11:30

IN SITU DETECTION OF EGF RECEPTOR mRNA IN HUMAN ATHEROMATOUS LESIONS: IMPLICATIONS FOR PROLIFERATION AND MIGRATION OF SMOOTH MUSCLE CELLS

Gerhard Bauriedel (1,2), Helga Riesemann (2), Ulrich Windstetter (1), Berthold Höfling (1), Reinhard Kandolf (1,2). Dept. Internal Med. I, University of Munich (1), Max-Planck-Inst. of Biochemistry, Martinsried (2), FRG

Local epidermal growth factor (EGF) receptor gene expression might be involved in the formation and progression of atherosclerotic plaques. Surgically removed specimens from advanced lesions (11 coronary, 3 aortic, 3 carotid, 2 femoral) of 19 patients were taken for in situ hybridization studies using a specific cDNA probe for EGF receptor mRNA. In serial vascular sections of 3/19 patients studied, autoradiographic silver grains were clearly localized to intimal cells adjacent to the internal elastic lamina. Positive signals were not seen in the fibrous cap, the plaque sholders, necrotic intimal areas and the media. In smooth muscle cells (SMC) cultivated from human plaques, EGF increased proliferation rate in a dose-dependant manner (control: 0.14 ± 0.01 population doublings/day; 10ng/ml EGF:0.20 ± 0.03; x ± SD, n=4). In addition, SMC migratory velocity assessed by a semi-automatic standardized video system was increased from 19.1 ± 1.6 (control; n=62 observed cells) to 29.0 ± 1.6 µm/h (10 ng/ml EGF; x ± SEM, n=66). Conclusions: EGF was shown to synergistically increase proliferative and migratory activity of plaque derived smooth muscle cells. The expression of EGF receptor mRNA in human atheromatous lesions may suggest corresponding stimulatory mechanisms in vivo.

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11:45

IN VIVO CARDIOVASCULAR GENE TRANSFER VIA INTERVENTIONAL TECHNIQUE: INITIAL EXPERIMENTAL RESULTS

Gregory D. Chapman, Chang S. Lim, Judith L. Swain, Roger S. Gammon, Robert P. Bauman, and Richard S. Stack. Duke University Medical Center, Durham, North Carolina

Gene therapy for treatment of coronary artery disease has potential application given recent advances in molecular biology and interventional cardiology. The ability to inhibit restenosis and thrombosis by gene products at the site of arterial lesions may greatly improve patient outcomes. The purpose of this study was to achieve in vivo gene transfer in coronary arteries via a catheter delivery system. The DNA transferred in these experiments was a non-retroviral plasmid coding for luciferase. This reporter was chosen since it is not present in mammalian cells, and a sensitive assay exists for detection of protein (light units generated by hydrolysis with ATP). Two techniques were evaluated for intra-arterial uptake and expression of this gene: 1) surgical exposure of femoral and coronary arteries with direct cannulation and perfusion with DNA and a lipid vehicle, and 2) exposure of the coronary arteries to the DNA and lipid vehicle via a percutaneous intracoronary delivery catheter. Mongrel dogs were treated and all survived until sacrifice at 3 days post-procedure. Luciferase activity (in light units) in treated arterial segments was compared to non-treated segments. Luciferase activity was not detected in the control segments. DNA mediated gene transfer and expression occurred in 6 of 8 femoral arteries and 2 of 2 coronary arteries for the surgical technique; it occurred in 4 of 6 coronary arteries treated percutaneously (Table).

	DNA treated segments	
Surgical technique:	Light units	picograms luciferase
Femoral arteries (n=8)	570±275	19±9
Coronary arteries (n=2)	899±38	30±1.3
Percutaneous catheter delivery:		
Coronary arteries (n=6)	98±49	3.3±1.6

Thus we demonstrate that DNA can be introduced into coronary and peripheral arteries in vivo, with resultant expression of gene products. In addition, we show that a percutaneous catheter approach can be used for localized gene transfer into coronary arteries. This method offers an interventional approach to the treatment of cardiovascular disease through gene therapy.

Monday, March 4, 1991
10:30AM–12:00NOON, Room 254, West Concourse
Thrombosis and Fibrinolysis

10:30

FIBRINOLYTIC ACTIVITY FOLLOWING VASCULAR WALL INJURY

Yi Shi, Andrew Zalewski, Donald Nardone, Paul Walinsky, Thorir D. Bjornsson, Thomas Jefferson University, Philadelphia, PA

Vessel wall injury in either acute coronary syndromes or during coronary angioplasty is associated with increased procoagulant activity and frequent intracoronary thrombi. Accordingly, the aim of this study was to assess plasma levels of tissue plasminogen activator activity (t-PA) and plasminogen activator inhibitor-1 activity (PAI-1) prior to and at 1, 6, and 24 hr after experimental vessel wall injury (INJ). In 26 atherosclerotic rabbits, INJ was produced by angioplasty of iliac arteries in 18, while 8 rabbits with no INJ served as controls (C). Histology in 9 rabbits from INJ group revealed medial INJ in all animals. t-PA levels (IU/ml, mean ± SEM) were as follows:

	Baseline	1 hr	6 hr	24 hr
C	13.53±2.07	11.97±1.81	14.16±0.99	12.67±1.32
INJ	14.89±1.02	13.57±0.87	14.73±0.86	16.64±1.05

t-PA levels were not different between C and INJ groups. PAI-1 levels (AU/ml, mean ± SEM) were as follows:

	Baseline	1 hr	6 hr	24 hr
C	21.80±1.34	22.72±2.50	21.66±3.57	22.28±2.67
INJ	21.79±1.27	22.61±1.11	32.05±1.47*	23.99±1.16

Thus, PAI-1 activity increased significantly at 6 hr following INJ (*$p<0.01$ vs INJ at baseline, 1, 24 hr and vs all times in C group). In contrast, C group showed no change throughout the study period.

Conclusions:

1) Fibrinolytic activity was transiently impaired following deep vascular wall injury as a result of an increase in PAI-1 activity.

2) In addition to other procoagulant mechanisms, PAI-1 elevation in the presence of vessel wall injury may play a role in the pathogenesis of acute coronary syndromes.

10:45

THE EFFECT OF TPA ON RESTENOSIS FOLLOWING BALLOON ANGIOPLASTY: A STUDY IN THE ATHEROSCLEROTIC RABBIT.

Joel Gellman, Stephen L. Sigal, Qingsheng Chen, Ernie L. Esquivel, Michael D. Ezekowitz, Yale U, New Haven, CT., and the VAMC, West Haven, CT.

To test the hypothesis that thrombosis and platelet derived growth factors contribute to restenosis after angioplasty (A) and that their removal using a thrombolytic agent will influence the restenosis rate, we performed a study in 17 NZW rabbits with bilateral focal femoral atherosclerosis who were undergoing A. Rabbits were randomized to either a 3 hr infusion of tissue plasminogen activator (tPA), 3mg/kg, or saline (S). Balloon A (2.5mm balloon; 3 x 60sec, 10 Atm inflations 60secs apart) was performed 60.1 ± 3 min (mean ± SD) after commencement of infusion of tPA or S. Angiograms were obtained at baseline, 6 ± 2 min. and 28 ± 1 days post-Balloon A. Rabbits were sacrificed after the final angiogram. Serum fibrinogen (mg/dl) was measured at baseline: 256 ± 132 in tPA vs. 209 ± 79 in S, NS, and after infusion: 108 ± 59 in tPA vs. 251 ± 158 in S, $p<0.05$. There were no acute occlusions, perforations or hemorrhages: (Hb; 10.1 ± 1.8 to 8.6 ± 0.2, NS, in tPA and 10.7 ± 1.3 to 10.9 ± 2.8, NS in S, over the infusion period). The minimum angiographic lumen diameter measured 28 days after A changed from 1.2 ± 0.1 to 1.7 ± 7 ($P<.11$) in the t-PA group and from 1.4 ± 0.3 to 1.0 ± 0.6 in the control ($P<.03$). Thus tPA at a dose which decreased serum fibrinogen by about 60% resulted in a decrease in the restenosis rate. We postulate that this observation may be related to the removal of thrombus derived growth factors by the thrombolytic agent.