia after stent placement or reduce the restenosis process after PTA or stent implantation.

Poster No. 401

Effects of Anthranilic Acid Derivatives on Human Aortic Vascular Smooth Muscle Cells Are Caused by the Downregulation of p44/42 MAP Kinases

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PURPOSE: The aim of the study was to examine the effects of meclofenamic acid and flufenamic acid on proliferation, clonogenic activity, migratory ability, cell cycle distribution, and p44/42 MAPK (mitogen activated protein kinase) expression on serum-stimulated human aortic smooth muscle cells (haSMCs).

MATERIAL AND METHODS: HaSMCs were treated in 3 different concentrations with meclofenamic acid (10μM, 100μM, 200μM) and flufenamic acid (40μM, 200μM, 400μM) for four days. Then meclofenamic/flufenamic acid free culture medium was supplemented every four days until day 20 after initial treatment. The growth kinetics of the treated cells were compared with non-treated cells. Cell cycle analysis was performed by flow cytometry. The clonogenic activity was evaluated with colony formation assays. The migratory ability was investigated by stimulation with platelet derived growth factor (PDGF-BB) in 24 well plates with 8 μm pores membrane inserts. The p44/42 MAPK was detected by Western blot technique.

RESULTS: Meclofenamic acid and flufenamic acid inhibited proliferation, clonogenic activity, and migratory ability of haSMCs in a dose dependent manner. Cell cycle analysis revealed a G2/M-phase block. The p44/42 MAPK was significantly reduced .

CONCLUSION: Meclofenamic acid and flufenamic acid inhibit the proliferation and migration of haSMCs. If a sufficient dose of meclofenamic acid or flufenamic acid can be applied systemically or by local drug delivery it could be a candidate to prevent restenosis after angioplasty.

Poster No. 402

Vascular Smooth Muscle Cell-specific Gene Targeting With the SM22 Promoter

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PURPOSE: Adenoviral-mediated gene transfer to the vessel wall holds promise as a therapeutic intervention for vascular occlusive diseases. However, myriad cell types comprise advanced human vascular lesions, thus complicating the rational deployment of therapeutic genes to the vessel wall. We have undertaken studies that couple cell-restricted gene targeting using a smooth muscle-specific promoter (SM22) with a local delivery catheter called the Infiltrator.

MATERIALS AND METHODS: We substituted the CMV promoter of the pCA3 shuttle plasmid with the mouse SM22 promoter followed by (a) a nuclear lacZ reporter gene; (b) a dominant negative fibroblast growth factor receptor (DNFGFR) or (c) a dominant negative serum response factor (DNSRF) cDNA. Shuttle plasmids were integrated into a replication-defective adenovirus (dL327) and titered for transduction efficiency using either rat carotid artery or rabbit iliac artery SMC. Rabbit studies utilized an intramural catheter device (Infiltrator) for local delivery (infusion time was 45 seconds) of adenovirus. End points measured included the histochemical staining of the lacZ product as well as FGF-induced MAPK activity and c-fos expression and SRF-dependent mRNA/protein expression.

RESULTS: At 30 MOI, SM22 directed specific, high level expression of each transgene in cultured SMC.