Background: It is well documented that endothelial nitric oxide synthase (eNOS) homozgyous knock out mice (eNOS-/-) develop sustained arterial hypertension. Because of controversy over the development of cardiac pathophysiology in these mice, we undertook to investigate the cardiac functional and biochemical effects of chronic eNOS ablation at different ages. Methods: We examined steady state levels of molecular markers of cardiac hypertrophy and heart failure in male eNOS-/- and control mice at various ages. Results: Histological examination at 40 weeks of age and cardiac functional analysis using the isolated work-performing heart preparation were performed at 52 weeks of age. Decreased in expression of heart of mice at 27-40 weeks, whereas hearts of eNOS-/- mice at 52 weeks of age demonstrated normal SERCA2a and slightly decreased phospholamban protein levels. An increased ratio of SERCA2a protein to phospholamban protein at this timepoint suggested normalized SERCA2a and slightly decreased phospholamban protein levels. Conclusions: Although eNOS-/- mice exhibited concentric left ventricular hypertrophy, multi-focal re-organization fibrosis and evidence of myocyte degeneration/death. As mice aged, re-induction of atrial natriuretic factor (ANF) and c-skeletal-actin mRNA correlated positively with the degree of cardiac hypertrophy. Significant increases in cardiac expression of tumor necrosis factor-α (TNF-α) mRNA and protein were detected at 27-30 weeks. Sarcoplasmic reticulum Ca'+-ATPase (SERCA2a) transcript levels were markedly decreased at 27-40 weeks, whereas hearts of eNOS-/- mice 52 weeks of age demonstrated normal SERCA2a and slightly decreased phospholamban protein levels. Although trend of eNOS-/- mice at 52 weeks of age demonstrated no deficit of basic function, there was a blunted response to β-adrenergic stimulation indicative of reduced contractile reserve. Conclusions: Although eNOS-/- mice exhibit myocardial remodeling, including reduction of cardiac fetal genes and dysregulation of TNF-α, there is no progression to failure before 52 weeks of age and in fact these hearts are hypercontractile, suggests a mechanism for the long-term physiological compensation that occurs in eNOS-/- hearts.

New Development in Lamin A/C Gene Associated With Severe Dilated Cardiomyopathy

Background: Idiopathic Dilated Cardiomyopathy (DCM) is familial in about 30% of the cases. Lamin A/C mutations have been identified for causing familial DCM, frequently associated with conduction system disease. We report here a novel lamin A/C mutation with features of newseve dilated cardiomyopathy.

Methods: After informed consent, we studied the lamin A/C gene in 17 patients of 14 different families with familial DCM. DNA was isolated from frozen blood samples and coding regions of lamin A/C were PCR amplified, studied by SSCP and cycle sequenced. Results: Three members of one of the families (mother and her two female identical twins) developed severe DCM and required cardiac transplantation at 36, 18, and 20 years old respectively. The father of the index case had died suddenly at 52 years old. At diagnosis, the mother was on aortic insufflation with slow ventricular responses. No conduction disturbance was present in the twins. A new mutation (Arg349Leu) was identified in exon 6 in the three patients. This mutation was not present in 22 unaffected relatives and in more than 100 healthy controls. This mutation was a highly conserved region identical in Xenopus laevis, Gallus gausus, Rattus norvegicus and humans.

Conclusions: The Arg349Leu mutation in LMNA A/C gene is associated with a severe form of DCM.

Cyclosporine A Treatment Decreases Left Ventricular Mass in Mice Expressing a FHC-Linked Troponin T (I79N) Mutation

Background: Cyclosporine (CyA) prevents cardiac hypertrophy in several animal models, and has been proposed as treatment for Familial Hypertrophic Cardiomyopathy (FHC). But with the recent report that CyA administration increased cardiac hypertrophy and mortality in a mouse model of FHC (eMHC+) [403], proposed clinical studies with CyA were abandoned. Because the CyA effect could be specific to this particular mouse model, we examined the effect of CyA in a different murine FHC model expressing a troponin T (I79N) mutation. Methods: Mice expressing human wild-type (Tg-WT), mutant (Tg-I79N) Troponin T and non-transgenic littermates (Non-Tg) were treated with CyA (18mg/kg/day) or vehicle for 4 weeks. LV dimensions, mass and function were measured with serial echocardiography in blinded fashion. Results: All mice tolerated CyA treatment. LV wall thickness and mass significantly decreased in CyA-treated Tg-I79N mice compared to all other groups (table). Systolic function was unchanged: on sacrifice, heart to body weight ratio was significantly decreased in CyA-treated compared to vehicle-treated Tg-I79N mice, (3.3±1mg% vs. 3.6±0.1mg%, p<0.05). No significant differences in tissue histology were found. Blood CyA levels were 45±3±124ng/ml. Conclusion: Unlike in mice expressing a troponin I assay, CyA treatment reduced LV mass in mice expressing a FHC-linked Troponin T mutation. Thus, any effect of CyA treatment should not be generalized across different FHC-linked variants or models.

Effect of Estrogen on Angiotensin Receptors, Matrix Metalloproteinases, and Left Ventricular Mass in a Transgenic Mouse Model of Human Hypertrophic Cardiomyopathy

Background: Mutations in α-tropomyosin (Asp175Asn) can cause familial hypertrophic cardiomyopathy (FHC). Estrogen has been shown to be cardioprotective in several disease states. This study was designed to determine whether estrogen in physiological dosages may modulate left ventricular (LV) function by hypertrophy by attenuating release of matrix metalloproteinases (MMPs) and by regulating angiotensin receptors (AT1) in a transgenic mouse model (TGM) expressing Asp175Asn. Methods: TGM and nontransgenic cohorts (NTGM) (20 each) were ovariectomized (OVX). Slow-release (90 days) estrogen pellets were implanted in 10 mice of each group; the other 10 received placebo. Echocardiograms were performed in awake mice at 2 weeks after OVX (baseline) and after 3 months of daily treadmill exercise. LV mass was assessed from M-mode tracings. Mice were then euthanized and some hearts were perfusion-fixed and embedded in paraffin, for sectioning; others were processed for frozen sections and tissue extracts. Immunohistochemical staining and immunoblotting were performed. Results: Total AT1 and NTGM had LV masses of 77 and 49 mg, and placebo-treated and estrogen-treated oVX TGM had LV masses of 79 and 60 mg, respectively (p<0.05, for both comparisons). AT1-receptors, MMP-3, and MMP-13 were increased in untreated oVX TGM compared to estrogen-treated oVX TGM. Conclusions: Estrogen replacement significantly reduced LV mass, AT1 receptors and MMP-3 and MMP-13 in a model of FHC. These findings suggest that estrogen can regulate expression of genes involved in matrix metabolism and cardiac fibrosis, which are of clinical importance in human patients with FHC.

Determinants of Exercise Capacity in Hypertrophic Cardiomyopathy: The Role of Left Ventricular Outflow Tract Obstruction

Background: The influence of left ventricular outflow tract obstruction (LVOTO) on exercise capacity in patients (pts) with Hypertrophic Cardiomyopathy (HCM) is poorly understood.

Methods: 82 pts with HCM (24+14 yrs, 77% symptomatic) underwent upright bicycle ergometry with expiratory gas analysis and echocardiography. Oxygen consumption at peak exercise (VO2peak), anaerobic threshold (AT), O2 pulse at peak exercise (pO2 pulse), anaerobic threshold (atO2 pulse), and peak VO2 were expressed as percentage of predicted values. Results: 37 pts with resting LVOTO >30mmHg (58±20mmHg, group A), had a lower pO2 peak vs those without resting LVOTO, 65±19 vs 74±19%, p<0.003. In pts without resting LVOTO, pO2peak correlated with peak exercise LVOTO (r=0.43, p<0.002) and change in LVOTO during exercise (r=0.4,-0.04, p=0.02). Of 49 pts without resting LVOTO, 16 developed LVOTO >30mmHg during exercise (group B), 33 did not (group B). Peak exercise LVOTO was lower in group B than group A (49±29 vs 81±27mmHg, p<0.001).

Table 1. There was no difference between the 3 groups for peak heart rate and respiratory quot.