

27. Prevention of Cardiac Remodeling and Heart Failure in Dahl-Salt Sensitive Rats by AAV Vector-Mediated Interleukin-10 Expression

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Background: Inflammation plays a crucial role in the pathogenesis of congestive heart failure (CHF). Enhanced plasma levels of proinflammatory cytokines in patients with CHF have been demonstrated by many other groups. TNF- α targeted approach was not sufficient to disrupt the activated network of inflammatory mediators in CHF. IL-10 exerts pleiotropic cardioprotective effects, such as anti-inflammatory effect, and improvement of the endothelial cell function. Previously, we reported that systemic expression of IL-10 ameliorated hypertension as well as cardiac hypertrophy in the Dahl salt-sensitive (DS) rats. In this study, we further investigated LV systolic function and remodeling to examine the preventive effects of IL-10 on CHF in the DS rats.

Methods: We constructed AAV type 1-based vectors that express rat IL-10 (AAV1-IL-10) or EGFP (AAV1-EGFP) driven by the CAG promoter. The DS rats were intramuscularly injected with the vector (1×10^{12} genome copies/body, n=10 each) at 5 weeks old. Systolic blood pressure (sBP) was measured by the tail-cuff method every 2 weeks. Transthoracic echocardiography was performed at 5, 11, and 19 weeks old. At 20 weeks old, heart tissue was examined under the light microscope following H&E, Azan-Mallory staining, and immunohistochemistry for ED-1.

Results: Six weeks after single intramuscular injection of the vectors, the serum concentration of IL-10 significantly increased in the IL-10-transduced group compared to that in the EGFP-transduced group (418 vs. 0 pg/ml, $p < 0.0001$). TNF- α concentrations in serum and TGF- β 1 levels in the heart decreased at 20 weeks old in the IL-10-transduced group (0 vs. 7.1 pg/ml, $p < 0.005$; 63.5 vs. 174.3 pg/mg protein, $p < 0.05$). Four weeks after injection of the vectors, sBP of the IL-10-transduced group significantly decreased compared to that of the EGFP-transduced group. This sBP reduction persisted over 2 months (at 18 weeks old; 175 vs. 231 mmHg, $p < 0.0001$). At CHF phase (19 weeks old), LV systolic dysfunction (percent fractional shortening; 59.2 vs. 32.9, $p < 0.0001$) and LV dilatation (LV end-diastolic dimension; 7.3 vs. 8.8 mm, $p < 0.0001$) improved in the IL-10-transduced group. Importantly, histological examination revealed that fibrotic changes and macrophage infiltration significantly increased in the heart of the GFP-transduced group. However, these cardiac remodeling were abolished in the IL-10-transduced group. Furthermore, survival rate also improved at 20 weeks old in the IL-10-transduced group (100 vs. 0%).

Conclusion: AAV vector-mediated systemic expression of IL-10 in the DS rats effectively improved inflammation, hypertension, and CHF. Protein supplementation therapy through AAV vector-mediated systemic IL-10 expression would be a promising strategy to prevent the development of cardiac remodeling and CHF in patients with genetic predisposition.

28. rAAV2/9 Mediated Gene Delivery of Acid α -Glucosidase Corrects the Cardiac Phenotype in a Mouse Model of Pompe Disease

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The long term goal of this project is to develop a clinically relevant gene therapy approach for the treatment of Pompe Disease. Pompe Disease is a form of muscular dystrophy and metabolic myopathy caused by mutations in the acid alpha glucosidase (GAA) gene. An insufficient amount of GAA leads to the accumulation of glycogen in lysosomes and consequent cellular dysfunction. Cardio-respiratory failure typically occurs in the early onset patients within the first year of life. We have characterized the cardiac phenotype in our mouse model (*gaa*^{-/-}) at various ages. Through ECG analysis we observe a shortened PR interval by 3 months of age (*gaa*^{-/-} 33.41 \pm 1.35ms, cont 44.95 \pm 1.58ms) mimicking the conduction phenotype in the human Pompe population. Abnormal amounts of glycogen are observed in lysosomes as demonstrated by the periodic acid shift (PAS) stain. MRI analysis shows a decrease in stroke volume (SV)(*gaa*^{-/-}36.13 \pm 1.19ul, cont 51.84 \pm 3.59ul) and a decrease in cardiac output (CO)(*gaa*^{-/-}7.95 \pm 0.26ml/min, cont 11.40 \pm 0.79ml/min) at 3 months and an increase in mass (*gaa*^{-/-}181.99 \pm 10.7mg, cont 140.79 \pm 5.12mg) by 12 months. This model of cardiac dysfunction is being used to develop a cardiac gene delivery technique which can be applied to many genetically inherited cardiomyopathies. Previously, we have shown that intra-venous (IV) delivery of recombinant adeno-associated virus type 1 (rAAV2/1) pseudotype capsid carrying the CMV-*hga*a construct to 1 day old *gaa*^{-/-} neonates restores GAA activity in mice. Also, LacZ transgene delivery using the IV administration route and rAAV2/9 pseudotype capsid resulted in 200 fold higher levels of expression in cardiac tissue than rAAV2/1. Additional experiments showed transduction following delivery to adults. We have now combined rAAV2/9 with the clinically relevant IV administration route in order to deliver the human GAA (*hga*a) gene to *gaa*^{-/-} mice. Neonates treated with rAAV2/9-CMV-*hga*a at a range of doses (4 \times 10⁵v_g; 4 \times 10⁸v_g; 4 \times 10¹⁰v_g) have demonstrated sustained PR interval correction (39.38 \pm 2.42 ms). PAS stains as well as NMR analysis have shown less glycogen accumulation in cardiac tissue of treated *gaa*^{-/-} mice as compared to untreated mice. MRI analysis shows an increase in SV and CO. Adult *gaa*^{-/-} mice have also been treated using this strategy and are being assessed in order to determine if we have the ability to reverse the effects of Pompe Disease in mice already presenting the cardiac phenotype. While the focus of this project is on correction of the cardiac phenotype of Pompe Disease, our systemic delivery route, use of the CMV promoter and the fact that GAA is a secreted enzyme all promote correction throughout the body. GAA activity is observed in other tissues of treated mice including skeletal muscles and liver. These studies have demonstrated the ability of rAAV2/9 to be administered systemically using the IV delivery route, transcend the vasculature, transduce tissues throughout the body and ultimately prevent presentation of the cardiac phenotypes of Pompe Disease.