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LOCAL ANGIOTENSIN II AND TRANSFORMING GROWTH FACTOR-BETA 1 IN RENAL FIBROSIS OF RATS

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Studies have demonstrated that local angiotensin II (AngII) generation is 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 (myoFb). 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 myoFb at these sites of fibrosis, 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

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

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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 develeloped gene therapy using adeno-associated virus (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_1R-AS. The rAAV contained a CMV promoter and neo[1] 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_1-R-AS. At both time periods, the AS-AT_1R 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 log doses of Ang II, compared to controls (n=6) ($p<0.01$). Autoradiography of AT_1-R showed a reduction in receptors in the rAAT-AT_1R-AS treated group only. **Conclusion:** The results demonstrated that a single systemic delivery of rAAV-AT_1R-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 AT_1R AS transgene inhibiting AT_1 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

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SYNERGISTIC INCREASE IN TISSUE DAMAGE CAUSED BY LOW DOSE ANGIOTENSIN II AND L-NAME INFUSION

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Excessive circulating angiotensin II (Ang II) and reduced nitric oxide (NO) are independently associated with end organ damage. However, low dose infusions of Ang II that only increase plasma Ang II levels 2-fold, cause no detectable end organ damage (Hu et al J Hyper 16: 1285, 1998). To determine possible interactions between the two systems, we coinfused low dose Ang II and L-NAME in chronically catheterized Sprague-Dawley rats.

After 5 days saline infusion, L-NAME (10 μg/kg/min) was infused for 20 days. After 6 days of L-NAME, Ang II (10ng/kg/min) was coinfused for 14 days (LA, 16 rats). Two other groups of rats were infused with L-NAME (L, 8 rats), or saline (C, 5 rats) alone.

Blood pressure and heart rate were monitored continuously. Plasma renin (PRC) was measured daily. Plasma cardiac troponin T (cTnT) and urinary albumin (UA) were measured as indices of cardiac and renal damage, respectively. At the end of the study, hearts and kidneys were assessed histologically.

cTnT rose and then fell sharply 1 to 4 days after the start of Ang II, and then remained elevated. cTnT was always undetectable in L or C rats. UA began to rise after 4-5 days Ang II infusion and continued to rise thereafter. L-NAME done did not increase UA. Unlike L rats, LA rats had extensive cardiac and renal perivascular fibrosis, glomerular damage, and an increase in the overall cardiac collagen volume. Renal parenchymal damage was increased in both LA and L rats, but was much greater in the LA group.

Taken together, these observations show that there is greatly increased renal and cardiac damage when Ang II and L-NAME are infused together over two weeks at doses that, when infused alone, produce relatively little injury. Thus, Ang II may cause greater end organ damage in a setting of reduced NO bioavailability.

Key Words: Angiotensin, Nitric Oxide, End Organ Damage

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MOLECULAR BASIS FOR THE INSURMOUNTABLE AT-1 RECEPTOR ANTAGONISM OF TELMISARTAN

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In vitro studies have shown that telmisartan is an insurmountable angiotensin II AT-1 receptor antagonist. In this study we have investigated the molecular basis of this insurmountable antagonism. The association and dissociation kinetics of telmisartan to angiotensin AT-1 receptors were

measured using an *in vitro* radio-receptor binding assay. These radioligand binding studies were conducted either directly on rat vascular (aorta) smooth muscle cells (RVSMC) expressing solely the AT-1 receptor or on membrane preparation obtained from the same cells.

The specific binding of ^{3}H-telmisartan to the surface of living RVSMC or membranes was saturable. From these data, a Kd value of 1.7 nM was estimated. Scatchard analysis of the ^{3}H-telmisartan binding on RVSMC indicated the existence of a single class of binding sites. The affinity of telmisartan for AT-1 receptor is only poorly affected by the presence of proteins (0.4% of rat plasma proteins) in the binding buffer, indicating that no great competition between telmisartan binding to its specific AT-1 receptor and to non-specific proteins binding sites occurs. In association experiments, the specific binding of ^{3}H-telmisartan increases quickly and reaches equilibrium within less than 1 hour, with an association rate constant calculated to be 0.006 $min^{-1}nM^{-1}$. Telmisartan dissociates very slowly from the AT-1 receptor, either in RVSMC membrane preparation or in living cells with a dissociation rate constant of *ca.* 0.01 min^{-1} resulting in a dissociation half-life ($t_{1/2}$) of about 60 min, which is comparable to the previously published data for candesartan in bovine adrenal cortical membranes and almost 5 times slower than that of ^{125}I-angiotensin II binding ($t_{1/2}$=12 min). In contrast to candesartan that has been shown to re-associate with the AT-1 receptor, telmisartan does not appear to re-associate. Indeed, when the dissociation of labeled-telmisartan from AT-1 receptors was induced by washing the cells with cold-binding buffer, followed by addition of fresh binding buffer containing either cold telmisartan, Ang II or losartan, or nothing, no difference were observed in the dissociation rate constants measured with telmisartan whatever the composition of the binding buffer after removal of labeled-telmisartan.

In conclusion, these results suggest that the insurmountable antagonism of telmisartan is due mainly to its very slow dissociation from angiotensin AT-1 receptors.

Key Words: telmisartan, Angiotensin II AT-1 receptor, association and dissociation rate constants