as additional controls. Results: Responses to acetylcholine (Ach) and A23187 were improved with spironolactone (Table 1) while responses to (endothelium independent) agonist nitroglycerin and phenylephrine remained unaltered (pk relaxation of 100 ± 3 vs. 100 ± 3% and constriction of 103 ± 22 vs. 108 ± 19% in placebo vs. spironolactone respectively.

Table 1.

	Ach		A23187	
	Pk Relaxation(%)	ED_{50}	Pk Relaxation (%)	ED_{50}
$LDLR^{-/-}$ Placebo	67.3 ± 8.1	7.5 ± 0.21	74.8 ± 2.6	7.4 ± 0.1
$LDLR^{-/-}$ Spiro	103.6 ± 6.4*	7.6 ± 0.10	92.0 ± 2.5*	7.6 ± 0.4

**= p<0.01 vs. placebo,*

Time to thrombosis was decreased with aldosterone infusion (13±2 vs. 23±2 minutes in controls, p<0.05 by ANOVA) while spironolactone administration prolonged time to thrombosis (28±2 vs. 23±2 minutes, p<0.05 by ANOVA).

Conclusion: MR antagonism improves endothelial function and attenuates thrombotic response to injury while aldosterone potentiates thrombotic response to injury. These findings indicate an emerging role for aldosterone receptor antagonists in atherosclerosis.

1032-130 Short-Term Effect of Oral Anticoagulant Therapy on Documented Left Atrial Thrombi in Candidates for Percutaneous Transvenous Mitral Commissurotomy

Songkwan Silaruks, Bandit Thinkhamrop, Songsak Kietchoosakul, Chaiyasith Wonvipaporn, Pyatat Tatsanavivat, Virat Klungboonklong, Khon Kaen University, Khon Kaen, Thailand

Background: The presence of left atrial thrombus (LAT) in mitral stenosis patients is a contraindication to percutaneous transvenous mitral commissurotomy (PTMC). Although resolution of LAT after long-term oral anticoagulant therapy has been documented, its short-term effect, which would be more clinically important, has been less clearly established.

Objectives: To estimate the disappearance rate of documented LAT among candidates for PTMC who were treating with oral anticoagulation for 6 months and to determine its significant predictors.

Design: Prospective cohort study.

Methods: Between August 1996 and February 2002, a total of 687 consecutive PTMC candidates underwent both transthoracic and multiplane transesophageal echocardiographic studies (TTE, TEE). Of these, 219 patients demonstrated LAT by TEE and were given oral anticoagulation (INR 2.0 to 3.0). The fate of LAT was studied at 6 months using both TTE and TEE.

Results: Among 219 PTMC candidates with LAT (mean age 39.6±7.4 years, range 19-62 years; 73% females), complete resolution of LAT was demonstrated in 53 cases at the first 6th-month follow-up, with an overall disappearance rate of 24.2% (95%CI: 18.5% to 29.9%). All 53 patients subsequently underwent successful PTMC. None of the cases, with LAT in the atrial body (n=27), had LAT resolution. Among the 166 patients whose LAT persisted, the LAT size had nevertheless been reduced by approximately 20% from the baseline (p<0.001). By using multiple logistic regression analysis, the significant predictors associated with LAT resolution included a NYHA Class of 1 or 2 (OR=11.11: 95%CI=3.23, 33.33), a LAT size <1.6 cm2 at the first study (16.67: 5.56, 50.00), a fixed LAT (4.35: 1.49, 12.5), a left atial spontaneous echo contrast of less than or equal to Grade 1 (3.45: 1.20, 10.0), and an INR of more than or equal to 2.5 (19.53: 3.37, 113.16).

Conclusions: About a quarter of PTMC candidates with LAT could avoid heart surgery by safely undergoing PTMC after 6 months of oral anticoagulant therapy as their LAT had disappeared. Less clinical severity, presenting as a small and fixed LAT, less grading of the left atrial spontaneous echo contrast, and a tolerance to high INR, could enhance such an outcome.

1032-131 The Role of Endothelial-Derived Hyperpolarizing Factor in Tissue-Type Plasminogen Activator Release

James A. Muldowney, III, John R. Falck, Nancy J. Brown, Douglas E. Vaughan, Vanderbilt University Medical Center, Nashville, TN, University of Texas-Southwestern, Dallas, TX

Physiologic release of tissue-type plasminogen activator (t-PA) from the endothelium is critical for vascular homeostasis, and is prostacyclin and nitric oxide-independent in humans. Previously, we have suggested that endothelial-derived hyperpolarizing factor (EDHF) may play a role in t-PA release. EDHF promotes endothelial-dependent smooth muscle relaxation and hyperpolarization, and is believed to be a cytochrome P450 (CYP450) metabolite of arachidonic acid (AA). Calcium-dependent potassium channel blockade with tetraethylamine (TEA), apamin and charybdotoxin, or CYP450 inhibition with miconazole, are indirect, but accepted methods of EDHF antagonism. In this study, we tested the hypothesis that thrombin-induced t-PA release is EDHF-dependent. Human microvascular endothelial cells were incubated in with 2mM sodium butyrate for 24 hours. Cells were washed and incubated for 30 minutes in M199+0.03% albumin and inhibitors in triplicate. The cells were then stimulated with 10 U/ml thrombin or vehicle. Medium was harvested at multiple time intervals and t-PA antigen was measured by ELISA. As expected, in the presence and absence of ASA and L-NAME, thrombin triggered a significant induction of t-PA release. TEA had no effect on t-PA release at any doses, nor did apamin and charybdotoxin. However, miconazole effectively inhibited thrombin-induced t-PA release in a dose-dependent manner (IC_{50}=8 nM). The AA epoxygenase inhibitor MS-PPOH inhibited both constitutive and thrombin-induced release of t-PA in a dose-dependent manner. These studies indicate that miconazole and MS-PPOH, but not potassium channel blockade, inhibit thrombin-induced t-PA release in the presence of ASA and L-NAME. While these methods of EDHF antagonism are indirect, we speculate EDHF induces t-PA release through a mechanism independent of calcium-dependent K^+ channel activation.

1032-132 Eptifibatide Blocks C-Reactive Protein Increase After Coronary Angioplasty

Alvaro Merino, Ignacio Segura, Bernat Vidal, Andres Grau, Cristina Imizcoz, Antonio Rodriguez, Eduardo Alegria, Clinica Rotger, Palma de Mallorca, Spain, Clinica Universitaria, Pamplona, Spain

Background: Intraarterial thrombosis and arterial wall inflammation interactions could be responsible for the sharp increase in C-reactive protein (CRP) observed in acute coronary syndromes. Thus, we tested the hypothesis that CRP increase in a model of acute coronary syndromes such as coronary angioplasty could be due to intraarterial thrombosis.

Methods: One hundred twenty-five patients who underwent a coronary angioplasty procedure at our cath lab were included. We excluded patients with inflammatory, metabolic or neoplastic diseases. Patients with post angioplasty CK/MB elevation were also excluded. Inflammation markers studied were: CRP, IL 6, IL-1b, and plasma macrophage colony stimulating factor (MCSF). Blood samples were collected pre angioplasty, 24 and 48 hours after the procedure. In 71 patients an eptifibatide perfusion was initiated immediately after the procedure, and continued for only 24 hours. The remaining 54 patients received standard antithrombotic therapy.

Results: CRP figures are shown in the table. IL-6, IL-1b and MCSF results will be presented at the meeting:

Conclusions: C-reactive protein increase after coronary angioplasty was suppressed by eptifibatide, a synthetic peptide which is a selective blocker of the platelet GP IIb-IIIa receptor with no known anti-inflammatory effects. Thus, it seems that CRP increase in acute coronary syndromes could be mostly due to intraarterial thrombosis.

C-reactive Protein After PTCA

	n	Pre-PTCA	24 h	48 h
Eptifibatide	71	0.76±1.15	0.69±1	1.14±1.13
Control	54	0.52±0.52	1.08±0.99	1.47±1.38
p		ns	p<0.001	ns

1032-133 Inhibition of Plasminogen Activator Inhibitor Type-1 Expression in Human Adipose Tissue: An Antithrombotic and Antiatherogenic Effect of ANP, CNP, and BNP

Andreas Zirlik, Anne Leughers, Sven von Saldern, Christoph Bode, Burton E. Sobel, Thomas K. Nordt, University of Freiburg, Freiburg, Germany, University of Vermont College of Medicine, Burlington, VT

Background: Human adipose tissue produces substantial amounts of plasminogen activator inhibitor type-1 (PAI-1), an established risk factor for cardiovascular disease. Little is known about natural regulators of PAI-1 in human adipose tissue. Therefore, the present study examines the possible effect of angiotensin II, atrial natriuretic peptide (ANP), C-type natriuretic peptide (CNP), and B-type natriuretic peptide (BNP) on PAI-1 expression in human adipose tissue.

Methods: Human preadipocytes in primary culture were exposed to selected concentrations of angiotensin II (100nM, 1μM, 10μM, 100μM) as well as ANP, CNP, and BNP (10nM, 100nM, 1μM) in the presence and absence of transforming growth factor-beta. PAI-1 protein was measured by ELISA, total protein colorimetically, PAI-1 mRNA by light cycler RT-PCR. Parallel experiments were performed in primary cultures of in vitro differentiated human adipocytes and in human adipose tissue explants.

Results: Incubation with angiotensin II up to unphysiological doses as high as 100μM did not have a significant effect on PAI-1 secretion in all cell types (n=3). However, ANP, CNP, and BNP reduced basal PAI-1 expression in the conditioned media by up to 21%±5, 16%±6, and 44%±8 in preadipocytes (n=7, P<0.05). Stimulation with TGF-beta resulted in a 10-fold increase of basal PAI-1 production (n=7, p<0.001). Under stimulated conditions ANP, CNP and BNP significantly suppressed PAI-1 levels by up to 25%±7, 31%±8, 20%±8 (n=7, P<0.05). In human adipocytes ANP downregulated TGF-beta induced PAI-1 expression by up to 31%±9, CNP by up to 22%±12, and BNP by up to 42%±13 (n=5, p<0.05). Similar results were found in human adipose tissue explants. Total protein quantification indicated persistent viability of cells. Quantification of PAI-1 mRNA suggested that regulation occured at the transcriptional level.

Conclusions: Angiotensin II does not influence PAI-1 expression in human adipose tissue. In contrast, ANP, CNP, and BNP significantly reduce PAI-1 expression in all cell types. These observations suggest that natriuretic peptides may have an antithrombotic and antiatherogenic effect by being natural downregulators of PAI-1.