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P459 -Aristolochic acid and cisplatin differentially regulate retinoic acid activities in collecting duct cells and in kidneys of RARE-Luciferase mice

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Introduction: Aristolochic acid I (AAI) is a natural nephrotoxicant and cisplatin is a nephrotoxic chemotherapeutic agent. In mice, acute kidney injury can be induced by a single high-dose injection of either agent. Emerging evidence suggests that the canonical vitamin A signalling mediated by retinoic acids (RAs) and RA receptors (RARs), particularly that in collecting ducts (CDs), may be a critical protector against injury. In this study, we set to examine how AAI and cisplatin affect the RA/RAR activities in cultured CD cells and in kidneys.

Methods: mIMCD-3 mouse inner medullary CD cells were transfected with a retinoic acid response element (RARE)-Firefly Luciferase reporter plasmid and a control Renilla Luciferase plasmid, treated by AAI, cisplatin or vehicle, and subjected to RARE dual-luciferase reporter assays to quantify RA/RAR activity. Effects on renal RA/RAR activities were examined in heterozygous RARE-Luc transgenic mice (C57BL/6, male, 22-28 weeks old, 30-50g, n=6/group), in which RA/RAR activation leads to RARE-driven expression of luciferase, which is visualised and quantified after i.p. injection of luciferin (150 mg/kg) under an IVIS Lumina III bioimager. Renal RA/RAR activities seven days after a single injection of AAI (10 mg/kg, i.p., vs vehicle) and three days after a single injection of cisplatin (20 mg/kg, i.p. vs vehicle) were examined. Kidneys were removed and cut into two halves horizontally and longitudinally, respectively, and subjected to ex-vivo imaging analysis. Photon emission from tissues was then integrated over a period of 1 min and recorded as pseudo-color images. Signal intensity was quantified as sum of all detected photon counts within region of interests.

Results: In mIMCD-3 cells, 10, 20 and 40 μM AAI dose-dependently induced RA/RAR activity, $p>0.05$, $p<0.05$ and $p<0.001$, respectively. In contrast, 5, 25 and 50 μM cisplatin dose-dependently repressed RA/RAR activity, $p<0.01$, $p<0.001$ and $p<0.001$, respectively. In RARE-Luc transgenic mice, the gross distribution of renal RARE-Luc activity was similar to that of kidneys of RARE-LacZ mice as we reported before, namely, decreasing from papilla, inner and outer medulla to cortex, where signal was hardly visible. Interestingly, AAI and cisplatin both induced expansion of RARE-Luc signal towards outer medulla and cortex. AAI induced a significant 6-fold rise of RARE-Luc activity in the outer medulla/cortex ($p<0.001$), while the signals of the entire kidney and inner medulla/papilla did not alter significantly; cisplatin, however, caused a significant increase of RARE-Luc activity in papilla and inner medulla (4-fold), outer medulla and cortex (20.6-fold), and indeed the entire kidney (6.2-fold), $p<0.001$.

Conclusions: AAI significantly increases RA/RAR activity in mIMCD-3 cells, in renal cortex and outer medulla, but does not significantly affect RA/RAR activity in papilla and inner medulla, where physiological RA/RAR is most abundant. In contrast, cisplatin significantly represses RA/RAR activity in mIMCD-3 cells and paradoxically induces RA/RAR activity across the entire kidney. Further studies are needed to determine mechanisms of the differing regulation, exact cellular location of the changed RA/RAR activity and roles for the RA/RAR activity, particularly that in CDs, in AAI- and cisplatin-induced kidney injury.