

ATTACHMENT C.3
INDUCTION STUDY: RESULTS

Results

Test System

CYP1A2 activity in cryopreserved human hepatocytes was quantified by measuring the formation of acetaminophen from phenacetin. Following treatment with 50 μ M omeprazole, a known inducer for CYP1A2, CYP1A2 activity was 2,027%, 854%, and 2,276% of the vehicle control (VC, 1% methanol) in human hepatocytes prepared from Donors 1, 2, and 3, respectively (Table 1). CYP3A4 activity in cryopreserved human hepatocytes was quantified by measuring the formation of 6 β -hydroxytestosterone from testosterone. Following treatment with 25 μ M rifampin, a known inducer for CYP3A4, CYP3A4 activity was >1,153%, >766%, and >1,511% of the VC in human hepatocytes prepared from Donors 1, 2, and 3, respectively (Table 2). The increase in activities of CYP1A2 and CYP3A4 following treatment with the known inducers met the criteria set in the protocol; therefore, the hepatocytes from these donors were considered inducible.

CYP1A2

Metaxalone at the tested concentration of 40 μ M induced CYP1A2 activity in human hepatocytes prepared from Donors 1, 2, and 3. This conclusion was based on CYP1A2 activity (110, 131, and 332% of the VC in donor 1; 108, 113, 197% of the VC in donor 2; and 107, 108, and 297% of the VC in Donor 3) in hepatocytes treated with 0.4, 4, and 40 μ M metaxalone (Table 3). The apparent increase of CYP1A2 activity by metaxalone at the concentrations of 0.4 and 4 μ M was not statistically significant ($p > 0.05$; unpaired two-tailed *t* test). The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP2A6

CYP2A6 activity in cryopreserved human hepatocytes was quantified by adding coumarin to the hepatocytes and measuring the formation of 7-hydroxycoumarin and its conjugated derivatives, 7-hydroxycoumarin glucuronide and 7-hydroxycoumarin sulfate. CYP2A6 activity in the VCs from Donors 1, 2, and 3 was below the lower limit of quantitation (Tables 4a-d). Following treatment with metaxalone, CYP2A6 activity was still below the lower limit of quantitation. Therefore, metaxalone at the concentrations tested did not induce CYP2A6 activity in human hepatocytes isolated from these donors. The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP2B6

CYP2B6 activity in cryopreserved human hepatocytes was quantified by adding *S*-mephenytoin to the hepatocytes and measuring the formation of its metabolite, nirvanol. Metaxalone at the tested concentrations did not induce CYP2B6 activity in human hepatocytes prepared from Donors 1, 2, and 3. This conclusion was based on CYP2B6 activity (107, 95.7, and 97.1% of the VC in donor 1; 106, 97.0, <86.5% of the VC in donor 2; and 99.1, 99.1, and 99.1% of the VC in Donor 3) in hepatocytes treated with 0.4, 4, and 40 μ M metaxalone (Table 5a). On the other hand, phenobarbital at the concentration of 1 mM induced CYP2B6 activity (335, 159, and >307% of the VC in hepatocytes from Donors 1, 2, and 3, respectively) (Table 5b). The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP2C9

CYP2C9 activity in cryopreserved human hepatocytes was quantified by adding tolbutamide to the hepatocytes and measuring the formation of its metabolite, 4'-methylhydroxytolbutamide. Metaxalone at the concentrations tested did not induce CYP2C9 activity in human hepatocytes isolated from Donors 1, 2, and 3. This conclusion was based on CYP2C9 activity (108, 100, and <73.2% of the VC in Donor 1; 96.6, 92.9, and 60.4% of the VC in Donor 2; and 111, 103, and <78.4% of the VC in Donor 3) in hepatocytes treated with 0.4, 4, and 40 μ M metaxalone (Table 7a). The apparent increase of CYP2C9 activity in Donors 1 and 3 following treatment with metaxalone was not statistically significant ($p>0.05$; unpaired two-tailed t test). On the other hand, rifampin at the concentration of 25 μ M induced CYP2C9 activity (213, 124, and 201% of the VC in hepatocytes from Donors 1, 2, and 3, respectively) (Table 7b). The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP2C19

CYP2C19 activity in cryopreserved human hepatocytes was quantified by adding *S*-mephenytoin to the hepatocytes and measuring the formation of its metabolite, 4'-hydroxymephenytoin. CYP2C19 activity levels in hepatocytes isolated from all three donors were below the lower limit of quantitation. Metaxalone at the concentrations tested did not induce CYP2C19 activity in human hepatocytes isolated from these donors since CYP2C19 activity in hepatocytes treated with 0.4, 4, and 40 μ M metaxalone was undetectable or below the lower limit of quantitation (Table 8a). Rifampin at the concentration of 25 μ M did not induce CYP2C19 activity in all donors either (Table 8b). The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP2D6

CYP2D6 activity in cryopreserved human hepatocytes was quantified by adding dextromethorphan to the hepatocytes and measuring the formation of its metabolite, dextrorphan. CYP2D6 activity was below the lower limit of quantitation in the VC from Donor 1. Metaxalone at the concentrations tested did not induce CYP2D6 activity since the activity following treatment with metaxalone was also below the lower limit of quantitation (Table 9). Metaxalone at the concentrations tested did not induce CYP2D6 activity in human hepatocytes isolated from Donors 2 and 3. This conclusion was based on CYP2D6 activity (95.9, 103, and <73.2% of the VC in Donor 2; and 98.8, 89.8, and 73.5% of the VC in Donor 3) in hepatocytes treated with 0.4, 4, and 40 μ M metaxalone (Table 9). The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP2E1

CYP2E1 activity in cryopreserved human hepatocytes was quantified by adding chlorzoxazone to the hepatocytes and measuring the formation of its metabolite, 6-hydroxychlorzoxazone. Metaxalone at the concentrations tested did not induce CYP2E1 activity in human hepatocytes isolated from all three donors. This conclusion was based on CYP2E1 activity (97.8, 104, and 104% of the VC from Donor 1; 104, 89.6, 99.6% of the VC from Donor 2; and 90.7, 87.7, and 93.9% of the VC from Donor 3) in hepatocytes treated with 0.4, 4, and 40 μ M metaxalone (Table 10). The apparent increase of CYP2E1 activity in Donors 1 and 2 following treatment with

metaxalone was not statistically significant ($p > 0.05$; unpaired two-tailed t test). The assay method detected no chromatographic interference from metaxalone or its metabolite.

CYP3A4

CYP3A4 activity in the VC from Donor 1 was below the lower limit of quantitation. Metaxalone at the concentrations tested did not induce CYP3A4 activity since the activity following treatment with metaxalone was still below the lower limit of quantitation (Table 11). Metaxalone at the tested concentration of 40 μM induced CYP3A4 activity in human hepatocytes prepared from Donor 2. The conclusion was based on CYP3A4 activity (>115 , >98.7 , and $>121\%$ of the VC) in hepatocytes treated with 0.4, 4, and 40 μM metaxalone (Table 11). The increase of CYP3A4 activity following treatment with metaxalone at the concentration of 0.4 μM was not statistically significant ($p > 0.05$; unpaired two-tailed t test). CYP3A4 activity in the VC from Donor 3 was below the lower limit of quantitation. Metaxalone at the tested concentrations did not induce CYP3A4 activity since the activity following treatment with metaxalone was still below the lower limit of quantitation (except in one of three replicated for the concentration of 40 μM). The assay method detected no chromatographic interference from metaxalone or its metabolite.

Conclusions

Metaxalone at the tested concentration did not induce the activities of CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP2E1. Metaxalone at the concentrations of 0.4 and 4 μM did not induce CYP1A2 activity. However, metaxalone at the concentration of 40 μM induced CYP1A2 activity in all three donors. The induction of CYP1A2 activity ranged from about 2- to 3.3- folds. Metaxalone at the concentration of 40 μM slight induced (about 21%) CYP3A4 activity in one of three donors tested. At the lower concentrations (0.4 and 4 μM), metaxalone did not induce CYP3A4 activity in all three donors.