

P014 The role of oxidative and endoplasmic reticulum stresses in the development of diabetic nephropathy

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High glucose concentrations, mimicking effects of hyperglycaemia, have been reported to cause oxidative stress in a variety of cells including kidney mesangial cells. We are investigating glucotoxicity *in vitro* in NRK-52E proximal tubular cells and whether endoplasmic reticulum (ER) stress is induced. Cytotoxicity assays were based on MTT reduction, LDH release or Hoechst-propidium iodide (HPI) staining; production of reactive oxygen species (ROS) was evaluated using nitroblue tetrazolium salt (NBT) reduction into blue formazan. ER stress was evaluated by Western blotting against the ER stress markers CHOP, GRP78 and GRP94. High glucose was toxic to NRK-52E cells in a dose-dependent and time-dependent manner. Cell death was by both necrosis and apoptosis as determined by HPI staining. Oxidative stress due to 72h treatment with 25mmol/l glucose lead to increase of NBT reduction. The antioxidants EUK-134 and tempol inhibited NBT reduction due to high glucose by 30% and 60% respectively ($P < 0.01$). Treatment with high glucose (25-35mmol/l) significantly increased GRP94 and CHOP, which was increased at 1, 3 and 7 days. We propose that rat kidney proximal tubular cells are sensitive to increasing glucose concentrations and undergo cell death mediated by both oxidative and ER stresses.