PTK 0796 (omadacycline) is a novel macrodilide now in Phase 3 clinical development. In an in vitro assessment of the potential for metabolism and/or drug-drug interactions, was undertaken.

**Objectives.** The in vitro stability and interaction of PTK with human cytochrome P450 isoforms were determined to assess the potential for in vivo or modified or a combination of both co-factors. Factors of 14C-PTK to liver microsomes was determined by ultrafiltration. The metabolism of 14C-PTK by human hepatocytes (Celsis, Baltimore, MD) was tested at 2.5 µM and 12.5 µM with 2 x 10^6 cells/ml at 37°C for 2-24 hrs. PTK and metabolites were detected by HPLC with radio-detection. CYP450 induction was determined in primary human hepatocytes (1 x 10^6 cells) incubated with 1-100 µM PTK 0796 and substrate probe for 24 and 48 hrs. Inhibition of CYP450 isoforms was determined using pooled human microsomes (BD Biosciences, Bedford, MA) with PTK (1-50 µM) and probe concentrations approximating the Km of each probe. Time-dependent inhibition was determined by preincubating microsomes with 1-10 µM PTK. Probe metabolism was determined by LC-MS.

**Results.** There was no detectable metabolism of omadacycline by human microsomes, hepatocytes, S9 cytosol, or recombinant flavin monooxygenases (FMO1, FMO3, FMO5). Metabolism of 14C-PTK (50 µM) was determined with either NADPH or UDPGA or a combination of both co-factors. The non-specific binding of [14C]PTK 0796 to liver microsomes was determined by ultracentrifugation. The metabolism of [14C]PTK by human hepatocytes (Celsis, Baltimore, MD) was tested at 2.5 µM and 12.5 µM with 2 x 10^6 cells/ml at 37°C for 2-24 hrs. 14C-[PTK 0796(1-50 µM)] and substrate probes were detected by HPLC with radio-detection. CYP450 induction was determined in primary human hepatocytes (1 x 10^6 cells) incubated with 1-100 µM PTK 0796 and substrate probe for 24 and 48 hrs. Inhibition of CYP450 enzymes was determined using pooled human microsomes (BD Biosciences, Bedford, MA) with PTK (0.1-100 µM) and probe concentrations approximating the Km of each probe. Time-dependent inhibition was determined by preincubating microsomes with 1-100 µM PTK 0796 up to 20 minutes prior to assay. Probe metabolism was determined by LC-MS.

**Conclusions.** PTK 0796 does not exhibit significant induction of CYP450 activity in human hepatocytes as shown in Table 5. Change of enzyme activity in cultured primary human hepatocytes.

**METHODS.** Metabolism assays were conducted using either pooled human liver microsomes, S9, liver cytosol, or recombinant flavin monooxygenases (FMO1, FMO3, FMO5). The metabolism of [14C]PTK 0796 (50 µM) was determined with either NADPH or UDPGA or a combination of both co-factors. The non-specific binding of [14C]PTK 0796 to liver microsomes was determined by ultracentrifugation. The metabolism of [14C]PTK 0796 by human hepatocytes (Celsis, Baltimore, MD) was tested at 2.5 µM and 12.5 µM with 2 x 10^6 cells/ml at 37°C for 2-24 hrs. The metabolism of [14C]PTK 0796 by human hepatocytes (1 x 10^5 cells) incubated with 1-100 µM PTK and substrate probe for 24 and 48 hrs. Inhibition of CYP450 isoforms was determined using pooled human microsomes (BD Biosciences, Bedford, MA) with PTK (1-50 µM) and probe concentrations approximating the Km of each probe. Time-dependent inhibition was determined by preincubating microsomes with 1-10 µM PTK. Probe metabolism was determined by LC-MS.

**RESULTS.**

**Table 6.** Induction of CYP450 mRNA by real-time PCR, from cultured primary human hepatocytes.

**Table 7.** Change of enzyme activity in cultured primary human hepatocytes.

**Table 8.** Inhibition of CYP450 mRNA by real-time PCR, from cultured primary human hepatocytes.

**Table 9.** Change of enzyme activity in cultured primary human hepatocytes.

**RESULTS.**

**Table 1.** Recovery of PTK 0796 after 30 minute incubation with Human Liver Microsomes

**Table 2.** Stability of PTK 0796 in the presence of Human Hepatocytes

**Table 3.** Lack of inhibition of cytochrome P450 isozyme activity by PTK 0796

**Table 4.** Absence of time dependent inhibition by PTK 0796

**Table 5.** Change of enzyme activity in cultured primary human hepatocytes.

**RESULTS.**

**Table 1.** Recovery of PTK 0796 after 30 minute incubation with Human Liver Microsomes

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