Accelerated Communication

Effect of Dimethyl Sulfoxide on In Vitro Cytochrome P4501A2 Mediated Phenacetin O-Deethylation in Human Liver Microsomes

Ramakrishna Nirogi, Vishwottam Kandikere, Gopinadh Bhyrapuneni, Ranjith Kumar Ponnamaneni, Raghava Choudary Palacharla, and Arunkumar Manoharan

Pharmacokinetics and Drug Metabolism, Discovery Research, Suven Life Sciences Limited, Hyderabad, India

ABSTRACT:
In this study, we report the effect of dimethyl sulfoxide (DMSO), acetonitrile, and methanol on the CYP1A2-mediated metabolism of phenacetin in human liver microsomes. Phenacetin O-deethylation is the preferred probe reaction for CYP1A2, in which the metabolite, acetaminophen, is quantified using liquid chromatography-tandem mass spectrometry. DMSO was found to inhibit CYP1A2-mediated phenacetin O-deethylation even at low concentrations (0.1%). Acetonitrile did not significantly change the phenacetin O-deethylation activity at concentrations up to 2%. There was no effect on the phenacetin O-deethylation when methanol was present at levels up to 2%. We found that the DMSO level should be kept lower than 0.05% because a concentration of 0.1% strongly affected the metabolism of phenacetin. These findings should be taken into consideration when designing in vitro metabolism studies, especially studies in which metabolism of the investigational compound needs to be evaluated, which would confound the results. The findings from this study indicate that methanol is the suitable solvent and has no significant effects on CYP1A2-mediated phenacetin O-deethylation.

Introduction
Cytochrome P450 (P450) enzymes play an important role in the metabolism of xenobiotics. The effects of new drugs on probe reactions specific for human drug-metabolizing enzymes are examined using in vitro approaches, and these approaches use human liver microsomes (HLM) routinely. CYP1A2 is one of the major metabolizing enzymes expressed in liver. CYP1A2 constitutes approximately 15% of the total microsomal P450 content in human liver (Lee et al., 2003).

Phenacetin O-deethylation and caffeine N3-demethylation are the two recommended probe reactions for detecting CYP1A2-based drug interaction potential in vitro. However, phenacetin is preferred over caffeine because caffeine N3-demethylation is sensitive to solvent effects (Yuan et al., 2002). Substrates and inhibitors that are used in metabolism studies need to be dissolved in organic solvents because of the solubility problem with physiological buffers.

Organic solvents can inhibit or stimulate the activity of P450 enzymes involved in the biotransformation of xenobiotics. It is always necessary to evaluate the potential effects of organic solvents for in vitro metabolism studies, especially when a new chemical entity is being evaluated. Numerous reports were published on the impact of organic solvents on phenacetin O-deethylation activities in HLM (Chauvet et al., 1998; Hickman et al., 1998; Busby et al., 1999). Chauvet et al., 1998 found that phenacetin O-deethylation is not sensitive to dimethyl sulfoxide (DMSO), acetonitrile, or methanol. Hickman et al. (1998) found that phenacetin O-deethylation is sensitive to methanol and DMSO at a concentration of 1%.

In this study, we report new findings on the inhibitory effects of DMSO, acetonitrile, and methanol on the hepatic activity of phenacetin O-deethylation mediated by CYP1A2 in HLM.

Materials and Methods
Materials. Human liver microsomes were obtained from XenoTech, LLC (Lenexa, KS). NADPH, acetaminophen, phenacetin, and 4-OH-butyranilide were purchased from Sigma-Aldrich (St. Louis, MO). All other reagents were of analytical grade or the highest purity commercially available.

Effect of Organic Solvents on Phenacetin O-Deethylation Assay. All incubations were performed under conditions shown to be linear with respect to time, protein, and substrate concentration. Phenacetin stock solution was prepared in water. Incubations were performed with 0.025 mg/ml microsomal protein in phosphate buffer (100 mM, pH 7.4) containing 50 μM phenacetin in a final volume of 0.2 ml. All incubations were performed in triplicates.

An appropriate volume of organic solvent (DMSO, acetonitrile, and methanol) was added to achieve the desired percentage (0.2, 0.5, 1, and 2%) of organic content, and control incubations contained no organic solvent. An aliquot (0.18 ml) of the incubation mixture was transferred to a reaction plate. Reactions were commenced with the addition of NADPH (1 mM) to final
incubation volume of 0.2 ml and maintained at 37°C for 18 min. The reaction mixtures were quenched with 1% formic acid: acetonitrile (70:30 v/v) containing 4-OH-butyranilide (1.08 μM) as internal standard (IS). The plates were then centrifuged at 4000g for 10 min at 4°C. An aliquot of the supernatant fraction was mixed with 0.1% formic acid in water, and an aliquot of the mixture was analyzed by liquid chromatography-tandem mass spectrometry.

**Liquid Chromatography-Tandem Mass Spectrometry Analysis.** The high-performance liquid chromatography system (Shimadzu Corporation, Kyoto, Japan) was equipped with two LC-20AD prominence pumps, a SIL-HTc auto sampler unit, CTO-10AS VP thermo stated column oven, and DGU20A3 degasser unit. Analysis was carried out with API-4000 Qtrap mass spectrometer (Applied Biosystems MDS Sciex, Toronto, Canada) coupled with turbo-spray ionization source. The chromatography was performed using symmetry C8, 3.5 μm, 4.6 × 100 mm ID column. Acetaminophen and IS (4-OH-butyranilide) were eluted with a mobile phase system consisting of water/acetonitrile/formic acid (80:20:0.1% v/v) and 20:80:0.1% v/v for mobile phase A and B, respectively. A constant flow of 0.750 ml/min was used with a gradient mobile phase system. Mobile phase B 10% was pumped for 0.8 min, and then increased to 90% in 2 min. Up to 3-min mobile phase B was pumped constantly and then reversed back to 10% after 3.5 min to restabilize the column for up to 4.5 min. During the run, column oven temperature was maintained at 40°C.

Analysis was performed on mass spectrometer in positive multiple reaction-monitoring mode with a dwell time of 150 ms for each transition. The mass transitions for acetaminophen and IS were as follows: m/z 152.1 → m/z 110.1 and m/z 180.2 → m/z 71.1. Declustering potential and collision energy values were set at 70 and 24 V for metabolite and at 60 and 27 V for IS. Typical source conditions were as follows: the curtain gas was set at 25, ion spray needle voltage was 5500 V, the turbo-gas temperature was set at 400°C, and nebulizer gas as well as turbo gas were set at 35 and 40 (arbitrary units). Data were collected and processed using Sciex Analyst 1.4.2 software (MDS Sciex, Concord, ON, Canada).

**Data Analysis.** Results are obtained from three different incubations and are represented as a mean ± S.D., with the exception of DMSO, the concentrations of which are ≈0.2% where results are obtained from six determinations (triplicates from two different experiments). The extent of inhibition in the activity is the ratio of the activity in the incubations containing organic solvent over the average activity in the controls containing no organic solvent. Data are expressed as percentage of control incubations.

**Results and Discussion**

The results of the effect of DMSO, acetonitrile, and methanol on phenacetin O-deethylation activity are summarized in Fig. 1. Phenacetin-O-deethylase activity is not affected by methanol at concentrations ≤2%, as was also observed by Chauret et al. (1998) and Busby et al. (1999) with HLM. This result is in contrast to the findings of Hickman et al. (1998), who found strong inhibition by methanol of CYP1A2 activity using caffeine as a substrate in liver microsomes. We found that acetoni-trile induced the activity of phenacetin O-deethylation at concentrations ≥0.5%. The inhibition or inducer effects of acetoni-trile on phenacetin O-deethylation are in line with the findings of Chauret et al. (1998), Busby et al. (1999), and Hickman et al. (1998).

We observed strong inhibition effects of DMSO at concentrations ≥0.1% (Fig. 2). Initially, DMSO was tested at concentrations ranging from 0.2 to 2%. We also observed that at concentrations of 0.2%, DMSO showed inhibitory effects on phenacetin O-deethylation. In another experiment, to determine the minimum percentage of DMSO that will not affect the phenacetin O-deethylation, DMSO was tested as low as 0.05%. We observed that at these levels, DMSO had no inhibitory effects (8% increase, compared with control incubations). This result is in contrast to the findings of Chauret et al. (1998) and Busby et al. (1999) and are in line with the findings of Hickman et al. (1998), showing CYP1A2 activity inhibition by DMSO (1%) when caffeine N3-demethylation was used as probe reaction. The difference in observations on the inhibitory effects of DMSO and methanol between different laboratories is not clear. The difference observed in inhibitory effects of methanol on CYP1A2 activity may be attributed to the different substrate used, caffeine by Hickman et al. (1998), who found strong inhibition by methanol of CYP1A2 activity using caffeine in liver microsomes. We found that acetoni-trile induced the activity of phenacetin O-deethylation at concentrations ≥0.5%. The inhibition or inducer effects of acetoni-trile on phenacetin O-deethylation are in line with the findings of Chauret et al. (1998), Busby et al. (1999), and Hickman et al. (1998).

Our findings show that the choice of solvent may affect the activity of phenacetin O-deethylation in liver microsomes. Use of DMSO at concentrations ≥0.05% in reaction phenotyping studies and enzyme kinetic determinations would lead to wrong interpretation of the data. Although
phenacetin is sufficiently soluble in water, the use of inhibitors and test compounds dissolved in dimethyl sulfoxide during reaction phenotyping studies may confound the results. The concentration of DMSO should be kept low as indicated in this study, or an alternate solvent may be used to solubilize the inhibitors and test compounds. The findings from this study also indicate that methanol appears to be the solvent with no effects on the activity of phenacetin O-deethylation.

Acknowledgments

We acknowledge the support from Venkateswarlu Jasti (CEO, Suven Life Sciences Ltd., Hyderabad).

Authorship Contributions

Participated in research design: Nirogi, Kandikere, Bhyrapuneni, Ponnamaneni, Palacharla, and Manoharan.

Conducted experiments: Ponnamaneni, Palacharla, and Manoharan.

Performed data analysis: Kandikere, Bhyrapuneni, Palacharla, and Manoharan.

Wrote or contributed to the writing of the manuscript: Nirogi, Kandikere, Bhyrapuneni, Ponnamaneni, Palacharla, and Manoharan.

References


Address correspondence to: Ramakrishna Nirogi, Suven Life Sciences Ltd., Serene Chambers, Road-5, Avenue 7, Banjara Hills, Hyderabad 500 034, India.

E-mail: Ramakrishna_nirogi@yahoo.co.in