A Comparison of the Effects of Minocycline and 5-Aminoisoquinolinone on Gentamicin-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 is able to nephroprotect against oxidant injury (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 gentamicin, an aminoglycoside antibiotic known to have oxidant-induced nephrotoxic effects.

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 gentamicin (0-12mg/mL) in Dulbecco's Modified Eagle's Medium (DMEM) for 72 hours. Cultures were also incubated with gentamicin in the presence of minocycline (10µM and 100nM) and 5-AIQ (100µM) for 72 hours. 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 presented as mean % cell viability±S.D. and analysed using one-way ANOVA followed by Bonferroni's post-hoc testing. All drugs (gentamicin, minocycline, 5-AIQ), DMEM and MTT were obtained from Sigma-Aldrich.

RESULTS: Gentamicin (Gm) produced a significant reduction in the viability of NRK-52E cells at a concentration of 8mg/mL (untreated cells: 100.0±2.6% *vs*. Gm only: 51.8±12.0%, $p<0.05$, n=12). Minocycline (MC) produced a significant reduction in gentamicin toxicity at the high concentration of 10µM (Gm only: 51.8±12.0% *vs*. Gm+MC: 72.1±9.2%, $p<0.05$, n=10), but no significant difference was observed at the lower concentration of 100nM (Gm only: 51.8±12.0% *vs*. Gm+MC: 42.4±13.3%, $p>0.05$, n=10). 5-AIQ also produced a significant reduction of gentamicin toxicity at a concentration of 100µM (Gm only: 51.8±12.0% *vs*. Gm+5-AIQ: 83.3±6.7%, $p<0.05$, n=12). Minocycline or 5-AIQ alone did not have any effect on NRK-52E viability at these tested concentrations (data not shown).

CONCLUSIONS: These results suggest that minocycline and 5-AIQ are able to reduce gentamicin toxicity significantly at µM concentrations, but nM concentrations of minocycline could not exhibit protection. The protective effects of minocycline at µM concentrations may be in part due to its recently proposed ability to inhibit endoplasmic reticulum stress (Huang *et al*., 2012) – an identified mechanism of genatmicin-induced cell death. This potential mechanism of protection from minocycline warrants further investigation in renal cells.

References:

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