PI-11

PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1) EXPRESSION IN VASCULAR SMOOTH MUSCLE CELLS INVOLVES A SRC/MEK/USF SIGNAL CASCADE. P. J. Higgins, PhD, Albany Medical College, Albany, NY.

BACKGROUND: High PAI-1 levels associate with increased cardiovascular disease risk. PAI-1 targeting may be an important approach to the therapy of vascular disorders.

METHODS: Pathway-specific pharmacologic/molecular agents were used to define signaling events associated with PAI-1 expression.

RESULTS: MEK (PD98059, U0126) or src family (PP1) kinase inhibitors attenuated growth factor-stimulated PAI-1 transcription in smooth muscle cells. PP1 blocked ERK1/2 activation suggesting that the required src kinase is upstream of ERK1/2. Transfection of a dominant-negative pp60c-src construct, moreover, reduced PAI-1 levels to that of PP1-treated controls implicating pp60c-src as the involved src kinase. An E box motif in the PAI-1 promoter was determined to be a USF-1 binding site. Mutation of this site or transfection of dominant-negative USF-1 constructs attenuated PAI-1 expression in smooth muscle cells. Nuclear USF-1 was determined to be a phosphorylation target of translocated ERK1/2 by both in situ co-localization microscopy and co-immunoprecipitation.

CONCLUSIONS: A major phenotypic characteristic of "activated" smooth muscle cells (i.e., transcription of fibrosis-associated target genes [e.g., PAI-1]) requires pp60c-src kinase activity and MEK signaling and involves activation of the USF transcription factor likely by ERK family MAP kinases. Pharmacologic or genetically-targeted manipulation of this pathway may have therapeutic usefulness in treatment of vascular disease.

PI-12

BEYOND HEMOSTASIS: THE ANTIFLAMMATORY AND ANTIOXIDANT EFFECTS OF DIPYRIDAMOLE. S. Chakrabarti, PhD, O. Vitseva, PhD, D. Iyu, BS, S. Varghese, MS, J. Freedman, MD, Boston University School of Medicine, Boston, MA.

BACKGROUND: Platelet stimulation leads to release of reactive oxygen species (ROS) that are known to influence platelet function and thrombosis. Dipyridamole is a vasodilator and platelet inhibitor that decreases adenosine uptake and may be a highly efficient chain breaking antioxidant. The antioxidant effects of dipyridamole on platelet-derived ROS and the potential (anti)inflammatory effects are not known.

METHODS: Human platelets were incubated with dipyridamole (0–100 μM) and specific antioxidant and antiflammatory mediators were measured.

RESULTS: Incubation with dipyridamole did not alter platelet release of nitric oxide but significantly attenuated superoxide release (17±3.7 a.u. for control vs. 7.4±1.1 a.u. for 20μM; n=5, $P<0.001$). Using flow cytometry, dipyridamole decreased intracellular levels of ROS, as measured by 2',7'-dichlorodihydrofluorescein diacetate fluorescence dye (1±0.34% control vs. 0.66±0.2% for 20μM, n=4, $P=0.03$). There was also a dose-dependent (0–100μM) suppression of soluble CD40 ligand release ($P<0.05$).

CONCLUSIONS: In summary, at a clinically relevant concentration, dipyridamole suppresses stimulation-dependent release of superoxide as well as the formation of ROS and leads to the attenuated release of sCD40L from platelets. These data suggest that dipyridamole, beyond inhibition of platelet aggregation, suppresses the platelet inflammatory reactions recently shown to be relevant in the development of atherothrombotic disease.

PI-13

COMPARISON OF IRBESARTAN VERSUS ATORVASTATIN THERAPY ON ANGIOTENSIN II (ANG II)-INDUCED VENOCONSTRICTION AND PLASMA LEVELS OF ANGIOTENSIN-(1–7) [ANG-(1–7)] IN HEALTHY VOLUNTEERS. C. Schindler, MD, K. B. Brosnihan, PhD, C. M. Ferrario, MD, W. Kirch, MD, Institute of Clinical Pharmacology, Medical Faculty, University of Technology, Dresden, Germany, Hypertension and Vascular Disease Center, Wake Forest University of Medicine, Winston-Salem, USA, Hypertension and Vascular Disease Center, Wake Forest University of Medicine, Winston-Salem, USA, Institute of Clinical Pharmacology, Medical Faculty, University of Technology, Dresden, Germany.

AIM: Experimental studies suggest interactions of statins with the renin-angiotensin-aldosterone-system. [Ang-(1–7)], the most pleiotropic metabolite of angiotensin II functions as a vasodilator by releasing prostaglandins and stimulating NO release.

METHODS: In a randomized double blind double crossover study (n=8) we compared the effects of 30 days systemic therapy with irbesartan (150 mg; IRB) versus atorvastatin (20 mg; STAT) on Ang II- induced venoconstriction, endothelium dependent histamine and endothelium independent glyceroltrinitrate [GTN]-induced dilation by the dorsal hand vein compliance method. Systemic treatments were separated by a 30 day washout period.

RESULTS: Constant infusion of Ang II caused rapid venous desensitization that peaked after 8 minutes of infusion. Ang II-induced constriction was 51±25% basal vein size (BVS) before and 36±28% BVS after 30 days of statin treatment ($p=0.16$) compared to 50±23% before vs 85±26% BVS after initiation of IRB treatment ($p=0.012$). There was no difference in histamine- and GTN-induced dilation between treatments. Ang II-levels were 26±13 before and 31±11 pg/mL after STAT (p=n.s.) and 35±12 before vs 329±285 pg/mL after IRB ($p=0.02$). Ang 1–7 levels were 9±6 before vs 11±9 pg/mL after STAT (p=n.s.) and 10±8 before vs 35±16 pg/mL after IRB ($p=0.01$).

CONCLUSION: A differential effect of STAT and IRB on venous compliance and plasma angiotensins in healthy volunteers suggests a venodilator action of [Ang-(1–7)] following AT_1-blockade.

PI-14

THE SELECTIVE ALPHA$_2$-ADRENERGIC RECEPTOR AGONIST DEXMEDETOMIDINE (DEX) DOES NOT AFFECT AORTIC PRESSURE AUGMENTATION. D. Kurnik, MD, G. G. Sofowora, MD, M. Muszkat, MD, P. A. Harris, PhD, A. J. Wood, MD, C. M. Stein, MD, Clinical Pharmacology and Medicine, Vanderbilt University, Nashville, TN.

BACKGROUND: Despite a similar reduction in peripheral blood pressure (BP), antihypertensive drugs have variable effects on central blood pressure and its augmentation by the reflected pulse wave, a measure of arterial stiffness. Infusion of norepinephrine (NE) increases arterial stiffness. Centrally acting alpha$_2$-AR agonists decrease NE concentrations and blood pressure, but their effects on aortic pressure augmentation are not known.

METHODS: In a single-blind, placebo-controlled study, 36 healthy subjects received sequential infusions of placebo and DEX (0.1, 0.15 and 0.15 mcg/kg). Brachial artery blood pressure, heart rate, and NE plasma concentrations were measured at baseline and after every infusion. Radial artery waveforms were recorded by applanation tonometry (Sphygmocor) and the central aortic waveform and augmentation index - the proportional increase in systolic pressure due to the reflected wave - calculated.

RESULTS: DEX reduced brachial and central systolic (−15.0±6.2 (SD) and −12.6±5.8 mmHg) and diastolic BP (−9.9±6.5 and −10.1±6.6 mmHg), respectively ($p<0.001$). NE levels decreased (−103±68 pg/mL, $p<0.001$) but augmentation index did not change (placebo 3.4±10.3% vs DEX 3.0±10.1%, $P=0.8$).

CONCLUSION: DEX reduced NE concentrations and central and peripheral blood pressure but did not alter aortic pressure augmentation. Pharmacological reduction of endogenous baseline NE concentrations did not alter arterial stiffness and pulse wave reflection.