Identification of the site of endothelin A receptor antagonist-induced fluid retention

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Endothelin (ET) receptor antagonist-induced fluid retention is a major clinical problem, being responsible for discontinuation and/or failure of several large clinical trials. Such fluid retention appears to occur with either ETA or ETB receptor blockade. ETB receptor inhibition is predicted to cause fluid retention though reduction of the known natriuretic effects of ET-1 on renal tubules, however how ETA receptor (ETAR) antagonism elicits fluid retention is uncertain. To begin to examine the mechanisms involved, the relatively ETAR-selective antagonist, ambrisentan (ETA:ETB selectivity ranging from 29-4000:1, depending on the assay), was administered to mice in which ETAR were selectively deleted in cardiomyocytes (CM ETA KO), vascular smooth muscle (VSM ETA KO), or the renal collecting duct (CD ETA KO), as well as mice with intact ETAR. All gene targeting utilized mice with loxP-flanked ETAR and Cre recombinase expressed in a tissue-specific manner. Myosin heavy chain-Cre conferred heart-specific expression, smooth muscle-22-Cre conferred vascular smooth muscle-specific expression, and aquaporin-2-Cre conferred renal collecting duct-specific expression. All mice were given a 4% NaCl diet throughout the course of the study to exacerbate Na retention. Animals were given no treatment for 1 week (baseline), followed by 2 weeks of 100 mg/kg/day ambrisentan or vehicle by oral gavage (N=5-10 per group). Baseline radiotelemetric blood pressure and pulse were not different between groups (averaging a systolic BP of 113 ± 7 mmHg). Ambrisentan did not significantly alter blood pressure or pulse in any of the mouse lines over the two weeks of treatment, although blood pressure did gradually decrease in all groups over the course of the study (final systolic BP averaging 102 ± 8 mmHg). Two weeks of ambrisentan treatment increased body weight in control and CM ETA KO (by an average of 1.6 ± 0.4 g), but not in VSM ETA KO or CD ETA KO. Ambrisentan decreased hematocrit in control, CM ETA KO and VSM ETA KO (an average decrease of 1.7 ± 0.5% with baseline hematocrit being 51 ± 4%), but not in CD ETA KO animals. Body fluid volume compartments were determined by impedence plethysmography. Ambrisentan tended to increase extracellular fluid volume in all mouse groups except in CD ETA KO mice. At the conclusion of the experimental study, plasma volume was determined by Evan's blue dye dilution; no alterations in plasma volume were detected, although the technique was unlikely to detect the magnitude of predicted changes. In summary, ambrisentan appears to cause fluid retention in control mice and animals deficient in ETAR in the heart or vascular smooth muscle, however absence of CD ETA receptors prevented the ambrisentan response. These data suggest that the CD is the site responsible for ETAR antagonist induced fluid retention, however additional studies will be needed using other ETAR antagonists, particularly those with very high ETAR selectivity. Nonetheless, these findings suggest that concurrent treatment with diuretics, particularly those targeting the collecting duct, may be beneficial in mitigating ETAR-induced fluid retention.