MRI-histopathology correlation of N-phenylanthranylic acid induced nephropathy in rats

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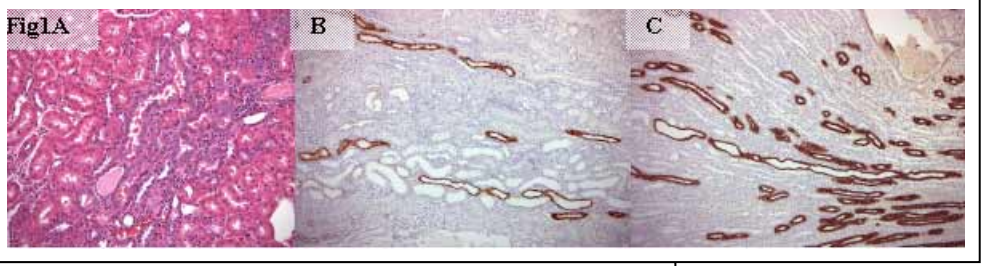
Introduction:
Analgesic-associated nephropathy and renal papilla necrosis is a common cause of renal failure in humans. It mainly develops as a consequence of chronic consumption or long-term abuse of single or mixed analgesics or non-steroid anti-inflammatory drugs. The mechanism of this analgesic-associated nephropathy is poorly understood, and its diagnosis remains difficult. This study investigated the correlation between histopathology of N-phenylanthranylic acid (NPAA) induced nephropathy in rats and its MRI appearance by ex vivo scan.

Materials and methods:
Twelve male Han: Wistar rats were used in this study. The age at start of dosing was 6-8 weeks. Animals were housed 4/cage with water from the site drinking supply and pelleted diet. Eight animals were dosed once daily, by oral gavage, of NPAA 700mg/kg/day, for 14 days. Four animals were treated with vehicle. All animals were killed by inhalation of halothane prior to necropsy approximately 24h after receiving the final dose. MRI was performed immediately after the euthanasia. The left kidney of the rats were removed from the abdomen and placed within a small vial containing 10% formalin. These kidneys were scanned using a Varian horizontal 9.4T magnet, equipped with a 40 Gauss/cm gradient with a rise time of 200 microseconds. The vials containing kidney were placed inside a 38 mm quadrature birdcage RF coil, with kidneys' longitudinal axis parallel to B0. MRI included transverse spin echo scan (TR/TE=1000/13msec, resolution=0.078*0.313mm), coronal spin echo scan (TR/TE=2000/30msec, resolution=0.313*0.313mm), and coronal gradient echo scan (TR/TE=35/6msec, Flip angle=40degree, resolution=0.313*0.313mm). The slice thickness was 0.5mm for all scans. After MRI scan, kidneys were processed to haematoxylin-eosin stained sections plus immunohistochemistry for collecting ducts.

Results:
1. Histopathology: Kidneys of all NPAA treated animals showed various nephropathy changes. Macroscopically the diseased kidneys increased in their size. Microscopically, NPAA induced nephropathy included cortical collecting tubular dilatation, tubular basophilia, corticomedullary interstitial nephritis, intratubular protein casts, and papillary collecting tubule dilation. One rat showed minimal degree of renal papilla necrosis.
2. MRI: MRI data with good signal-to-noise ratio were acquired. In control animals, the medulla showed higher signal than the cortex both on spin echo and gradient echo images, with a clear corticomedullary junction. MRI revealed an increase in longitudinal and axial dimensions of the NPAA induced kidneys compared with the control kidneys. A loss in the conspicuity of the normal corticomedullary junction was seen. Additionally, fine radial bands pointing to the renal papilla with alternating high and low signal were observed primarily in the cortex. These radial bands were aligned with the medulla rays seen longitudinally. These changes can be appreciated in both spin echo images and gradient echo images, though spin echo images tends to show richer tissue contrast than gradient echo images.



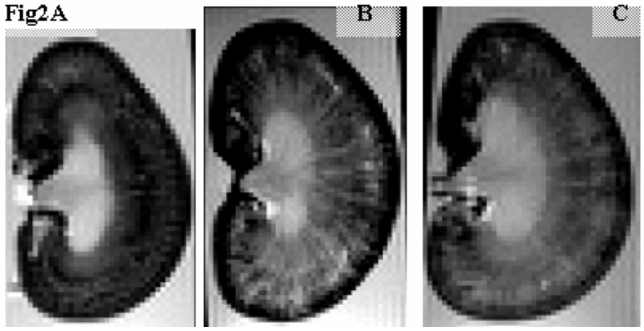


Fig 1: a. HE stain shows inflammatory changes in corticomedullary junction region; b (cortex) & c (outer medulla) Immunochemistry showed dilated collecting ducts (brown) and distal tubules (pale blue).
Fig 2: a. Coronal spin echo MR image of a left kidney from a control animal; b&c, Coronal spin echo image of left kidney from NPAA treated animals. The kidneys in Fig b, c show increased size, loss of conspicuity of corticomedullary junction, and fines radial bands with alternating high and low signal. Fig c shows an swollen medulla.

Discussion:
NPAA is a known inducer of nephropathy and renal papillary necrosis in the rat (1,2,3). There are literature reports of using H1 MR spectroscopy for the analysis of urine sample from NPAA induced rat nephropathy (2,3). To our knowledge there is no literature report on MR imaging study in this nephropathy model.
This study demonstrated that MRI was able to detect NPAA induced nephrotoxic changes in rats ahead of renal papillary necrosis. Besides that MRI demonstrated the increase in longitudinal and axial dimensions of kidneys, the pre-necrotic changes observed in the medulla (increased interstitial matrix, hypertrophy of collecting ducts) were consistent with the swollen medulla as demonstrated by MRI. Particularly, it is interesting to observe that grouping of collecting tubular dilatation in medullary rays was reflected on MR images as fine radial bands pointing to the renal papilla. This increased the water content and the MR signal intensity. Histopathology showed that the corticomedullary junction region was the site of degenerative and regenerative inflammatory changes. This was shown by MRI as loss of conspicuity of the corticomedullary junction. It has long been recognised that the changes in the corticomedullary junction is an indicator of kidney diseases (4,5). This was believed being partly due to the changed status of the relative hydration of the medulla and cortex.
With the magnet field and gradient strength used in this study, higher spatial resolution could be achieved. However, during the study this was constrained by the requirement that the MR scan duration needed to be minimised so that the kidney specimens could be processed quickly for further histology.
Our ex vivo MRI preliminary results appear to suggest that MRI could provide sensitive imaging markers for NPAA induced kidney damages. These findings could potentially be translated to non-invasive in vivo animal studies and clinical studies in humans.

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