Formation of Free Radicals in Rat Erythrocytes Exposed to 6-Methoxy-8-Hydrolylaminoquinoline, A Model of Primaquine-Induced Hemolytic Anemia

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Electron Paramagnetic Resonance (EPR) spectroscopy was used to study the effects of the 6-methoxy-8-hydroxylamino-quinoline (MAQ-NOH) on rat erythrocytes. MAQ-NOH is a N-hydroxy metabolite of primaquine that has been shown recently to be a direct-acting hemolytic agent in rat red cells [1] and that the hemolytic activity of this metabolite is associated with GSH oxidation and oxidative damage to both membrane lipids and skeletal proteins [2]. To determine whether the formation of free radicals could be involved in this process, rat red cells (40% suspensions) were incubated with hemolytic concentrations of MAQ-NOH (150-750 µM) in the presence of the radical spin trap, 2-ethoxycarbonyl-2-methyl-3,4-dihydro-2H-pyrrole-1-oxide (EMPO). Addition of MAQ-NOH to red cell suspensions gave rise to concentration dependent multiline EPR spectra having hyperfine coupling constants consistent with those of an EMPO-hydroxyl radical adduct. Formation of EMPO-OH was constant for up to 20 min and dependent on the presence of erythrocytic glutathione. Although no other radical adduct signals were definitively detected in the cells by EPR spectroscopy, spectrophotometric analysis revealed the presence of ferryl hemoglobin, which indicates hydrogen peroxide is generated under these experimental conditions. These data support the hypothesis that reactive oxygen species, giving rise to oxygen and possibly other free radicals, are involved in the mechanism underlying MAQ-NOH-induced hemolytic anemia.

[1] Bolchoz LJ, Budinsky RA, McMillan DC and Jollow DJ *J.Pharmacol.Exp.Ther.*, 297,509-515, 2001.

[2] Bolchoz LJ, Morrow JD, McMillan DC and Jollow DJ *J.Pharmacol.Exp.Ther.*, in press.