VASCULAR RESPONSES TO ACTIVATION OF LEUKOCYTES IN VIVO AFTER REGRESSION OF ATHEROSCLEROSIS

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We have shown previously that activation of leukocytes in vivo with the chemotactic peptide fMLP produces constriction of large arteries in atherosclerotic (AS), but not normal monkeys. Regression of AS in primates restores endothelium-dependent responses to normal, and abolishes the hyperresponsiveness to serotonin. We tested the hypothesis that vascular responses to activation of leukocytes in vitro may normalize after regression of AS. fMLP (0.1 μmol) was injected i.a. into the blood-perfused hind limb of normal cynomolgus monkeys (n=12), monkeys fed with an atherogenic for 4 months (hypercholesterolemic, n=12), 18 months (AS, n=12), and 12 AS monkeys that were given a normal (regression) diet for 18 months. Injection of fMLP did not change resistance of large arteries in normal or hypercholesterolemic monkeys. In contrast, fMLP increased resistance of large arteries by 3.9±1.0 (mean ± SE, mmHg/ml/min/100g tissue) in AS and by 1.6±0.5 in regression monkeys. In AS monkeys, leukocytes were found in the intima and the adventitia by esterase staining. In regression monkeys, leukocytes were virtually absent in the intima, but present in the adventitia.

Conclusion: Regression of AS does not eliminate the abnormal vascular response to activation of leukocytes in vivo. We speculate that abnormal adherence of leukocytes to endothelium may persist despite regression, or that adventitial leukocytes may be the source of vasoconstrictor factor(s).

tPA STIMULATION OF LOCAL PROSTACYCLIN PRODUCTION IN THROMBOSED CORONARY ARTERIES

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Although tissue plasminogen activator (tPA) is known to augment thromboxane (Tx) A_2, a platelet aggregator and vasoconstrictor, its influence on local release of prostacyclin (PGI_2), a platelet disaggregator and vasodilator, is uncertain. Enhancement of PGI_2 levels near thrombi (Th) would favor thrombolysis, particularly of resistant, platelet-rich Th. We measured 6-keto-$PGF_{1\alpha}$ and TxB_2, stable metabolites of PGI_2 and TxA_2, respectively, in anterior coronary veins (CV) and systemic veins (SV) of 15 pigs given tPA after anterior coronary occlusion by platelet Th. Six other pigs received tPA plus the Tx synthetase inhibitor OKY-046 (OKY). The following results are means (ng/dl)±SE; *=p <0.05 vs Th; §=p <0.05 vs Th+tPA.

	TxB_2 (SV/CV)	6-keto-$PGF_{1\alpha}$ (SV/CV)
Pre-Th(n=15)	34±7 /40±9	20±4/ 33±5
Th (n=15)	31±6 /40±8	16±4/ 42±10
Th+tPA(n=15)	55±8*/74±11*	20±3/ 77±17*
Th+tPA+OKY(n=6)	30±6 /37±7§	35±8/101±11§

Thus, tPA raises local PGI_2 metabolite levels in thrombosed coronary vessels, an action magnified by OKY and not reflected in samples from SV. Preferential enhancement of PGI_2 synthesis near Th may be important for tPA-induced thrombolysis. Further increase in local PGI_2 by TxA_2 synthetic blockade may add to thrombolytic efficacy while avoiding adverse effects of systemic increases in PGI_2.

Tuesday, March 5, 1991
Poster Displayed: 2:00PM–5:00PM
Author Present: 3:00PM–4:00PM
Hall F, West Concourse
Metabolic Insights of Ischemic Injury

CORRELATION OF MYOCARDIAL ADENOSINE WITH POST-ISCHEMIC RECOVERY

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Adenosine triphosphate (ATP) depletion occurs during ischemia, however ATP content does not predict myocardial recovery following ischemia. Augmenting ATP precursor availability, such as adenosine (ADO) during ischemia improves myocardial recovery. To determine if myocardial adenosine is an important determinant of ischemic tolerance, and predicts functional recovery, 86 isolated, crystalloid perfused adult rabbit hearts were subjected to 120 minutes of 34° C global ischemia. Each group (n=7-10 each) was treated with interventions designed to modify ADO levels during ischemia. Hearts received St. Thomas cardioplegia (CP); or cardioplegia containing 1 μM deoxycoformycin (DCF), which inhibits adenosine breakdown, 200 μM adenosine (ADO), 25μM phosphate (PO) or both (AP), as well as 15, 5, 2.5, or 0.025 μg/ml adenosine deaminase (ADA), which results in adenosine depletion. ADO and ATP (μM/mg protein) were measured in tissue biopsies (by HPLC) during and at the end of ischemia. Baseline nucleotide levels were equivalent for all groups. Functional results are 45 minutes after reperfusion. Results are mean ± SEM.

	End Ischemic ADO	*End Ischemic ATP*	*%DP*
ADA 15	*0*	*0*	*0*
ADA 5	*0*	*0*	*0*
ADA 2.5	*0*	*0*	*0*
ADA .025	*1.6+.03*	*1.1+.01*	*29+5*
CP	*2.0+.12*	*0.8+.01*	*38+4*
PO	*2.1+.07*	*0.7+.15*	*50+4*
DCF	*2.9+.09*	*0.8+.01*	*59+2*
AP	*3.1+.13*	*0.8+.05*	*63+4*
ADO	*5.6+.17*	*0.8+.01*	*68+7*

Postischemic functional recovery (% of baseline developed pressure, DP) closely paralleled the availability of myocardial adenosine, but not ATP content. End-ischemic ADO levels averaged from 0 to 5.6 ± .17 μM/mg protein and correlated closely (r^2=0.96) with functional recovery. This relationship was best described by the equation $y = -2.8x^2+28.7x-2.6$. End-ischemic ATP levels averaged from 0.8 to 1.1± 0.1 μM/mg protein and did not predict or correlate with functional recovery. These results demonstrate that myocardial adenosine levels at end ischemia are important determinants of functional recovery. Consequently, maintaining or augmenting myocardial adenosine during ischemia should be of clinical benefit

A NOVEL ADAPTATION OF POLYMERASE CHAIN REACTION PROVIDING FOR PRECISE QUANTITATION OF MESSENGER RNA IN HUMAN HEART

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Polymerase chain reaction (PCR) amplification can be used to detect minute amounts of DNA or RNA sequences and is rapidly becoming a very important qualitative and semiquantitative diagnostic tool. However, polymerase chain reaction at high cycle numbers enters a plateau phase during which reactants become rate limiting and the rate of amplification is variable and no longer exponential thus, precise quantitative analysis of the tissue mRNA content is yet to be achieved. To avoid this limitation, conditions were established at low cycle numbers to ensure that the rate of amplification is consistent and exponential. An external standard of *in vitro* transcribed mRNA for M and B creatine kinase (CK) and an internal standard of 18s rRNA were used for assay standardization and calibration. The detection was performed with radiolabelled internal primers after a fixed number of PCR cycles. Both species of mRNA were resolved simultaneously on a sequencing gel. The labelled PCR products were sectioned from dried gels after autoradiography and quantitated in a scintillation counter. 18s rRNA was detected and quantitated without amplification. Amplification of CK mRNA standards and cDNA synthesized from mRNA isolated from normal human left ventricle increased in uniform exponential progression with identical slopes of one and were identical to the predicted concentrations. B and MCK mRNA were quantified in samples from 12 human hearts starting with 1 μg of RNA as template for cDNA synthesis. The results were expressed as molar ratio of B or M CK mRNA to 18s rRNA. The ratios ($\times 10^{-10}$) were 398±66 and 2738±279 for B and MCK respectively from 9 hearts with terminal ischemic heart disease and were 434±103 and 3956±1470 from 3 hearts with normal LV function. Creatine kinase M and B mRNA in hearts from patients with terminal ischemic heart disease were not significantly different from that of observed in normal human left ventricle. The methods described provide precise quantitation of mRNA and can be easily applied to other species of mRNA in any tissue under different experimental conditions.