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A Comparison of the Effects of Minocycline and 5-Aminoisoquinolinone on Paraquat-induced Oxidant Injury in Renal Epithelial Cells

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INTRODUCTION: Oxidant injury is implicated in the development of acute kidney injury (AKI). During severe oxidative stress, the generation of reactive oxygen species (ROS) leads to the over-activation of the DNA repair enzyme poly(ADP-ribose) polymerase-1 (PARP-1) resulting in ATP depletion and cell death. The tetracycline antibiotic minocycline has been reported to inhibit PARP-1 activation (Alano *et al*., 2006) and can protect against oxidant injury to the kidney (Xia *et al*., 2011).

AIM: The aim of this study was to investigate and compare the effect of minocycline and 5-aminoisoquinolinone (5-AIQ), the latter being an established PARP-1 inhibitor which has been shown to protect the kidney *in vitro* and *in vivo* (Chatterjee *et al*., 2004), on oxidant injury caused by paraquat, a potent pro-oxidant which causes ROS-induced renal cell death (Samai *et al*., 2007).

METHODS: Confluent cultures of NRK-52E cells, a rat proximal tubular cell-line obtained from the Health Protection Agency Culture Collections, were incubated with increasing concentrations of paraquat (0-5 mM) in Dulbecco's Modified Eagle's Medium (DMEM) for 24 hours. Cultures were also incubated with paraquat in the presence of high and low concentrations of minocycline (10 μM and 100 nM) and 5-AIQ (100 μM) for 24 hours. Cell viability was then 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 presented as mean % cell viability±S.D. analysed using one-way ANOVA followed by Bonferroni's post-testing. All drugs (paraquat, minocycline, 5-AIQ), DMEM and MTT were obtained from Sigma-Aldrich.

RESULTS: Paraquat (PQ) produced a significant reduction in the viability of NRK-52E cells at a concentration of 3 mM (untreated cells: 100.0±1.7% *vs*. PQ only: 38.0±7.4%, $p<0.05$, n=12). Minocycline (MC) produced a significant reduction in paraquat toxicity both at a high concentration of 10 μM (PQ only: 38.0±7.4% *vs*. PQ+MC: 49.0±6.0%, $p<0.05$, n=12) and at a lower concentration of 100 nM (PQ only: 38.0±7.4% *vs*. PQ+MC: 50.2±11.5%, $p<0.05$, n=8-12). 5-AIQ was also able to produce a significant reduction of paraquat toxicity at a concentration of 100 μM (PQ only: 38.0±7.4% *vs*. PQ+5-AIQ: 73.9±8.9%, $p<0.05$, n=12). Minocycline or 5-AIQ alone did not have any effect on NRK-52E cell viability at the concentrations tested against paraquat (data not shown).

CONCLUSIONS: These results suggest that minocycline is able to reduce paraquat toxicity significantly at nM concentrations. The PARP-1 inhibitor 5-AIQ was also able to protect against paraquat toxicity but at a much higher (μM) concentration. Minocycline may also be able to provide protection via its ability to inhibit endoplasmic reticulum stress which has recently been proposed as a mechanism of paraquat-induced cell death (Huang *et al*., 2012). This potential mechanism of protection warrants further investigation in renal cells.

References:

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