Analysis of Different Nrf2 Inducers on Reversal of Stress-induced Changes in Renal Tubule Cells

Wadhwa, Shruti

Oxidative stress is present during kidney disease. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor which combats oxidative stress and compounds inducing Nrf2 reduce kidney injury in experimental animals. Preliminary observations in this study showed different morphology of cultured kidney tubule cells when treated with two different Nrf2 inducers. This study addressed the hypothesis that different Nrf2 inducers reverse stress-induced changes in renal tubule cells by different mechanisms. Cultured human proximal tubule cells (HK11 cells) were treated with five different concentrations of Nrf2 inducers Dimethyl Fumarate (DMF) or Protandim (made up of milk thistle, Bacopa, Ashwagandha, green tea, and turmeric), or respective diluent controls. Cell viability was analyzed by reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and Trypan Blue dye exclusion. Cellular extract of DMF or Protandim treated HK11 cells were analyzed by Western blotting for protein expression of Nrf2 and its transcriptional targets NQO1 (NADPH dehydrogenase quinone 1) and SOD1 (superoxide dismustase). MTT reduction was decreased at 40uM DMF and 40ug/ml Protandim and Trypan blue positive cells increased with higher concentrations of each compound. Combined the results show decreased viability with higher concentrations of both compounds. DMF caused more cells to round up, while Protandim treated cells were smaller. Treatment of cells with both inducers led to increased expression of NQO1 whereas expression of SOD1 was induced more by DMF. The results suggest that differential induction of Nrf2 transcriptional targets and cellular morphology by DMF and Protandim may result in different tubule cell responses to stress stimuli.