11:00

790-3 Induction of Programmed Cell Death in Human Vascular Smooth Muscle Cells Following Exposure to β Irradiation

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Relevant biological mechanisms by which endovascular irradiation may prevent neointimal hyperplasia following angioplasty remain unclear. Radiation-induced programmed cell death may play an important role in this phenomenon. Accordingly, the purpose of this study was to verify if ^{32}P irradiation induces apoptosis in vascular smooth muscle cells (VSMC). Human VSMCs were irradiated with sealed sources of ^{32}P with activity levels of 0.2, 0.5, 1, 2, and 5 μCi, for continuous periods of 6, 12, and 24 hours. Apoptosis was evaluated by labelling the cell's DNA with ^{14}C-thymidine and then comparing the level of incorporated ^{14}C in fragmented and intact DNA. The irradiation effect on cellular proliferation was also assessed under the same conditions using the ^{3}H-thymidine assay. No significant apoptosis was detected for irradiation periods of 6 and 12 hours. Similarly, no inhibition of cellular proliferation was detected for the same exposure time to ^{32}P. After 24 hours of irradiation, however, apoptosis was detected for the 2 and 5 μCi activity levels, with respective values of 23.5 ± 3.8% (P = 0.008 vs control) and 26.4 ± 5% (P = 0.002 vs control). Inhibition of VSMC proliferation was significative after the 24 hour exposure period with inhibition values of, respectively, 31.9 ± 4.9% (P = 0.001 vs control) and 62.5 ± 6.3% (P = 0.0002 vs control) for the 2 and 5 μCi activities. No significant apoptosis was detected for the lower activity of ^{32}P source. *Conclusions:* 1. Exposure of human VSMCs to β radiation originating from a ^{32}P source results in a significant reduction in their proliferation index. 2. Concomitant with this proliferation inhibition, β irradiation induces apoptosis in VSMCs. 3. The occurrence of apoptosis and inhibition of proliferation in VSMCs by β irradiation, appears to be dependent on a threshold of source activity and exposure time.

11:15

790-4 Atherectomy Specimens Obtained From Patients With Restenotic Lesions Reveal Higher Monocyte Chemoattractant Protein-1 Levels Than Those With De novo Lesions

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This study sought to study the presence of monocyte chemoattractant protein 1 (MCP-1) in the human atherectomy specimens derived from both de novo and restenotic lesions.

Methods: A total of 24 specimens (12 de novo and 12 restenotic) were used in this study. Frozen sections (5 μ) were used for immunostaining (IHC), using an immunoenzymatic staining kit (DAKO). As a primary antibody rabbit anti baboon MCP-1 polyclonal antibody was used in 1:750 dilution. Counterstaining was performed with Mayer's hematoxylin. IHC staining was evaluated by light microscopy, grading on a semiquantitative scale from 0 to 4 in a blinded manner, corresponding to the estimated fraction of positive staining cells and the estimated average staining intensity of positive cells, respectively. Staining was assessed and recorded according to a proportion and intensity scoring system developed for IHC staining of tumors (Allred et al, 1993).

Results: staining for MCP-1 was present in 25% of the specimens obtained from de novo lesion, staining index was between 1 and 2. On the other hand, MCP-1 stain in restenotic lesion was present in all specimens, with the intensity of stain 2–3. Cells producing MCP-1 were identified by monoclonal antibodies as smooth muscle cells and monocytes/macrophages.

Conclusions: MCP-1 is produced by smooth muscle cells and monocyte/macrophages in coronary atheromas. Since a significant difference was shown between de novo and restenotic lesions, the pivotal role of monocyte/macrophages in restenosis processes is suggested.

11:30

790-5 Adenoviral Gene Transfer of Human Constitutive Endothelial Nitric Oxide Synthase to Injured Coronary Arteries

O.H. Varenne, S. Pislaru[1], H. Gillijns, R.D. Gerard, F. Van de Werf[1], D. Collen, S.P. Janssens[1]. *Center for Transgene Technology and Gene Therapy, Katholieke University of Leuven, Leuven, Belgium, [1] Department of Cardiology Katholieke University of Leuven, Leuven, Belgium*

Coronary gene transfer to prevent restenosis has been hampered by inefficient local delivery systems. We assessed the ability of the Infiltrator catheter to deliver adenovirus to the porcine LAD after overstretch injury (20 mm balloon, 3 inflations, 10 atm). Virus encoding either β-galactosidase (AdCMVβgal) or nitric oxide synthase (AdCMVceNOS) was injected (0.3 ml of 5 × 10^9 pfu/ml) over 10–15 sec. Histological staining for β-galactosidase and ceNOS showed homogeneous transgene expression in medial smooth muscle cells (SMC) and in adventitial cells adjacent to the media. A maximum of 41 ± 10% of medial SMCs and 23 ± 3% of adventitial cells expressed the transgene. Thus, the Infiltrator catheter enables highly efficient intramural adenovirus-mediated gene transfer. No staining was observed in the distal LAD, unrelated coronary arteries, or arteries infected with control adenovirus lacking a transgene (AdRR5). AdCMVceNOS infection markedly reduced platelet adhesion at the site of injury as studied by anti-platelet glycoprotein Ib immunostaining. Neointima formation was assessed at 28 days by computer-assisted planimetry on 6 μm sections. Balloon injury was similar in the two groups (balloon:artery ratio of 1.60 ± 0.08 vs 1.65 ± 0.09, p = NS). Initial studies (being extended) suggest that the neointimal area was reduced in AdCMVceNOS-treated vessels (0.75 ± 0.22 mm^2 vs 1.24 ± 0.36 mm^2 in AdRR5, $p = 0.058$, $n = 8$). Overexpression of recombinant ceNOS in balloon-injured coronary arteries may be a promising therapeutic strategy for restenosis.

11:45

790-6 Urokinase Plasminogen Activator Expression After Balloon Injury is Associated with Adventitial Cell Migration and Angiogenesis of the Vasa Vasorum

K. Pels, M. Labinaz, M.R. Garvin, C. Hoffert, E.R. O'Brien. *Univ. Ottawa Heart Inst., Ottawa, Canada*

In a porcine model of coronary artery angioplasty, we have observed angiogenesis of the vasa vasorum and adventitial myofibroblast proliferation and apparent inward migration. Before a cell can migrate local tissue barriers must be broken down by proteases, such as urokinase plasminogen activator (uPA). *Purpose:* Determination of the temporal relation between uPA expression and adventitial cell migration. uPA and plasminogen activator inhibitor (PAI-1) expression was studied by *in situ* hybridization and immunocytochemistry on days 3, 7, 14 and 28 after single and double injury of porcine coronary arteries. Incorporation of BrDU was used to assess cell proliferation. *Results:* Maximal levels of proliferating cells (e.g., ≤ 4%) were found in either the combined intima + media, or adventitia 3 days after single or double injury. Adventitial microvessel number and area increased dramatically 3 days after injury – only to later undergo resorption. Despite an absence of proliferation, intimal and medial cell number peaked on day 28 after second injury – probably due to inward migration of adventitial cells. PAI-1 mRNA was expressed in both normal and diseased arteries. uPA mRNA and protein expression showed a discrete interval of upregulation on days 3 and 7 after balloon injury – particularly in adventitial myofibroblasts and endothelial cells, and then returned to near baseline levels.

Conclusions: After angioplasty, uPA over-expression coincides with adventitial angiogenesis and the apparent inward migration of myofibroblasts. Antagonism of the uPA receptor may provide insight into the role of uPA in vascular cell migration and lesion formation.

791 Unstable Angina: Pathophysiology II

Wednesday, March 19, 1997, 2:00 p.m.–3:30 p.m.
Anaheim Convention Center, Room C2

2:00

791-1 Circadian Variation of Nitric Oxide Production in Normal Subjects

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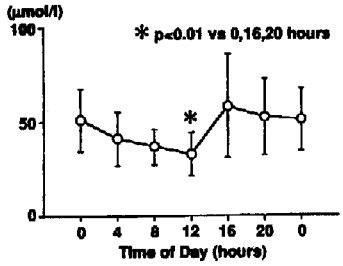
The time of the onset of acute myocardial infarction has circadian variation, i.e., the incidence of acute myocardial infarction is highest during the period from 6 a.m. to noon. Nitric oxide (NO) plays an important role in preventing against thrombosis by regulating platelet-vessel wall interaction. Therefore, if NO production is decreased in the morning, it may be a pivotal role for the high incidence of acute myocardial infarction in this period. The aim of this study was to determine whether a circadian variation is detected in the plasma NO levels. We studied 10 healthy male volunteers, aged 23–40. Blood was sampled from the peripheral vein at 4-hour intervals for 24 hours. We measured the plasma concentration of nitrate plus nitrite (NO^{2-} + NO^{3-}), the end-product of NO, by Griess method. NO^{2-} + NO^{3-} concentration was decreased in the morning and was lowest at 12:00. The lowest concentration was about a half as compared with a peak at 16:00 (Fig.).



Thus, a marked circadian rhythm was detected in the plasma concentration of $NO^{2-} + NO^{3-}$. Since this morning decrease in $NO^{2-} + NO^{3-}$ is consistent with the period of highest incidence of acute myocardial infarction, the morning decrease in NO production may contribute to the higher morning incidence of acute myocardial infarction.

2:15

791-2 Early Non-Invasive Assessment of Reperfusion by Myocardial Protein Release Patterns

K. Ramanathan [1], J.T. Stewart [1,2], J.K. French [1], P. Théroux [2], H.D. White [1]. [1] *Green Lane Hospital, Auckland, New Zealand,* [2] *Montreal Heart Institute, Montreal, Canada*

Prognosis after acute myocardial infarction is related to TIMI flow in the infarct-related artery (IRA) 90 minutes after thrombolytic therapy. To determine the efficacy of non-invasive assessment of reperfusion we measured CKMB mass, cardiac troponin T and myoglobin in 105 patients (59.1 [10.1] years), at 0, 30, 60 and 90 minutes after intravenous streptokinase (1.5×10^6U over 30–60 minutes), and assessed angiographic patency of the IRA at 90 minutes. The median time from symptom-onset to treatment was 2.8 hours. The myocardial protein levels were compared to pre-thrombolytic values to determine an index which identified TIMI flow grade <3. TIMI 3 flow in the IRA was seen in 59 patients, TIMI 0 or 1 in 26, and TIMI 2 in 20. A troponin T ratio $\leq$ 10 at 90 minutes detected failure to achieve TIMI 3 flow with 95% sensitivity, 58% specificity, 65% positive predictive accuracy (PA), and 94% negative PA. The corresponding values for myoglobin and CKMB mass ratios of 10 were 88% and 91% sensitivity, 65% and 49% specificity, 69% and 61% positive PA, and 86% and 87% negative PA, respectively.

Conclusions: Following streptokinase for acute myocardial infarction, comparison of myoglobin, troponin T and CKMB mass levels at 90 minutes with initial values, provides an accurate non-invasive means of detecting incomplete reperfusion. This may identify patients who would benefit from early catheterization and further reperfusion therapies.

2:30

791-3 The Predictive Value of Fibrinogen, C-Reactive Protein and Interleukin-6 on Admission in Severe Unstable Angina

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Recent studies suggest that inflammation is involved in unstable angina (UA). We studied the relation between markers of inflammation and in-hospital prognosis in 211 consecutive pts with severe UA. The clinical endpoint was persistent UA for which urgent coronary angiography was indicated, despite standard oral and intravenous anti-anginal therapy. Medical therapy alone was successful in 135 pts (64%). Persistent UA was present in 76 pts (36%); of these, 6 died, 10 had a myocardial infarction and 63 needed urgent PTCA or CABG. Fibrinogen levels on admission were significantly higher in the group with persistent UA; 3.70 v 3.28 g/L, $p < 0.001$. Levels of C-Reactive Protein and Interleukin-6 were 3.12 v 2.45 mg/L and 3.42 v 3.22 pg/mL (NS).

We separately studied the effects of duration of angina prior to admission on the relation between inflammatory markers and prognosis. In a predefined subgroup of 116 pts with recent onset UA (angina at rest that started <24 hrs before admission) those with persistent UA (35 of 116 pts = 30%) had significantly higher levels of Fibrinogen (3.63 v 3.22 g/L, $p < 0.05$), as well as CRP (3.97 v 1.97 mg/L, $p < 0.01$) and IL-6 (4.05 v 2.88 pg/mL, $p < 0.05$) compared with those who could be stabilised with medical therapy. All values are geometric means and adjusted for age, sex, body mass index and smoking behaviour.

We conclude that in patients with UA, higher levels of Fibrinogen – and in recent onset UA also higher levels of CRP and IL-6 – are associated with adverse in-hospital prognosis, underscoring the role of inflammation in the pathogenesis of unstable angina.

2:45

791-4 Soluble E-Selectin, ICAM-1 and VCAM-1 Levels in Systemic and Coronary Circulation in Patients With Variant Angina

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In order to assess the plasma levels of soluble adhesion molecules including E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), antecubital venous plasma samples were collected from 18 patients with variant angina (VA), 14 patients with stable effort angina (SA) and 16 control subjects (C). Samples were also collected from the aortic root (AO) and coronary sinus (CS) in 13 VA at baseline and after the resolution of spasm in the left coronary artery induced by intracoronary injection of acetylcholine.

	E-selectin	ICAM-1	VCAM-1
C	38 ± 4	182 ± 18	699 ± 45
VA	50 ± 4*	285 ± 15**	887 ± 88
SA	39 ± 4	187 ± 11	737 ± 38

mean ± SE ng/ml, * $p < 0.05$, ** $p < 0.01$ vs C and SA

	Baseline		After spasm	
	AO	CS	AO	CS
E-selectin	52 ± 4	53 ± 5	53 ± 6	59 ± 6
ICAM-1	293 ± 24	236 ± 16*	260 ± 16	287 ± 24*
VCAM-1	833 ± 90	676 ± 83**	760 ± 66	867 ± 113

ng/ml, *$p < 0.05$, ** $p < 0.01$ vs AO

The CS-AO differences in ICAM-1 and VCAM-1 levels were significantly increased after spasm as compared with the baseline, respectively. In conclusions, venous plasma E-selectin and ICAM-1 levels were higher in VA, indicating an association of an inflammatory reaction with coronary spasm. In VA, both soluble ICAM-1 and VCAM-1 appeared to be trapped in the coronary circulation at baseline, suggesting an autoprotective mechanism, and released into coronary circulation following spasm and reperfusion.

3:00

791-5 Evidence of Diffuse Myocardial β-adrenoceptor Downregulation after Myocardial Infarction in the Absence of Cardiac Failure

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Myocardial ischemia leads to activation of the autonomic nervous system. In the setting of acute myocardial infarction (AMI) there is evidence of sustained sympathetic overactivity. Acutely the net balance of this activation may be beneficial. Whether this also leads to abnormalities of myocardial β-adrenoceptor (βAR) density is not known. This density can be determined non invasively using positron emission tomography (PET). The aim of this study was to assess whether there are significant changes in myocardial βAR density in the subacute phase of infarction. We studied 25 patients (mean age 52 ± 11 years, range 31–72) whose first manifestation of ischemic heart disease was an AMI. All patients had single vessel disease and did not suffer from diabetes, hypertension or renal disease. Results in patients were compared with those obtained in 18 age matched controls (mean age 48 ± 14 years, range 23–65, p = NS vs patients). All patients were in NYHA class 1, they were studied between 1 and 2 months post AMI and none was on chronic β blockade. Regional myocardial βAR was measured by PET using ^{11}CGP 12177 as the ligand. *Results:* There was a significant difference in whole heart βAR density in patients compared to controls (5.78 ± 0.90 vs 8.35 ± 2.00 pmol/g, $p < 0.001$). In the patients, βAR density was reduced to a comparable extent both in the infarcted and the remote non-infarcted regions (5.70 ± 1.30 vs 5.79 ± 1.00 pmol/g, p = NS) supplied by angiographically normal or non significantly diseased coronary arteries.

Conclusions: 1- In the subacute phase of infarction there is a significant downregulation of myocardial βARs suggesting sustained sympathetic overactivity. 2- The reduction of βAR density affects both infarcted and remote non-infarcted myocardium to a similar extent. This suggests a diffuse myocardial autonomic dysfunction which might play a role in the genesis of left ventricular remodelling.