Evidence for cyclosporin A-induced free radical production in the rat kidney

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One major side effect of the immunosuppressive agent, cyclosporin A (CsA), is severe nephrotoxicity. It was reported that CsA causes vasoconstriction. Accordingly, these experiments were designed to investigate the role of hypoxia and free radical production in kidney injury caused by CsA. Rats were treated daily with CsA (25mg/kg, i.g.) for 5 day, and pimonidazole, a hypoxia marker, was injected 2 hours after the last dose of CsA. Spin trap, 4-POBN (α-(4-pyridyl 1-oxide)-*N*-*tert*-butylnitrone), was injected 3 hours after CsA to trap free radicals. CsA doubled serum creatinine and decreased glomerular filtration rates by 65%. Pimonidazole adduct binding in the kidney was increased nearly 3-fold by CsA, providing physical evidence for tissue hypoxia. Moreover, CsA increased 4-POBN/radical adducts 5-fold in the urine but did not alter levels in the serum. Dimethyl sulfoxide (DMSO) attacked by the hydroxyl radical releases a methyl radical; administration of CsA with ^{12}C-DSMO followed by 4-POBN produced two free radical species in urine, one with hyperfine coupling constants similar to the 4-POBN/methyl radical adduct. CsA given with ^{13}C-DSMO produced a 12-line spectrum, confirming the formation of hydroxyl radicals. The other free radical adduct found in urine had coupling constants similar to lipid derived 4-POBN radical adducts (a^N=15.6 G, a_β^H=2.5 G) but partitioned primarily into the aqueous phase in organic extractions of urine. In contrast, 4-POBN/methyl radical adducts partitioned primarily into the organic phase, leading to the conclusion that the unknown radical adduct was either highly polar or charged. Taken together, these data are consistent with the hypothesis that CsA-induced hypoxia results in increased hydroxyl radical formation in the kidney and subsequent tissue injury.