OR-1
PLACEBO CONTROLLED STUDY OF AN ANGIOTENSIN IMMUNOTHERAPEUTIC VACCINE (PMD3117) IN HYPERTENSIVE SUBJECTS
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Objective: Immunisation against the renin system might obviate the need for daily drug administration. No previous study has determined whether a sustained antibody titre can be achieved, and whether this might have pharmacological activity. Our primary objective was to determine the antibody response to two PMD3117 vaccine dosing regimes. Secondary objectives were to assess tolerability, and to determine if immunisation changes renin and aldosterone secretion, or attenuates the BP rise after withdrawal from an ACE inhibitor.

Methods: PMD3117 is a 12 amino acid analogue of angiotensin-I linked to Keyhole Limpet Haemocyanin, and adsorbed onto aluminium hydroxide (Alhydrogel). 24 patients responsive to an ACE inhibitor or angiotensin blocker were randomly assigned to 3 or 4 subcutaneous injections over 6 weeks with 100 mg peptide equivalent of PMD3117 or Alhydrogel. Blood was drawn at each visit for estimation of renin, aldosterone, and the IgG antibodies to angiotensin. Existing treatment was withdrawn for 2 weeks before and after the 6 weeks of vaccination. 24-ABPM was performed at the start and finish of the withdrawals.

Results: Median titres of 6739 and 11548 respectively for the 3- and 4-dose regimen were present by day 64 (left panel), and declined thereafter with median half-life of 85 days (95% CI[41,153] days). Clinic BP did not differ between PMD3117 and Alhydrogel, but 24-hour SBP on day 64 was 7.5 mmHg lower in the 4-dose than 3-dose PMD3117 subjects (p<0.01). PMD3117 reduced 24 h aldosterone excretion at 6 weeks to 6% (95% CI[1,31]%) of control (p<0.01) (middle panel) and plasma renin was elevated ~2-fold (right panel). Transient local swelling and itching occurred after some of the final PMD3117 injections. One patient from each group withdrew.

Conclusions: PMD3117 offers a well tolerated means for inducing a prolonged antibody titre against the renin system. Although a higher titre is likely to be required for BP reduction, the secondary endpoints already show evidence of pharmacological blockade.

Key Words: vaccine, angiotensin, antibodies

OR-2
MAXIMAL SUPPRESSION OF RENIN-ANGIOTENSIN SYSTEM IN PATIENTS WITH REFRACTORY PROTEINURIA
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Elimination of residual proteinuria (Uprot) is the novel target in renoprotection. Whether this goal can be achieved by maximal suppression of renin-angiotensin system (RAS) remains ill-defined. We evaluated the effects of stepwise increasing suppression of RAS on quantitative and qualitative proteinuria in ten patients with stable non-nephrotic Uprot (2.55±0.94 g/24h), due to primary nonproliferative glomerulonephritis (NPGN), unresponsive to standard therapy. Patients showed normal values of creatinine clearance (CrCl: 103±17 mL/min) and blood pressure (BP: 118±7 / 75±4 mmHg). The study was divided in 3 consecutive phases: (A) four subsequent one-month periods of ramipril at the dose of 2.5, 5.0, 10 and 20 mg/day, (B) two months of ramipril 20 mg/day + irbesartan 300 mg/day and, (C) two months of irbesartan 300 mg/day alone. Maximizing RAS suppression was not coupled with any major effect on CrCl and serum potassium; conversely, a decrement in hemoglobin (Hb) levels, of 0.8 g/dL on average (P<0.005), was observed during up-titration of ramipril dose. The 2.5 mg dose of ramipril decreased Uprot by 29±8% (P<0.05). Similar effects were detected after irbesartan alone (−28±5%). The antiproteinuric effect was not improved by the higher ramipril doses (−30±3% after the 20 mg dose) or after combined treatment (−33±6%). BP was reduced to 103±3 / 68±2 mmHg after ramipril 2.5 mg/day and did not change in the subsequent experimental steps; however, no significant correlation was found between the changes of proteinuria and BP values. The reduction of Uprot led to amelioration of the markers of tubular damage, as testified by the significant decrement of a,microglobulin excretion and of the tubular component of proteinuria at sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Therefore, in non-nephrotic NPGN patients: a) standard doses of either ramipril or irbesartan lead to significant reduction of Uprot and amelioration of the qualitative features suggestive of tubular damage, b) the change of Uprot is independent from the BP lowering effect, c) the enhancement of RAS suppression up to the maximal degree does not improve the antiproteinuric response, d) high-dose ramipril, at variance with irbesartan, decreases Hb levels.

Key Words: proteinuria, converting enzyme inhibitor, angiotensin II receptor antagonist

OR-3
INTRACELLULAR ANGIOTENSIN II ALTERS AT1 RECEPTOR DISTRIBUTION AND ACTIVATES CREB
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Hypothesis: Intracellular interaction of angiotensin II with the AT1 receptor invokes functional consequences.

Background: Our previous studies indicate that angiotensin II (AII) generated intracellularly from a signal peptide sequence-depleted angiotensinogen cDNA is growth stimulatory for H4-II-E-C3 rat hepatoma cells and that the mitogenic effect is, at least in part, due to increased production and secretion of PDGF A-chain.

Methods and Results: COS-7 cells were transfected, independently and concurrently, with fluorescent fusion proteins of angiotensin II and the AT1a receptor (AT1-R), and fluorescent microscopy was performed. In both expression plasmids, the sequence encoding the functionally relevant protein is fused, in-frame, upstream of the sequence encoding the fluorescent protein. The distribution of AII/ECFP (fusion product of AII and cyan fluorescent protein) is diffusely cytoplasmic. In those cells that express only AT1-R/EYFP (fusion product of the AT1-R with yellow fluorescent protein), protein accumulates in the endoplasmic reticulum, Golgi, and on the plasma membrane. However, in those cells that express both AII/ECFP and AT1-R/EYFP, the receptor is poorly expressed on the plasma membrane and is largely retained intracellularly.
Co-expression of AII/ECFP and AT1R/EYFP in COS cells also mediates phosphorylation of the CREB (cAMP response-element binding protein) activation domain in a GAL4-DNA-binding domain/CREB activation domain fusion protein. Luciferase activity from GAL4 upstream activator sequence/luciferase constructs was elevated 5-fold (p < 0.05) over control. This suggests that intracellular interaction of AII with the AT1-R enhances transcription of genes possessing cAMP response elements.

**Conclusions:** Intracellular AII results in intracellular retention of the AT1 receptor. Intracellular AII interacts with the AT1-R to mediate CREB activation and downstream transcriptional changes. In summary, AII which is either internalized or generated intracellularly can have functional consequences by altering signal transduction.

Key Words: angiotensin, intracrine, growth factor