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Reducing urate oxidase mRNA alters the oxidative stress response in a mouse hepatic cell line

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Abstract

Humans, birds, and higher primates do not express the uric acid degrading enzyme urate oxidase and, as a result, have plasma uric acid concentrations higher than urate oxidase expressing animals. Although high uric acid concentrations are suggested to increase the antioxidant defense system and provide a health advantage to animals without urate oxidase, knockout mice lacking urate oxidase develop pathological complications including gout and kidney failure. As an alternative to the knockout model, RNA interference was used to decrease urate oxidase expression in a mouse hepatic cell line (ATCC, FL83B). Cells were antibiotic selected for stable expression of a transfected plasmid that expresses a short hairpin RNA for urate oxidase knockdown. Urate oxidase mRNA was reduced 90% compared to wild type, as measured by real time RT-PCR. To determine if urate oxidase knockdown resulted in enhanced protection against oxidative stress, cells were challenged with hexavalent chromium (Cr(VI)), which generates an increased production of reactive oxygen species. Hydroxyl radicals, and possibly decomposed superoxide radicals, were quantified using electron spin resonance (ESR). Cells with urate oxidase knockdown demonstrated a 37.2 ± 3.5% reduction ($p<0.05$) in the ESR signal compared to wild type cells, consistent with a lower exposure to free radicals and decreased oxidative stress.

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