1063-105 Ribozymes to Conserved Human-Porcine PCNA Reduce In-stent Restenosis in a Porcine Model

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We designed chimeric DNA-RNA hammerhead ribozymes (RZ) to conserved mRNA for porcine and human PCNA. In vitro studies included RZ cleavage and serum stability. In vivo we assessed RZ effect on DNA synthesis and proliferation of cultured porcine and human vascular SM cells. We then studied, in a stent model, 18 non lipemic, adult pigs (26 coronaries) treated by local infusion of 180 μgm at the angioplasty site of either: 1) scrambled RZ control (SRZ), 2) RZ to PCNA or 3) stent alone (STE) (8 arteries/group). At 4 weeks angiography, sacrifice and histomorphometry were done.

Results: RZ cleaved >50% mRNA and persisted in serum up to 5 minutes. DNA incorporation/cell proliferation were both significantly reduced. Quantitative coronary angiography (% MLD stenosis) and histomorphometric analysis (% Area stenosis) data are:

	% MLD stenosis	*p	#p	% Area stenosis	*p	#p
STE†	61 ± 12	-	0.68	74 ± 12	-	0.38
SRZ‡	63 ± 9	0.68	-	71 ± 16	0.38	-
PCNA	34 ± 12	<0.0001	<0.0001	57 ± 17	<0.0001	<0.0001

Conclusions: RZs to PCNA are biologically active in-vitro and in-vivo. They markedly reduce angiographic and histomorphometric in-stent stenosis in a porcine model.

1063-106 Adenovirus-mediated Gene Transfer of Recombinant Tissue Factor Pathway Inhibitor in Vitro and in Vivo

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Local upregulation of tissue factor contributes to the thrombogenicity of the atherosclerotic wall. To assess whether the gene encoding human tissue factor pathway inhibitor (TFPI) can be transferred to vascular cells and the atherosclerotic vessel wall, we have constructed a recombinant adenovirus (Ad) carrying the cDNA encoding TFPI. TFPI was measured by ELISA in the conditioned medium of dog vascular smooth muscle cells (VSMC) infected with Ad.TFPI at a multiplicity of infection (MOI) of 500. Secretion of TFPI (ng/10^6 cells/24 h) was 1098 ± 292 (mean ± SD) on day 2, 6145 ± 1125 on day 4 (peak), and 826 ± 77 on day 7. Four days after infection with Ad.TFPI at MOI 20, 100, 200, and 500, VSMC produced, respectively, 61 ± 51, 197 ± 205, 405 ± 387 and 6145 ± 1125 ng TFPI/10^6 cells/24 h, which was, on a molar basis, >90% active in inhibiting tissue factor/factor VIIa in a factor X activation assay. Control Ad (no transgene) did not induce expression of TFPI in dog VSMC. No TFPI was detected by ELISA (detection threshold 0.4 ng/mL) in the conditioned medium of human VSMC, but was 347 ± 80 ng/10^6 cells/24 h three days after infection with Ad.TFPI at MOI 100. TFPI was visualized by immunohistochemistry in Ad.TFPI-infected cultured dog VSMC and in the balloon-injured femoral artery of a hypercholesterolemic dog infected 4 days before sacrifice by local dwell with Ad.TFPI (6×10^{10} pfu/mL). TFPI was observed by immunohistochemistry in severely atherosclerotic carotid arteries of Watanabe rabbits infected at the time of balloon injury with Ad.TFPI (but not control virus) at 1×10^{10} pfu/mL. Our findings show that adenovirus-mediated gene transfer of TFPI can achieve expression of TFPI within the atherosclerotic vessel wall and indicates the potential of local TFPI gene transfer to prevent thrombin generation and thrombosis at the site of balloon injury to atherosclerotic plaques.

1064 Genetic and Epidemiologic Determinants of Coronary Artery Disease

Monday, March 30, 1998, 3:00 p.m.–5:00 p.m.
Georgia World Congress Center, West Exhibit Hall Level
Presentation Hour: 3:00 p.m.–4:00 p.m.

1064-1 Thermolabile Methylenetetrahydrofolate Reductase in Coronary Artery Disease

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Mild hyperhomocysteinemia is an independent and graded risk factor for coronary artery disease (CAD), and may result from environmental and hereditary factors. A recently detected 677C→T transition in the methylenetetrahydrofolate reductase (MTHFR) gene has been associated with elevated plasma homocysteine concentrations. We assessed the frequency of this common mutation in 735 male patients from the Dutch REGRESS (Regression Growth Evaluation Statin Study) study and in 1,250 Dutch controls. The frequency of the homozygous (+/+) mutation was 9.5% among (CAD) patients versus 8.5% among controls (OR 1.21 [95% CI: 0.87–1.68]). Plasma homocysteine concentrations were significantly elevated in both (+/+) and (+/−) individuals compared with (−/−) subjects. For a reliable estimation of the risk of the (+/+) genotype in CAD, we performed a meta-analysis on 8 case control studies. In this analysis the (+/+) genotype was present in 299 (12.1%) out of 2,476 patients and in 257 (10.4%) out of 2,481 controls. This resulted in a significant OR of 1.22 [95% CI: 1.01–1.47], demonstrating that the (+/+) genotype is a modest but significant genetic risk factor for CAD, which is likely modulated by folate status.

1064-2 E-Selectin Genotypes in Patients With Documented Severe Coronary Artery Disease

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Background: The relevance of the E-selectin genotypes as an indicator of atherosclerotic risk is under debate. We studied the genotype frequency of the 128-serine/arginine polymorphism of the EGF-like domain of the E-selectin in 54 consecutive patients with coronary artery disease (CAD, 76% males) who underwent coronary angioplasty and in 102 control subjects (57% males) without clinical evidence of CAD.

Methods: The E-selectin polymorphism was analysed by PCR, single strand conformation polymorphism analysis and direct sequencing of PCR products.

Results: The frequencies of the arginine/arginine, arginine/serine and serine/serine genotypes were 0%, 9%, and 91% (1-vessel-disease, n = 11), 0%, 30%, and 70% (2-vessel-disease, n = 27) and 0%, 25%, and 75% (3-vessel disease, n = 16) in these groups (p = 0.0008, for intergroup differences) and 2%, 14%, and 84% in the control subjects (p = 0.08, controls vs all patients). There was an excess in the arginine/serine genotype among patients with multivessel disease vs patients with 1-vessel disease (28% vs 9%, p = 0.0005) and vs control subjects (28% vs 14%, p = 0.02) as well as in one subgroup of 12 patients with a positive family history for CAD in contrast to the remaining 42 patients (42% vs 19%, p = 0.03).

Conclusion: The arginine/serine genotype was significantly related to multivessel CAD and could help to predict the individual risk of development of severe manifestation of coronary artery disease.

1064-3 Oxidative Stress Is Genetically Influenced by the Haptoglobin Polymorphism

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Background: Haptoglobin (Hp) is a hemoglobin-binding plasma protein with three genetic phenotypes: Hp 1-1, Hp 2-1, Hp 2-2. The protein is an antioxidant protecting against the hemoglobin/iron-driven oxidative stress. As the Hp 2-2 type is an independent risk factor in coronary artery disease and peripheral arterial occlusive disease, we investigated the iron-driven oxidative stress according to Hp phenotype.

Methods: The Hp type was determined using starch gel electrophoresis in serum samples from 205 healthy Caucasians (108 males, 97 females). Iron metabolism and vitamin C antioxidant status were studied by measuring serum iron, transferrin (Tf) saturation, ferritin, and vitamin C levels.

	Hp 1-1 (n = 35)	Hp 2-1 (n = 98)	Hp 2-2 (n = 72)	ANOVA (P-value)
Haptoglobin (g/l)	1.26 ± 0.43	1.08 ± 0.50	0.84 ± 0.42	0.005
Iron (μmol/l)	16.6 ± 6.1	18.6 ± 7.7	21.3 ± 8.6	0.04
Tf-saturation (%)	27 ± 9	30 ± 10	35 ± 12	0.03
Ferritin (μg/l)	51 ± 35	64 ± 45	85 ± 59	0.003
Vitamin C (μmol/l)	57.7 ± 17.7	60.2 ± 19.3	46.4 ± 13.9	0.001

Data are mean ± S.D.

Results: The serum Hp concentrations correspond to the type-related reference values in Caucasians, showing lowest levels in Hp 2-2 subjects. The Hp 2-2 type is associated with higher iron, transferrin saturation, and ferritin levels and with low vitamin C concentrations.

Conclusion: Hp 2-2 subjects have a lower hemoglobin-binding capacity and hence a higher degree of heme iron accumulation. This iron excess on its turn induces an oxidative vitamin C depletion. Our results are in agreement with the clinical association of Hp 2-2 with atherosclerosis, and further support