Abstract: P4477

**Editing of myosin phosphatase as a novel approach for sensitization of vascular smooth muscle to vasodilators (NO/cGMP/ROS) and lowering of blood pressure in hypertension**

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Background: Despite the many drugs for treatment of hypertension, it remains inadequately treated in >50% of patients and the number one contributor to cardiovascular mortality world-wide. Thus new targets and treatment strategies are badly needed. Myosin Phosphatase (MP) is a viable target: it is the primary effector of vascular smooth muscle relaxation and a critical mediator of signaling pathways regulating vessel tone. Purpose: We are using complementary/ translatable approaches to test the hypothesis: editing of the Myosin Phosphatase Regulatory (Targeting) subunit (MYPT1), by shifting the expression of naturally occurring isoforms, will sensitize vascular smooth muscle to NO/cGMP/ROS mediated vasorelaxation and thereby lower BP in models of hypertension. A further goal is to determine mechanisms by which these signals activate MP thereby causing vasorelaxation. Methods: LoxP sites were inserted in introns flanking alternative Exon24 (E24) of Mypt1. Mice were crossed with smMHCCreER mice and treated with Tamoxifen for smooth muscle specific deletion of E24 (SMcKO E24). Skipping E24 codes for a Mypt1 isoform that contains a C-terminal leucine zipper (LZ) motif required for cGMP-dependent protein kinase (cGK1) binding and NO/cGMP/ROS activation of MP. Second, we developed and tested guide RNAs for the purpose of AAV-CRISPR/CAS9 editing of Mypt1 E24 as a treatment for hypertension. Effect of editing is tested in otherwise normal mice and in the AngII sub-pressor model of hypertension. Results: SMcKO E24 mice had mean BP that was 15±3 mmHg lower than control (n=5; p<0.05). Mesenteric arteries from these mice were significantly more sensitive to DEA/NO mediated relaxation (EC50: 2.1±0.5 nM vs 18.2±5.6 mM; n=5-6, p<0.05). Experiments testing response to AngII infusion are in progress and will be presented at the meeting. Preliminary biochemical assays support a 2-pool model, in which NO/cGMP/ROS activates the LZ+ pool, while contractile agonists inhibit the LZ- pool of MP, in the control of BP/ blood flow. We have generated a number of AAV Crispr/Cas9 gRNAs and validated their efficacy of editing of Mypt1 E24 in vitro. Experiments are in progress to test their efficacy and effect on BP in vivo Conclusion: These studies support that editing of Mypt1 E24 could be a novel strategy for vasodilator sensitization and effective lowering of blood pressure in humans with hypertension, thereby having a substantial impact on CV mortality world-wide.