Results:

Compared to controls, in ALDO-treated rats we found: 1) significantly enhanced in repairing kidney and angiotensin converting enzyme (ACE) inhibition or AT1 receptor blockade attenuates renal fibrosis. The localization of ACE and AngII receptors and their relationship to collagen synthesis in the injured kidney, however, remains uncertain. Using a rat model of renal injury with subsequent fibrosis created by chronic elevations in circulating aldosterone (ALDO), we examined the distribution and binding density of ACE and AngII receptors in repairing kidneys, as well as their anatomic relationship to transforming growth factor (TGF)-beta 1 mRNA, type I collagen mRNA, collagen accumulation and myofibroblasts (myoFbs). Two groups of animals (n=7/group) were studied: 1) normal rats served as controls; and 2) uninephrectomized rats received ALDO (0.75mg/hr sc) and 1% NaCl in drinking water for 6 weeks. Compared to controls, in ALDO-treated rats we found: 1) significantly (P<0.01) increased blood pressure and reduced plasma renin activity and increased plasma creatinine; 2) diffuse fibrosis in both renal cortex and medulla; 3) abundant myoFbs at these sites of fibrosis, and 4) significantly increased (P<0.01) binding density of ACE and AngII receptors (60% AT1: 40% AT2) at sites of fibrosis; and 5) markedly increased (P<0.01) expression of TGF-beta 1 and type I collagen mRNAs at these same sites. Thus, in this rat model of renal repair, enhanced expression of ACE, AngII receptors and TGF-beta 1 are associated with renal fibrosis. AngII generated at sites of repair appears to have autocrine/paracrine functions in regulating renal fibrous tissue formation alone or through its stimulation of TGF-beta 1 synthesis.

Key Words: Renal fibrosis, TGF-beta 1, Angiotensin II

SOMATIC GENE THERAPY FOR HYPERTENSION WITH ADENO-ASSOCIATED DELIVERY OF ANTISENSE TO ANGIOTENSIN TYPE 1 RECEPTOR mRNA

1Dept. of Physiology, College of Medicine, University of Florida, Gainesville, FL, United States

Introduction: The goal of gene therapy for hypertension is to produce safe, prolonged reductions of high blood pressure with a single administration of a transgene. We have developed gene therapy using adenovirus (AAV) antisense (AS) as a vector because it is safe, stable and effective. To test systemic injection in an adult hypertensive model, this study uses double transgenic (dt) mice, with human renin (hr) and human transgenes. In these mice, plasma Ang II levels are elevated and blood pressure increased (~140-160 mmHg), compared to controls (~100 mmHg). Methods: Therefore, dt mice with established baseline BP of ~140-160 mmHg (n=5) were systemically injected (100 µL) with a single dose of 4x10^10 infectious particles of rAAV-AT,R-AS. The rAAV contained a CMV promoter and neo reporter gene. Control (n=5) received the rAAV vector without AS. Blood pressure recordings by the tailcuff method were made once per week for up to 6 months. Results: One week after injection, BP decreased by 35-50 mmHg (p<0.001, compared to baseline). The normalized blood pressure persisted for the full length of the study. Individual mice were sacrificed at 14-28 weeks and tissues taken for detection of rAAV-AT,R-AS. At both time periods, the AS-AT,R transgene was present in lung, kidney, liver, heart, adrenal gland and fat. The rAAV was not detected in the brain. Renal arterioles (n=6) showed a reduction (50%) contractile response to increasing low doses of Ang II, compared to controls (n=6) (p<0.01). Autoradiography of AT1,R showed a reduction in receptors in the rAAV-AT,R-AS treated group only. Conclusion: The results demonstrated that a single systemic delivery of rAAV-AT,R-AS in adult, hypertensive mice produces a profound decrease in blood pressure for at least 6 months. The prolonged effect is due to the continuous expression of the AT1,R AS transgene inhibiting AT1 receptors. The results encourage further development of the rAAV with engineering to make AS transgene expression tissue-specific and switchable on or off for safety.

Key Words: AAV (Adeno associated virus), AT1 receptor, Antisense