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P161 -RAPID APPEARANCE OF MEMBRANE-FREE, INTRACYTOPLASMIC INCLUSIONS IN THE RENAL PROXIMAL TUBULE EPITHELIAL CELL AFTER ISCHEMIA-REPERFUSION: A PATHOLOGICAL INVESTIGATION IN A PORCINE MODEL

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Objectives: Acute kidney injury (AKI) impacts renal proximal tubule epithelia cells (RPTEC), resulting in a loss of cell function and various types of programmed cell death (apoptosis, ferroptosis, necroptosis, necrosis). Temporal decline in renal function is usually marked by a time-dependant rise in plasma or urinary biomarkers, but the parallel change in renal histopathology is often not known. Here, using an established large animal (porcine) model of ischaemia-reperfusion induced AKI, we report sequential histopathology from 2 to 48h reperfusion, and report very early histopathological change and, for the first time, appearance of periodic acid-Schiff (PAS)-positive intracytoplasmic droplets from as early as 2h reperfusion.

Methods: Female pigs (at least n=5 at each timepoint; 50-70 kg) were subjected to an ischemic AKI protocol under anaesthesia in which either both, or a single, renal artery is clamped for 40 mins. Suitable controls were used whom had no renal artery clamped. Animals for whom only a single renal artery was clamped (right kidney), the left kidney was used as an internal control and the animal euthanased at the end of the procedure e.g. 4h. At post-mortem, kidney tissue was harvested for histology, transmission electron microscopy (TEM) and further studies.

Results: Ischaemic kidneys in which no reperfusion occurred i.e. time zero, had no evidence of histopathology. By 2h reperfusion, focal simplification with loss of brush-border was clearly evident. At 4, 24 and certainly by 48h reperfusion, kidney histopathology had progressed to marked acute tubular damage and necrosis in all animals. At 2h, 4h and 24h intracytoplasmic droplets appeared primarily, but not exclusively, in proximal tubules. They clustered to certain tubules and were on visible with PAS. The droplets had the appearance of non-membrane bound lipid-rich inclusions, confirmed by TEM and were IHC positive for p62, a marker of intracellular autophagy, and ubiquitin.

Conclusion: We have shown for the first time in a large animal model of AKI evidence for some of the first indicators of histomorphological damage to the renal proximal tubule, prior to obvious cell death. These changes were evident as early as 2h after reperfusion. Based on TEM findings and PAS staining characteristics, the intracytoplasmic inclusions would appear to consist of lipids and sugars and perhaps evidence disrupted autophagy and protein degradation pathways given positivity for p62 and ubiquitin, respectively.