FINDING A ROLE FOR NECROPTOSIS IN RENAL ISCHEMIC REPERFUSION INJURY IN HUMAN RENAL PROXIMAL TUBULAR (HK-2) CELLS

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Abstract: Despite substantial advances in our understanding of the pathogenesis of acute renal failures, advances in treatments have been limited and morbidity and mortality remains high. Recent studies have made it abundantly clear that necroptosis is associated with the pathologies such ischemia and ischemic reperfusion injury which is one of the leading causes of acute renal failures. Hypothesis: HK-2 cells will undergo necroptosis Necrostatin 1, a potent inhibitor of necroptosis will inhibit cell death by death by an in-vitro model of ischemic reperfusion injury in human renal proximal tubular HK-2) cells. necroptosis is defined as a caspase independent form of cell death that is inhibited by necrostatin 1. To test our hypothesis we used western blot analysis to test for the protein requirements of RIPK1 (Receptor Interacting Protein Kinase 1) and RIPK3 (Receptor Interacting Protein Kinase 3). The Annexin V assay was used to validate necroptosis, and an in-vitro model requiring the use Antimycin A, a complex II inhibitor of mitochondrial electron transport, 2-deoxyglucose, a non-metabolizable form of L-glucose that depletes glucose, and a calcium ionophore, A23187 that mimics the ischemic phase of renal ischemic reperfusion injury was used to mimic ischemic reperfusion injury in HK-2 cells. The western blot analysis clearly showed that HK-2 cells meet the protein requirements for necroptosis. In the Annexin V assay cell were treated with 0, 0.5μg/mL of TNFα, TNFα and CHX, TNFα, CHX, and ZVAD. Our results from the flow show 98% cell viability in the control, 92% with TNFα, 88% with TNFα and CHX, and 74% with TNFα/CHx/and ZVAD. This clearly show that compared to our control the cell death increased in a dose dependent manner. In the in-vitro model of renal ischemia reperfusion we used three groups of cells, the control, one pretreated with necrostatin 1, and one with no treatment. The cells were treated with 10 μM of Antimycin A, 10mM of 2-deoxyglucose, and 5μM Ca++ Ionophore A23187 for 1 hour. The trepan blue exclusion ass was used and the results showed that the control and the pretreated group showed greater than 95% viability while the untreated group showed less than 10 % viability. er than control and the

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