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An Investigation of the Effects of the Uraemic Solute Indoxyl Sulphate on Paraquat-Induced Oxidant Injury in Rat Renal Epithelial Cells

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INTRODUCTION: Indoxyl sulphate (IS) is considered to be a uraemic toxin which accumulates in the serum of patients with chronic kidney disease (CKD). High serum levels (100 μM to 1 mM) are associated with the progression of CKD and can cause renal injury (Niwa, 2010). Recently, IS at a normal physiological concentration range (0.1 to 10 μM), has been shown to possess antioxidant properties via its ability to scavenge superoxide anions *in vitro* (Miyamoto *et al.*, 2010).

AIMS: The aim of this study was to investigate the effects of IS at concentrations associated with normal physiology and CKD on oxidant injury caused to rat renal proximal tubular cells by paraquat, which generates superoxide anions and thereby causes oxidant injury (Samai *et al.*, 2007).

METHODS: Confluent cultures of NRK-52E cells, a proximal tubular cell-line, were incubated with increasing concentrations of paraquat (0-5 mM) in Dulbecco's Modified Eagle's Medium (DMEM) for 24 hours. Cultures were also pre-incubated with either a high (1 mM) or low (10 μM) concentration of IS for 24 hours followed by 24 hour incubation with a sub-lethal concentration of paraquat (3 mM). Cell viability was assessed via spectrophotometric measurement of the mitochondrial-dependent conversion of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) into formazan.

Data are shown as mean ± S.D. and analysed using one-way ANOVA and a Bonferroni test, N=6-9.

RESULTS: Paraquat produced a significant reduction in the viability of NRK-52E cells at a concentration of 3 mM (Table 1). Pre-incubation with IS at a low concentration (10 μM) for 24 hours did not affect paraquat toxicity (Table 1). However, pre-incubation with a high concentration of IS (1 mM), produced a significant reduction in subsequent paraquat toxicity (Table 1). Incubation of NRK-52E cells with IS only for 24 hours did not have a significant effect on cell viability (data not shown).

Treatment	**Untreated cells (0 mM PQ or IS)**	**PQ only (3 mM)**	**IS (10 μM) then PQ (3 mM)**	**IS (1 mM) then PQ (3 mM)**
Cell viability (% untreated)	100.0 ± 2.0 #	11.8 ± 6.9 ★	15.7 ± 10.8 ★	43.0 ± 11.3 ★#

Table 1: Effects of IS on paraquat (PQ) toxicity. ★P<0.05 *vs.* untreated cells, #P<0.05 *vs.* PQ only

CONCLUSIONS: These results suggest that 24 hour pre-incubation of NRK-52E cells with IS at a high concentration can provide significant protection against oxidant injury. The cellular mechanisms underlying this protection by IS warrant further investigation.

REFERENCES: Miyamoto Y *et al.*, *FEBS Lett.* 584: 2816-2820, 2010. Niwa T. *Nagoya J Med Sci.* 72: 1-11, 2010. Samai M *et al. Free Radic Biol Med.* 43: 528-534, 2007.