

HEPATIC BIOTRANSFORMATION OF THE ANTICANCER AGENT NEMORUBICIN TO PNU-159682: AN INTERSPECIES COMPARISON

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Background and aims: Nemorubicin (methoxymorpholinyl doxorubicin; MMDX), a doxorubicin derivative currently undergoing Phase I/II clinical trials for the intrahepatic artery chemotherapy of hepatocellular carcinoma is converted by human liver cytochrome P450 (CYP) 3A4 to a more cytotoxic species, PNU-159682. Preclinical toxicology studies have revealed that male and female dogs and female rats are considerably less susceptible to drug-induced toxicity than male rats and mice of both sexes. As species- and sex-related differences in biological responses to several xenobiotics can be connected to those in hepatic expression of certain CYP forms, we investigated and compared the biotransformation of MMDX to PNU-159682 by liver microsomes from untreated mice, rats, dogs and humans of both sexes. Additional experiments were conducted using liver microsomes from various CYP inducer-treated animals to obtain information on the CYP enzyme(s) responsible for PNU-159682 formation in the investigated laboratory animal species.

Methods: MMDX was incubated with liver microsomes and NADPH for an appropriate time at 37°C, and supernatant analyzed for PNU-159682 content by HPLC with UV detection. In inhibition experiments, incubation mixtures included a CYP3A chemical inhibitor (troleandomycin or ketoconazole) or an anti-rat CYP3A1 polyclonal antibody. Estimates of kinetic parameters (K_m and V_{max}) were obtained by non-linear curve fitting.

Results and conclusions: In all species, PNU-159682 was the major liver microsomal metabolite of MMDX, and its formation was consistent with Michaelis-Menten kinetics for a single enzyme. Quantitative sexual differences in MMDX bioactivation were observed in the rat, with the male having a metabolic activity (V_{max}) about 10-fold greater than female, and similar to that observed in human liver microsomes. When expressed as intrinsic clearance (V_{max}/K_m) the catalytic efficiency of animal liver microsomes (male rat \approx mouse \gg female rat \approx dog) was found to correlate inversely with literature data of i.v. MMDX LD₅₀ values. Liver microsomes from rats, mice or dogs treated with prototypical inducers of CYP3A enzymes catalyzed PNU-159682 formation at a higher rate than corresponding control microsomes. Moreover, in all species examined, ketoconazole, troleandomycin and an anti-CYP3A1 polyclonal antibody, concentration-dependently inhibited PNU-159682 formation. These results suggest that in all the investigated laboratory animal species, PNU-159682 is formed mainly (if not exclusively) via a CYP3A enzyme, and mediates the *in vivo* activity of MMDX.