POSTER SESSION

1183 Myocardial Function: Basic

Tuesday, April 01, 2003, Noon-2:00 p.m.
McCormick Place, Hall A
Presentation Hour: 1:00 p.m.-2:00 p.m.

1183-62 Altered Aldosterone Disposition in P-Glycoprotein Knockout Mice

Robert B. Parker, C. Ryan Yates, S. Casey Laizure, University of Tennessee, Memphis, TN

Background: Elevated aldosterone concentrations play an important role in heart failure pathogenesis affecting the heart, kidneys, and brain. P-glycoprotein (P-gp), a cell membrane efflux pump expressed by the human MDR1 gene, actively secretes aldosterone from the adrenal cortex. P-gp is also expressed on the apical surface of aldosterone target tissues including the brain and heart, where it impedes cellular substrate uptake. In this study we compared the plasma and tissue distribution of aldosterone in wild-type (WT) and P-gp knockout (KO) mice.
Methods: Adult male P-gp KO and genetically matched WT mice received 1.0 μCi i.v. [^{3}H]-aldosterone. Groups of 3-4 mice were sacrificed at various times after aldosterone injection. Plasma, brain, and heart samples were collected and assayed for plasma and tissue radioactivity.
Results: Compared to WT mice, [^{3}H]-aldosterone activity [disintegrations per min (dpm)] in plasma and brain was higher in KO mice (data 45 minutes after injection shown in table). Area under the curve ($AUC_{0\text{-}90\ min}$) for plasma (1.8×10^6 vs 2.9×10^6 dpm•min/ml), brain (8.1×10^5 vs 1.6×10^6 dpm•min/g), and heart (2.0×10^6 vs 3.2×10^6 dpm•min/g) was also higher in KO mice.

	WT mice	KO mice	p value
Plasma (dpm/ml)	25915 ± 1781	32145 ± 3705	0.02
Brain (dpm/g)	10789 ± 2363	20092 ± 1543	0.001
Brain/Plasma	0.42 ± 0.10	0.63 ± 0.11	0.03
Heart (dpm/g)	30772 ± 9248	31847 ± 6381	NS
Heart/Plasma	1.09 ± 0.25	0.89 ± 0.10	NS

Mean ± SD
Conclusion: P-gp enhances aldosterone plasma clearance and limits its brain uptake. Overall exposure ($AUC_{0\text{-}90\ mins}$) to aldosterone in plasma and tissues is increased in the absence of P-gp. P-gp may play an important role in aldosterone tissue accumulation and activity.

1183-63 Glu298Asp Variant of the Endothelial Nitric Oxide Synthase Gene in Patients With Left Ventricular Dysfunction

Maria Giovanna Colombo, Maria Grazia Andreassi, Nicoletta Botto, Serena Masetti, Samantha Manfredi, Umberto Paradossi, Andrea Biagini, CNR, Institute of Clinical Physiology, G. Pasquinucci Hospital, Massa, Italy

Background. An early reduction of exercise capacity after endothelial NO Synthase (eNOS) inhibition has been recently reported. Moreover, ventricular dysfunction and remodeling after myocardial infarction have been shown to be markedly increased in eNOS-deficient as compared to wild-type mice. Therefore, functionally important variants of eNOS could influence left ventricular (LV) systolic performance.
Objective. To assess whether the Glu298Asp polymorphism (G894T) of the eNOS gene is associated with the occurrence of LV systolic dysfunction.
Methods. We performed PCR/restriction fragment length polymorphism analysis to detect the missense Glu298Asp variant in exon 7 of the eNOS gene in 73 patients with LV dysfunction (LV ejection fraction <40% assessed by echocardiography) consecutively admitted to our institute and 87 control subjects free of cardiovascular disease. Causes of LV dysfunction included coronary artery disease (n=49), valvular (n=14) and idiopatic (n=10).
Results. The frequencies of the eNOS Glu/Glu, Glu/Asp and Asp/Asp genotypes in the patients' group were significantly different from those of controls (45.2%, 34.2% and 20.6% vs 35.6%, 55.2% and 9.2%, respectively; c2=8.278, p=0.016). In comparison to Glu298 carriers, homozygosity for Asp298 was associated with an Odds Ratio of 2.5 (95% CI, 1.01 to 6.43, p = 0.04) for LV dysfunction.
Conclusions. The Glu298Asp polymorphism of the eNOS gene is associated with the occurence of LV systolic dysfunction suggesting that this genetic variant could be involved in the pathogenesis of heart failure.

1183-64 Abnormal Skeletal Muscle Mitochondrial Oxidative Phosphorylation in Severe Chronic Heart Failure

Pascal Vantrimpont, Kees Schoonderwoerd, Gerard De Jong, Jasper Scholte, Aggie Balk, Maarten Simoons, Erasmus Medical Center, Rotterdam, The Netherlands

Background: Skeletal muscle dysfunction plays an important role in the exercise intolerance of chronic heart failure (CHF) and several mechanisms have been invoked to explain it. However, little is known about the mitochondrial oxidative phosphorylation (OXPHOS) of skeletal muscle in CHF patients. We hypothesized that skeletal muscle OXPHOS is abnormal in these patients.
Patients and methods: Eleven patients with severe CHF underwent skeletal muscle biopsy. Protein content (mg/g wet weight), citrate synthase activity (CS, in mU/mg protein) and mitochondrial complex I to IV activity (CI to IV) were measured in the biopsy samples and compared with values obtained in a control group free of heart and muscle disease. To correct for a possible difference in number of mitochondria, CI to IV activities were expressed as percentages of CS activity. Statistical significance level was set at 0.05.
Results: As shown in the Table, protein content, CII activity and CIII activity were significantly lower in CHF (data given as mean ± standard deviation).
Conclusion: Both OXPHOS and protein content are abnormal in skeletal muscle of patients with severe CHF. The lower CII and CIII activities could be related to oxidative stress. The low protein content reflects malnutrition.

Results

	CHF (n=11)	Control (n=20)	P
Protein content	109 ± 31	186 ± 32	<0.001
CS	177 ± 94	135 ± 40	NS
CI/CS ratio	17 ± 6 %	21 ± 8 %	NS
CII/CS ratio	16 ± 3 %	21 ± 6 %	0.016
CIII/CS ratio	48 ± 35 %	101 ± 39 %	0.001
CIV/CS ratio	106 ±33 %	106 ± 30 %	NS

1183-65 Mitotic Cell Death as a Result of Cell Cycle Re-Entry Is a Unique Option for Senescent Cardiomyocytes in Addition to Apoptosis and Mitosis

Hiroaki Shimomura, Fumio Terasaki, Tetsuya Hayashi, Yasushi Kitaura, Masanori Hosokawa, Osaka Medical College, Takatsuki, Japan, Kyoto University, Kyoto, Japan

Background: It is believed that cardiomyocytes loose potential for proliferation after birth and stay at the G0 phase. However, binucleation of cardiomyocytes increases during aging, which possibly results from cell cycle re-entry and mitosis. Cell cycle re-entry may play one of key roles in myocardial remodeling during aging. **Methods:** To clarify the possibility of cell cycle re-entry and related morphological alterations in aging cardiomyocytes, we studied young (7 week-old) and old (90 week-old) mouse hearts both immunohistochemically and ultrastructurally. All data were quantitatively evaluated by the percentage index. **Results:** Immunohistochemically, Ki-67-positive nuclei were 6.07% in young mice and 9.88% in old mice ($p<0.001$). Ultrastructurally, metaphase mitotic nuclei were observed in a few cardiomyocytes of old mice, where nuclear envelopes had disappeared and nuclear chromatin was compacted as segments of homogenous electron-dense mass. In some mitotic cardiomyocytes, cytoplasm was severely degenerated with disorganized intracellular organelles and cytoplasmic vacuolization, but plasma membrane was well preserved. The mitotic cardiomyocytes with cytoplasmic degeneration seemed to undergo mitotic cell death. Apoptotic nuclei were also observed in aging heart, where nuclear chromatin was condensed and marginated beneath the well-preserved nuclear membrane. In old mice, each index was 0.22% in mitosis, 0.15% in apoptosis and 0.07% in mitotic cell death. All indexes were 0% in young mice. In aging heart, potential of cell cycle re-entry may be amplified in cardiomyocytes. It is possible that, as the result of cell cycle re-entry, myocardial cell death also occur in the mitotic phase. **Conclusion:** We conclude that cardiomyocytes in agirig heart potentially re-enter cell cycle, and apoptosis, mitotic cell death and mitotic nuclear division are the options for cardiomyocytes having re-entered the cell cycle in aging heart. However, it is not clear whether mitotic cell death is a part of apoptosis or is another distinct form of myocardial cell death.

1183-66 Upregulation of eNOS Contributes to the Control of Myocardial Oxygen Consumption During Pregnancy in the Dog

Jeffrey G. Williams, Fabio Recchia, Axel Linke, Gautam Churla, John McClung, Thomas H. Hintze, New York Medical College, Valhalla, NY

Pregnancy is associated with an increase in eNOS and NO production in almost all vascular beds in the mother, however the potential role of eNOS to regulate myocardial oxygen consumption during this high cardiac output state has not been addressed. Echocardiography was performed at 40, 50, and 60 days in awake pregnant dogs and the heart harvested for measurement of: 1) eNOS and 2) the ability of drugs that stimulate NO production to regulate myocardial oxygen uptake. Stroke volume (34 ± 1 to 38 ± 1 ml), heart rate (70 ± 5 to 98 ± 4 b/min) and cardiac output (2.3 ± .1 to 3.8 ± .2 L/min) increased from control to 60 days whereas ejection fraction did not change (63 to 66%). LV mass increased from 72 ± 2 to 90 ± 4 grams. ENOS protein increased by 179%. Bradykinin, ramaprilat and amlodipine all reduced myocardial oxygen consumption in normal heart (maximum of 22, 22, and 21%) and this was enhanced during pregnancy (27, 30 and 28%, $p<0.05$). The NO donor SNAP reduced oxygen consumption by a similar amount in both normal and pregnant dogs (30%). All of these changes reversed post partum. Thus there is an upregulation of eNOS during pregnancy at a time where cardiac output is chronically increased and this is associated with enhanced NO dependent control of myocardial oxygen consumption.