OXIDATIVE STRESS AND APOPTOSIS IN INTRINSIC RENAL CELL POPULATIONS - AN IN VITRO STUDY

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We have been studying the interaction between incidence of apoptosis and expression of selectd oncogenes and cytokines in an *in vivo* rat model of ischaemia-reperfusion injury. The ischaemia itself, and the reperfusion, induce oxidative damage to the tissues, including damage from oxygen-derived free radicals. The scenario is therefore similar to radiation-induced injury. In the *in vivo* studies, we often observed increased expression of bcl-2, a cell survival or anti-apoptosis gene, in the distal segment of the nephron. If this occurred, not only was cell viability maintained in the distal segment of the nephron, with only very little apoptosis and no necrosis observed, but the proximal nephron segments in the vicinity of the protected distal nephron were also protected. The proximal nephron segments, especially the pars recta, are usually acutely sensitive to ischaemia-reperfusion injury, undergoing necrosis in preference to apoptosis. We have formed the hypothesis that Bcl-2 protection of the distal nephron, a segment of the nephron known as a reservoir for many growth factors or cytokines, allows increased production of growth factors during oxidative stress, which then act in a paracrine manner to protect the nearby proximal tubule.

To test this hypothesis, we have commenced using an *in vitro* model of oxidative stress on either distal (Madin Derby Canine Kidney, MDCK) or proximal (human kidney-2, HK-2) established renal cell lines. We grow the cells as "coverslip cultures" in 12-well plates in Dulbecco's Modified Eagle's Medium or serum free medium. The treatments we have used to date are either hydrogen peroxide (a gradation of concentrations from 1mM to 50mM), tumour necrosis factor-alpha (TNF-alpha) or hypoxia, as inducers of oxidative stress. The parameters analysed in the present study were (i) cell death (apoptosis or necrosis, using histology, *in situ* end labelling, and electron microscopy) (ii) cell proliferation (morphology and proliferating cell nuclear antigen (PCNA)) and (iii) Bcl-2 expression (immunohistochemistry). We have found all treatments increase levels of apoptosis in both cell lines, and TNF-alpha also causes increased cell proliferation. At the higher concentrations of hydrogen peroxide, however, the HK-2 (proximal) cells have more of a tendency to undergo necrosis than do the MDCK (distal) cells, mimicking the *in vivo* situation. Bcl-2 expression is low in both cell lines, and does not appear to be affected by the treatments in this model, and this is a divergence from the *in vivo* results.

Although results are preliminary, we have believe the model will prove to be relevant to the *in vivo* situation, and useful for analysing growth factor production from either cell line after stress, and/or improvement in pathology after oxidative stress by addition of relevant growth factors.