

wall injury in C57BL/6J mice and ApoE^{-/-} mice. Treatment with MEG reduced neointimal thickening only in C57BL/6J mice, suggesting a selective role for iNOS. In addition, cigarette smoking and hypercholesterolemia may have a synergistic effect on oxidative stress response to arterial injury in mice.

POSTER SESSION

1177 Platelets, Fibronolysis, and Arterial Thrombotic Disease

Tuesday, March 19, 2002, Noon-2:00 p.m.

Georgia World Congress Center, Hall G

Presentation Hour: Noon-1:00 p.m.

1177-87

The Protective Effect of the T(-107)C Promoter Polymorphism of the Paraoxonase Gene in Arterial Thrombotic Disease

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Background: Approximately one-third of patients with arterial thrombotic disease do not have traditional cardiovascular risk factors. Serum paraoxonase (PON) is an HDL-associated esterase that hydrolyzes products of lipid peroxidation and prevents the oxidation of LDL. PON levels are significantly reduced in patients with coronary heart disease. Recently, two PON promoter polymorphisms, T(-107)C and G(-824)A, were identified that are consistently associated with lower PON expression and activity; however, it is not known if these polymorphisms are a risk factor for arterial thrombotic disease.

Methods: We studied the PON promoter genotypes and vascular risk factors of 115 patients (63 women, mean age: 35.8 ± 6.9 years) with a clinically and radiologically defined arterial ischemic stroke occurring before the age of 45 years and 117 age- and gender-matched controls. To identify the T(-107)C polymorphism, we used non-radioactive single-strand conformational polymorphism analysis of a 129 bp-sized fragment containing the polymorphic site.

For detection of the G(-824)A polymorphism, a 200 bp fragment was amplified using a mutagenic primer, resulting in an artificial restriction site for *Pvu II* when the polymorphic G nucleotide was present.

Results: When analyzing the PON T(-107)C polymorphism, we found that the TT genotype was significantly less frequent among patients than controls (12.2% vs. 22.2%, P=0.041). In a logistic regression model adjusting for arterial hypertension, smoking, diabetes mellitus and hyperlipoproteinemia, this association remained significant (P=0.0297, OR=0.41, 95% CI 0.19 to 0.9). Regarding the PON G(-824)A polymorphism, there was no significant difference between groups (P=0.47).

Conclusion: These findings suggest that the PON -107T allele may have a protective effect against arterial thrombotic disease in young adults. Studies including a larger number of patients and analyzing the effect of PON on atherothrombosis are necessary to understand better the mechanistic role of these observations in the development of arterial disease in the young.

1177-88

Variability in Inhibition of Platelet Aggregation With Clopidogrel and Correlation With ADP-Induced P-Selectin Expression on Platelets

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Background: Thienopyridines inhibit platelet activation and aggregation by blocking the ADP P2Y₁₂ receptor. We investigated the correlation between platelet aggregation and activation in patients treated with clopidogrel.

Methods: Patients (n=19) with stable angina were assessed before and after 1 week treatment with 75 mg of clopidogrel given daily. Blood samples were anticoagulated with PPACK-hirudin. Platelet aggregation (TA) in stirred platelet rich plasma (PRP) and micro-aggregate formation (PA) in 1:10 diluted PRP at a shear stress of 1000s⁻¹ were studied. In addition, flow cytometry was used to measure platelet activation, (P-selectin for secretion; and PAC-1 for free activated GPIIb/IIIa) after 10 μM ADP stimulation.

Results: Treatment by clopidogrel significantly reduced aggregation for both TA (65±8 vs. 45±15%, p<0.0001) and PA (84±8 vs. 49±18 %, p<0.0001). Inhibition varied widely, from 0 to 65% for TA; and from 0 to 70% for PA. Clopidogrel significantly decreased P-selectin expression (40±15 vs. 14±8%, p<0.0001) as well as PAC-1 binding (50±30 vs. 23±20, p<0.001). By Linear regression analysis the magnitude of inhibition of platelet aggregation correlated weakly with PAC-1 levels (R-square=0.17, p=0.08 for both TA and PA), but strongly with inhibition of P-selectin expression (R-square=0.28, p<0.02 and R-square=0.40, p<0.004, respectively). When inhibition of aggregation attained levels greater than 30% for TA and 25% for PA, P-selectin was always expressed by less than 20% of platelets.

Conclusion: Inhibition of platelet aggregation by clopidogrel was found to be highly variable. Inhibition may depend less on reduced free activated GPIIb/IIIa, where few receptors may be required to support aggregation, and more on reduced secreted adhesive ligands. Determination of P-selectin may be helpful to assess the anti-aggregant efficacy of clopidogrel.

1177-89

The Relationship Between Serotonin 2A Receptor Gene Polymorphisms and Serotonin Induced Platelet Aggregation

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Background: Serotonin (5-HT) induces platelet aggregation (PA) and increases the vascular tone through 5-HT_{2A} receptor activation. Recently two common polymorphisms in the 5-HT_{2A} receptor gene, T102C in exon 1 and -1438A/G in the promoter, was identified. We and others showed that these polymorphisms were associated with myocardial infarction or psychological disorder. However, these functional significance remains unknown.

Methods: We investigated 5-HT_{2A} receptor gene polymorphisms by PCR-RFLP and PA by using a novel laser-light scattering method in 33 young healthy volunteers (mean age 26.4±1.5SD). PA was induced by 5-HT 0.3, 1.0μM and ADP 0.1, 0.3μM, and evaluated by the peak of small aggregation counts.

Results: (1)T102C polymorphism: The peak of PA induced by 5-HT in TT genotype was significantly higher compared with TC and CC genotype (Table). However, this polymorphism did not influence ADP-induced PA. 5-HT concentration in platelet rich plasma (PRP) was not different between 2 groups.

(2)-1438A/G polymorphism: A allele frequencies were 0.59 (AA: n=9, AG: n=21, GG: n=3). 5-HT-induced PA was not different between 2 groups (AA vs. AG+GG: 5-HT 0.3μM; 30.8±11.4SEM×10³ vs. 21.5±6.6SEM×10³, NS, 5-HT 1.0μM; 128.7±17.6SEM×10³ vs. 90.9±12.2SEM×10³, NS). In addition, this did not influence ADP-induced PA.

Conclusion: The T102C polymorphism was associated with 5-HT-induced PA, but the -1438A/G was not. This is the first report demonstrating the functional significance of the T102C polymorphism.

T102C polymorphism, platelet aggregation, and 5-HT concentration in PRP

	TT(n=5)	TC+CC(n=22+6)	p
5-HT 0.3μM, ×10 ³	51.2±5.1	19.2±5.8	<0.05
5-HT 1.0μM, ×10 ³	168.0±10.2	89.3±10.6	<0.005
ADP 0.1μM, ×10 ³	15.5±6.7	15.8±5.4	NS
ADP 0.3μM, ×10 ³	161.2±25.9	92.7±14.3	NS
5-HT in PRP, μg/ml	0.28±0.01	0.26±0.02	NS

1177-90

Increased Tissue Plasminogen Activator Antigen Is Predictive of Cardiovascular Events in the Veterans Affairs HDL Intervention Trial (VA-HIT)

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Background: Hemostatic factors have been shown to be predictive of future cardiovascular disease events (CVD), although this has not been well studied in patients with known CVD and low HDL-Cholesterol.

Methods: We prospectively studied 829 subjects who were enrolled in the Veterans Affairs HDL Intervention Trial (VA-HIT). Enrollment criteria included known CVD and low HDL-cholesterol. Tissue plasminogen activator (tPA) antigen, factor VII antigen and fibrinogen were determined at baseline and after 1 year of therapy with placebo or gemfibrozil. There was no effect of gemfibrozil on these measures of hemostasis. Thus, data from the gemfibrozil and placebo arms were combined.

Results: During follow-up (median 5.1 years), 140 patients had as a first event nonfatal MI or cardiac death. Relative risks (RR) were determined by multivariate Cox models, adjusting for age, hypertension, diabetes, smoking, body mass index, compliance and treatment.

Results: Higher tPA antigen levels were associated with increased cardiovascular risk (4.7% for each unit tPA increase), whereas fibrinogen and factor VII were not significant predictors.

Conclusion: Reduced fibrinolytic potential, as evidenced by increased tPA antigen levels, was a significant predictor of cardiovascular events in patients with known CVD and low HDL. Measurement of tPA may provide prognostic value in this subjects. Strategies to improve fibrinolytic potential in this population warrant further investigation.

	RR	95% CI	p-value
tPA antigen (ng/ml)	1.047	1.004-1.092	0.03
Factor VII antigen (%)	1.002	0.990-1.014	0.78
Fibrinogen (mg/dl)	0.999	0.996-1.002	0.90

1177-91

Investigation of Thrombocytopenia in Individuals Receiving GPIIb-IIIa Antagonist Therapy

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Background: The use of GPIIb-IIIa antagonists for treatment of acute coronary syndromes continues to rise, as studies consistently demonstrate a significant benefit in reducing ischemic endpoints. Thrombocytopenia (TCP) is an infrequent, yet serious potential side effect of GPIIb-IIIa antagonists. Little is known about the mechanism behind its development, therefore it is often impossible to predict which patient is at risk at the treatment onset. These patients are also concurrently treated with several drugs