PATIENTS WITH HIGH BLOOD PRESSURE SHOULD AVOID ASPIRIN: REDUCED RENAL PERFUSION IN HYPERTENSIVE EP4 KNOCKOUT MICE

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Target audience: Clinicians and basic scientists interested in hypertension and kidney blood flow

Purpose: In hypertensive mice, to investigate the role of the E-prostanoid-4 (EP4) receptor in the maintenance of renal perfusion

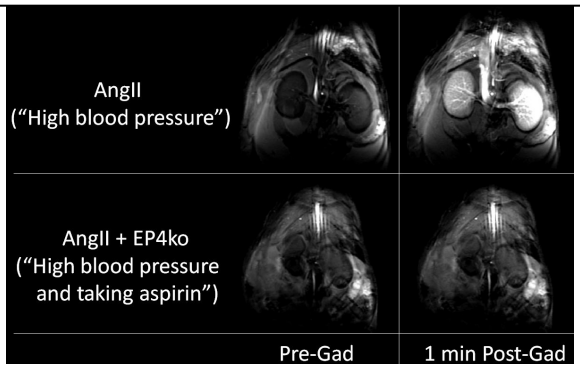
Introduction: Cyclooxygenase (COX) inhibition with nonsteroidal anti-inflammatory drugs (e.g. aspirin, ibuprofen, naproxen) is contraindicated in patients with hypertension as it may predispose them to kidney damage via decreased renal blood flow and glomerular filtration rate (1). COX-derived prostaglandin E2 activates EP receptors throughout the kidney and regulates a variety of physiological functions including glomerular and medullary microcirculation and electrolyte balance. Activation of the EP4 receptor in the kidney pre-glomerular vasculature leads to vasodilation and buffers the actions of potent vasoconstrictors including angiotensin II (AngII). In other words, hypertension vasoconstricts renal vessels while NSAIDs suppress EP compensatory vasodilation, thus making hypertension and NSAIDS a potentially dangerous combination. Direct, in-vivo evidence of this phenomenon is lacking, however. We conjectured that the EP4 receptor will maintain renal perfusion in a hypertensive setting where AngII levels are chronically elevated. We therefore generated inducible vascular-specific EP4 knockout mice and challenged them to a model of AngII-dependent hypertension. ***Our hypothesis was that mice with AngII-induced hypertension will suffer impaired renal perfusion if their EP4 receptors are suppressed.***

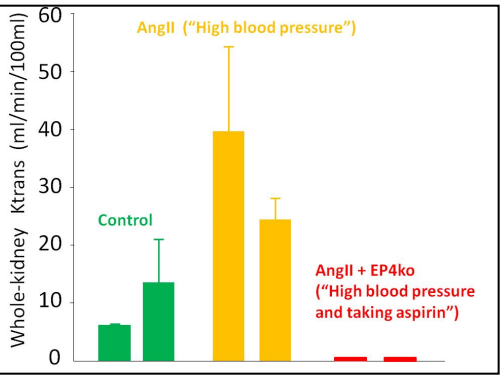
Methods: Tamoxifen treatment (5 day, IP, 1 mg/day) in mice expressing the Sma-Cre-EP4flox/flox transgene leads to excision of the EP4 sequence via activation of Cre-recombinase specifically in smooth-muscle actin (SMA) expressing cells (i.e. vasculature). After 4 weeks post-tamoxifen (corn oil as vehicle), subcutaneous osmotic minipumps were implanted in these mice, which deliver 1000 ng/kg/min of angiotensin II at a constant rate. Six mice were studied: **two regular control mice which underwent sham surgery**; **two regular mice that underwent AngII treatment**, and **two EP4 knockout (EP4ko) mice that underwent AngII treatment**. In-vivo renal perfusion experiments were performed at the University of Ottawa pre-clinical imaging core using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) on a 7 Tesla GE/Agilent MR 901. A single oblique coronal slice was prescribed through the kidneys. For DCE-MRI, a multi-phase fast spoiled gradient echo (FSPGR) sequence was used with thickness=2 mm, FOV=5x3.75 cm, matrix=256x144, TE=1.3 ms, TR=4.1 ms, flip=60 deg, bandwidth= 62 kHz, 1 average, temporal resolution = 0.6 s, total DCE imaging time = 30 minutes. During DCE, at 2.5 min, a 66:1 aqueous solution of Gadovist (Bayer) was used to deliver 3 uL of Gadovist intravenously (~ 0.1 mmol/kg). Before and after DCE, T1 maps of the kidneys were generated using the DCE sequence with an inversion pulse added (TI=0.3,0.4,0.5,0.75,1,2,3,6 sec; readout flip reduced to 5 deg; wait time between images = 10 sec). These "Bookend" T1 maps were used to convert kidney signal-vs-time to concentration-vs-time (relaxivity assumed to be 4.7 $mM^{-1}s^{-1}$) (2-3). The arterial input function (AIF) was estimated using phase in major arteries (4). This AIF procedure failed in two mice, therefore a population AIF was used from the average of the other four. Whole-kidney volume transfer coefficient (Ktrans), which provides an index of renal perfusion, was estimated using freely available "PMI" software by Dr. Steven Sourbron, University of Leeds, UK (https://github.com/plaresmedima/PMI-0.4-Runtime-Ottawa).

Results: Ktrans values in whole kidney (mean ± stdev for two kidneys per mouse, units of ml/min/100ml) were **6.3 ± 0.0 and 13.5 ± 7.5 for control** mice; **39.7 ± 14.6 and 24.4 ± 3.6 for AngII** mice; **0.3 ± 0.0 and 0.4 ± 0.0 for AngII+EP4ko** mice.

Figure 1 (left): Pre- and post-gadolinium images for an **AngII** mouse (upper row) and an **AngII+EP4ko** mouse (bottom row).

Figure 2 (right): Ktrans values for **control** mice, **AngII** mice, and **AngII+EP4ko** mice.





Conclusion: In mice with AngII-induced hypertension, suppression of the EP4 receptor leads to a catastrophic reduction in renal perfusion. This supports the idea that NSAIDs are bad for hypertensive patients and hints that EP4-selective activation (pharmacologically) may be beneficial.

References: 1) Whelton, AJ Therapeutics 7.2 (2000): 63-74. 2) Cron et al, MRM 51.5 (2004): 1066-1070. 3) Noebauer-Huhmann et al, Invest Radiol 45.9 (2010): 554-558. 4) Garpebring et al, Magn Reson MPBM 24.4 (2011): 233-245.