onary artery smooth muscle cells (HCASMC), respectively. **Methods**: HACM were isolated from recipients' hearts after heart transplantation and characterized by positive staining for troponin-I and cardiotin. The cells were negative for two fibroblast-specific antibodies as well as for desmin and vWF indicating the absence of fibroblasts, smooth muscle cells and endothelial cells. HCASMC were isolated from recipients' coronary arteries using the explant-technique and stained positive for alpha-smooth muscle actin. Such characterized cells were treated with PGE1 and effects on VEGF-A expression were studied using an ELISA and RT-PCR. **Results**: When HACM or HCASMC were treated with PGE1, a significant up to threefold increase in VEGF-A production could be observed. These results could be confirmed on the level of specific mRNA expression as determined by RT-PCR. The effect of PGE1 on VEGF-A expression could be reversed by pertussis toxin. **Conclusion**: We conclude from our data that PGE1-induced expression of VEGF-A in cardiac myocytes and smooth muscle cells might contribute to its beneficial effects seen in patients with ischaemic heart disease. It should, however, be mentioned that increased production of VEGF in smooth muscle cells could potentially also induce angiogenesis within the atherosclerotic plaque and thereby could contribute to plaque destabilization.

1054-143 **Different Cell Death Rates Determine Growth of Vascular Smooth Muscle Cells From Human Aortocoronary Bypass Vessels: Implications for Patency of Radial Artery Grafts**

Karin Frischknecht, Helen Greutert, Thierry P. Carrel, Felix C. Tanner, University Hospital Bern, Bern, Switzerland

Long-term patency of aortocoronary bypass grafts is determined by vascular smooth muscle cell (VSMC) proliferation leading to neointima formation. Radial artery (RA) patency rates seem to be in between those from mammary artery (MA) and saphenous vein (SV). We therefore examined whether and, if so, why proliferation of VSMC from these bypass vessels differs accordingly. After 6 days of serum stimulation, RA-VSMC number (51'520±7'538) was lower than SV (69'750±11'588), but higher than MA (28'365±6'324) (SV vs. MA: p<0.01; SV or MA vs. RA: p=n.s.; n=4). In contrast, RA-VSMC exhibited only minimal proliferation to PDGF-BB, (15'493±2'116), which was comparable to MA (15'446±2'768; MA vs. RA: p=n.s.) and differed from SV (33'094±3'028; SV vs. RA or MA: p<0.01; n=4). Analogous results were obtained by 3H-thymidine incorporation (n=4). As determined by FACS analysis, PDGF receptor β expression was similar (p=n.s.; n=5), whereas PDGF receptor β expression was higher in RA as compared to MA or SV (RA vs. MA or SV: p<0.05; MA vs. SV: p=n.s.; n=5). Western blotting confirmed these findings. Propidium iodide incorporation after PDGF stimulation showed identical cell cycle distribution in VSMC from all three vessels. Similarly, Western blotting for cell cycle proteins after PDGF treatment revealed identical expression: the cyclin-dependent kinase inhibitor (CKI) p27 was downregulated, the CKI p21 was slightly induced, while the CKI p57, cyclin-dependent kinase 2 (cdk2) and cyclin E did not show any change. Cdk2 kinase assay confirmed that G1 progression was identical in VSMC from all three vessels. While neither the visual aspect nor trypane blue staining were different, LDH release was higher in VSMC from MA as compared to RA or SV (SV vs. MA: p<0.05; RA vs. MA or SV: p=n.s., n=5). Thus, cell cycle progression is identical in VSMC from human bypass vessels, while different cell death rates determine growth, suggesting that VSMC death rather than proliferation may account for the different patency rates of bypass grafts. The weak effect of PDGF in RA as compared to SV encourages the clinical use of RA grafts.

1054-144 **Activated Protein C Inhibits the Release of Proinflammatory Cytokines and Chemokines From the Monocytic Cell Line THP-1**

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Background: It has been suggested that the activated protein C (APC) pathway serves as a common link between coagulation and inflammation. This has been further supported by reports showing that APC could inhibit or induce cytokine production in endothelial cells. In addition to endothelial cells, monocytes are also important in the inflammatory response. In order to determine what effect APC may have on cytokine and chemokine production in monocytes, experiments were conducted using the monocytic cell line THP-1. We investigated the effect of human APC on the monocytic release of the proinflammatory cytokines macrophage inflammatory protein-1-alpha (MIP 1-alpha) and tumor necrosis factor-alpha (TNF-alpha) as well as the effect of APC on the release of the chemokine monocyte chemoattractant protein-1 (MCP-1). All of these mediators may be important for the interaction of monoytes at the endothelium during initiation and progression of atherosclerosis. **Methods and Results**: We established a monocytic cell model of inflammation by the addition of lipopolysaccharide (LPS from E.coli 026:B6; 0.1-1µg/ml) and examined the effect of APC on cytokine/chemokine release. As a further extension of the effect of APC on cytokines, we found that human APC (2.5-10µg/ml) inhibited LPS-induced release of MIP-1-alpha and MCP-1 from THP-1 cells, as measured by an Enzyme-linked immunosorbent assay at 6 up to 24 h. Furthermore APC inhibited the LPS-induced relase of TNF-alpha, time- and dose-dependently.**Conclusions:** The ability of APC to inhibit the release of the proinflammatory cytokines MIP-1-alpha and TNF-alpha and of the chemokine MCP-1 provides further evidence that APC may modulate initiation and progression of atherosclerotic lesions through the control of cytokine and chemokine release from monocytes.

1054-145 **The Coexpression of the Migration Inhibiting Factor 1-Alpha With CD40l in Human Atherosclerotic Plaques Is Associated With Advanced Plaque Stages and Extent of Intraplaque Inflammation**

Alexander Schmeisser, Thomas Illmer, Christiane Graffy, Herribert Loesel-Sadee, Dittmar Boeckler, Dittmar Menschikowski, Gerhard Ehninger, Christoph Garlichs, Werner G. Daniel, Ruth H. Strasser, Technical University of Dresden, Dresden, Germany, University Erlangen-Nuernberg, Erlangen, Germany

Background: MIF is a hormone and cytokine with pivotal importance in inflammatory and immune responses. Little is known about the role of MIF in atherosclerotic plaque progression and inflammation.
Methods: Carotid endartherectomy and vessel samples (n=46) were collected from 36 patients and 4 young accident victim controls and histological classified by Stary: minimal plaques (MP) were defined as Stary: 0-III and advanced plaques (AP) as Stary: IV-VIII. The immunhistochemical detection of MIF and CD40l in macrophages (Ma), microvascular endothelial cells (MEC), vascular smooth muscle cells (VSMC) and T-lymphocytes (TL) was perfomed. The relative extent of MIF expression was graded on scales of d0 to d3 in terms of specific staining intensity. In addition, the number intraplaque microvessels and inflammatory foci (>10 CD45-positive mononuclear cells (MNC)/field) were counted. In vitro, the monocyte cell line THP-1 was stimulated with a CD40l-fusion protein (1microg/ml), 1mg/microl LPS or with heat denaturated CD40l.
Results: APs (n=36) shows sign. (p<0.01) more intimal MNC and intraplaque microvessels than MPs from patients/controls (n=10), and higher MIF expressing intensitiy by MA/TL and MVEC. Strong MIF staining (d2/d3) was detected in Ma/TL in 70% and MEC in 80% of AP, and only in 10% and 0% of MP, respectively. Strong MIF staining was colocalized with CD40l expression in foci with Ma/TL staining. VSMC stained only weak or no for MIF. CD40l upregulated THP-1 MIF expression after 12h (mRNA) and 48h (protein) sign. (p<0.01) and comparable to TNF-alpha in vitro.
Conclusion: The results reveal a marked upregulation of migration inhibitory factor 1-a in close correlation to macrophage/T-cell infiltration and intraplaque microvessel extent in advanced plaques. Additionally, the coexpression with CD40l suggests a pivotal role of MIF in the inflammatory process and intimal neoangiogenesis of atherosclerotic plaques.

POSTER SESSION

1055 Basic Mechanisms of Angiogenesis and Arteriogenesis

Sunday, March 30, 2003, 3:00 p.m.-5:00 p.m.
McCormick Place, Hall A
Presentation Hour: 4:00 p.m.-5:00 p.m.

1055-117 **Hammerhead Ribozymes Directed Against Cyclin E and E2F1 as Tools to Prevent In-Stent Restenosis**

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In-stent-restenosis is associated with prolonged cellular proliferation in stented arteries. We explored the possibility to specifically inhibit vascular smooth muscle cell (VSMC) proliferation via hammerhead ribozymes. Two ribozymes, targeted against cyclin E and E2F1 mRNAs, were used. As these two genes co-operate to promote the transition from G1 to S phase of the cell cycle, they represent ideal targets to arrest cells in G1 phase thus blocking proliferation.
Methods: Phosphorothioate-modified ribozymes complexed with liposomes were administered to human VSMC. Cell proliferation and distribution throughout the phases of the cell cycle were analyzed by a double DNA staining procedure. The antiproliferative effect of the specific ribozymes were evaluated in comparison to scrambled binding arm ribozymes (RzScr) liposomes alone and non-treated cells. Different ribozyme concentrations and times after transfection were tested. In addition, the effect of a combined administration of the two ribozymes was evaluated. Finally, a comparison between the efficacy of ribozymes and the corresponding antisense oligonucleotides (ODNs) was performed.
Results: Two days after transfection, each of the two specific ribozymes showed a dose-dependent inhibition of proliferation in comparison to non-treated, liposome-treated and RzScr treated cells. Ribozyme effect peaked at 420 nM, reducing cell growth down to 34 % of non treated cells. At the same final concentration (420 nM), a combined administration of the two ribozymes (210 nM each) resulted in an even more pronounced proliferation inhibition (25 % of non treated cells). Time course experiments showed that ribozyme activity persists for at least four days following transfection. In addition, ribozymes were more effective than the respective antisense ODNs. Finally, we proved that growth inhibition was due to an increase in G1 phase cells, paralleled by the concomitant decrease in S phase cells.
Conclusion: Ribozymes against cyclin E and E2F1 reduced VSMC proliferation up to four days by arresting cells in G1 phase. The dramatic and specific decrease in cell growth provides the basis for the development of a ribozyme therapy for in-stent restenosis.