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Posttranscriptional Elevation of Cytochrome P450 3A Expression

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Human CYP3A, the most abundant hepatic and intestinal cytochrome P450, catalyzes the metabolism of a diverse array of xenobiotics. Dimethyl sulfoxide is a commonly used solvent which has been used therapeutically. Dimethyl sulfoxide effects on CYP3A, CYP2E1, CYP2B and NADPH cytochrome P450 reductase expression in rat liver and in primary cultured rat hepatocytes were examined. Dimethyl sulfoxide increased immunodetectable hepatic CYP3A and CYP2E1 levels ~2.5 to 3-fold in the absence of any change in the respective mRNA levels. No change in CYP2B or P450 reductase expression was observed, indicating that dimethyl sulfoxide effects were selective. Dimethyl sulfoxide also increased CYP3A protein in rats pretreated with dexamethasone. In primary cultured rat hepatocytes, dimethyl sulfoxide increased CYP3A and CYP2E1 protein without increasing the respective mRNA levels. These results show that dimethyl sulfoxide, at levels relevant to human exposure, enhances CYP3A and CYP2E1 expression by posttranscriptional mechanisms.

The human CYP3A subfamily constitutes the most abundant hepatic and intestinal cytochromes P450 and catalyzes the oxidation of steroids, numerous therapeutic agents, carcinogens, including the aflatoxins and polycyclic aromatic hydrocarbons, as well as other environmental agents (4-6). Human CYP2E1 catalyzes the oxidation of a large number of low molecular weight lipophilic compounds, carcinogens and therapeutic agents (4-6). Although several solvents have been reported to posttranscriptionally increase CYP2E1 expression, most agents, including pyridine (7) and ethanol (8) and glucocorticoids such as dexamethasone, increase CYP3A expression by a pretranslational mechanism. Macrolide antibiotics, such as troleandomycin, have been reported to increase CYP3A levels by decreasing protein degradation as well as increasing synthesis (9). This decrease in CYP3A protein degradation, however, is associated with the covalent binding of troleandomycin metabolites to the CYP3A protein and loss of CYP3A-associated enzymatic activities (9).

Dimethyl sulfoxide (DMSO) is used as a solvent in industry and in experimental and therapeutic drug administration. DMSO has also been used therapeutically in humans to treat interstitial cystitis, scleroderma, elevated intracranial pressure, gallstones, extravasation caused by certain antineoplastic agents, and other diseases (1). Clinical use of DMSO has been reported to result in human serum levels greater than 0.1% for at least four days following cessation of treatment (2). A single oral treatment of 1 g DMSO/kg resulted in human serum DMSO levels in excess of 0.1% for 24 h or more (3).

Low molecular weight solvents have been reported to increase cytochrome P450 expression by a number of mechanisms. For example, pyridine increased CYP2E1 protein levels in the absence of any change in CYP2E1 mRNA levels, while CYP3A and CYP2B levels were elevated at both the mRNA and protein levels (7). Therefore, we examined the effects of single and repeated DMSO administration on CYP3A, CYP2E1 and CYP2B expression. We found that DMSO rapidly enhanced both CYP3A and CYP2E1 protein levels *in vivo* and in primary cultured rat hepatocytes, but failed to enhance CYP2B or P450 reductase expression. CYP3A and CYP2E1 mRNA levels were not increased, indicating that DMSO enhanced the posttranscriptional expression of these proteins. DMSO also increased CYP3A-associated catalytic activity, indicating that, unlike troleandomycin, DMSO does not inactivate CYP3A.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats, ~200 g, were obtained from Harlan Sprague Dawley (Indianapolis, IN). Animals were injected i.p. with 100, 300 or 1000 μl of undiluted DMSO (approximately equivalent to 500, 1500 or 5000 mg/kg, respectively) and sacrificed

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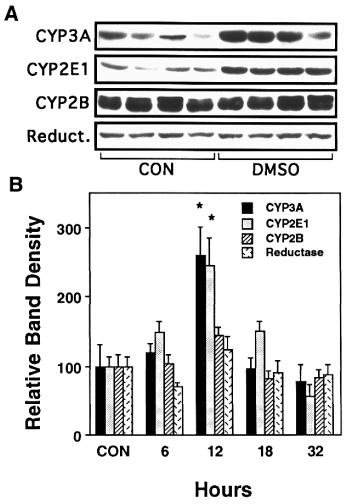

FIG. 1. Time-course of hepatic CYP3A, CYP2E1, CYP2B and P450 reductase protein levels following a single administration of 300 μl DMSO. Panel A: Representative Western blot analysis of hepatic microsomal protein 12 h after treatment with DMSO. Panel B: Summary of densitometric analysis of the Western blots. Each column and crossbar represents the mean and S.E. of 3 or 4 samples. *Values significantly different from control values ($p < 0.05$).

at the times indicated. Hepatic microsomes and total RNA were prepared as described (7).

Primary cultured rat hepatocytes. Hepatocytes were isolated and cultured on Vitrogen-coated dishes in the presence of modified Chee's medium, as described previously (10). Treatment with 0.1% or 1% DMSO was begun 72 h after plating of the hepatocytes and continued for 48 h thereafter. Total RNA and microsomal protein were prepared from the cultured cells as described (10).

Western and Northern blot analyses. Western and Northern blot analyses were undertaken as described previously (10,11). The Western blot procedures are linear over at least a 10-fold range of antigen concentration (11).

RESULTS AND DISCUSSION

The time-dependent effects of DMSO were examined by treating rats with a single DMSO administration and determining hepatic cytochrome P450 expression at 6, 12, 18 and 32 h following treatment. CYP3A and CYP2E1 protein levels were increased ~2.5-fold at 12 h and returned to basal levels at 32 h (Fig 1). CYP3A and CYP2E1 mRNA levels were not significantly altered in response to DMSO (Fig. 2), indicating that

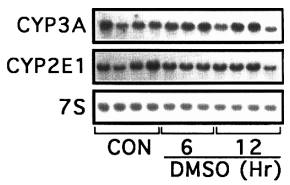

FIG. 2. Hepatic CYP3A and CYP2E1 mRNA and 7S RNA levels following a single administration of 300 μl DMSO. The 7S RNA was used to normalize for total RNA loading.

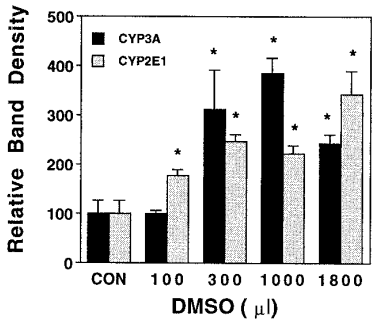

FIG. 3. Dose-dependent effects of DMSO on hepatic cytochromes P450 3A, 2B, 2E1 and NADPH-cytochrome P450 reductase protein levels. Animals either were treated with a single dose of 100, 300 or 1000 μl DMSO or were treated twice per day for 3 consecutive days with 300 μl DMSO (1800 μl total). Each column and crossbar represents the mean and S.E. of 3 or 4 samples. *Values significantly different from control values ($p < 0.05$).

DMSO altered the posttranscriptional regulation of these cytochromes P450. Neither CYP2B nor P450 reductase expression was altered in response to DMSO treatment (Fig. 1 and 2), showing that the effects of DMSO on CYP3A and CYP2E1 were selective.

DMSO volumes of 100 to 1000 μl are typically used as a solvent for the administration of xenobiotics to laboratory animals. Therefore, the effects of a single administration of 100, 300 or 1000 μl DMSO at 12 h after injection, or repeated administration of 300 μl at 12 h intervals for 3 days (total dose of 1800 μl) at 6 h after the final treatment were examined. CYP3A expression increased ~3.1-, 3.9- and 2.4-fold following treatment with 300, 1000 or 1800 μl DMSO, respectively (Fig. 3). CYP2E1 protein levels were increased ~1.8-, 2.5-, 2.2- and 3.4-fold by 100, 300, 1000 or 1800 μl DMSO, respectively (Fig. 3). In contrast, no change in CYP3A or CYP2E1 mRNA levels was observed under any of these conditions (data not shown). Neither CYP2B nor P450 reductase expression was altered by these treatments (data not shown). These data show that DMSO volumes commonly used as a solvent for administration of xenobiotics in animal experiments increase CYP3A and CYP2E1 expression and, hence, potentially may alter the metabolism, pharmacokinetics or toxicity of the co-administered therapeutic agents or xenobiotics.

Since DMSO enhances CYP3A expression by a post-

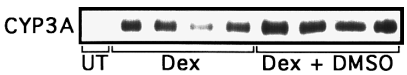

FIG. 4. Effects of DMSO treatment in combination with dexamethasone treatment on CYP3A protein levels. All lanes were loaded with 20 μg of microsomal protein. UT—untreated; Dex—40 mg dexamethasone/d for 3 days; Dex + DMSO—Dex-treated animals co-treated with 300 μl DMSO on the third day. Animals were sacrificed 12 hr after the final treatment.

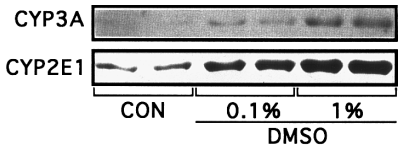

FIG. 5. DMSO effects on CYP3A and CYP2E1 protein levels in primary cultured rat hepatocytes. Cells were cultured for 72 hr prior to a 48 hr treatment with 0.1 or 1% DMSO.

transcriptional mechanism distinct from the transcriptional activation observed with dexamethasone, synergistic effects on CYP3A protein levels would be expected when DMSO and dexamethasone treatments are combined. As expected, dexamethasone treatment dramatically increased CYP3A protein when compared to untreated male rats (Fig. 4). Co-administration of DMSO elevated CYP3A protein levels an additional ~1.9-fold over that observed in animals treated with dexamethasone alone (Fig. 4). No additional change in CYP3A mRNA levels or in the DEX-induced CYP2B protein or mRNA levels was observed in response to DMSO treatment. These data show that DMSO posttranscriptionally regulates CYP3A expression even under conditions in which expression was already highly elevated through transcriptional activation.

Since serum DMSO levels of 0.1% or greater have been reported in humans (2,3), we examined the effects of 0.1% and 1% DMSO on cytochrome P450 expression in primary cultured rat hepatocytes. CYP3A protein levels were barely detectable in untreated cells, but were clearly increased in a concentration-dependent manner by 0.1% or 1% DMSO[2] (Fig. 5). CYP2E1 protein levels were increased ~4- and 6-fold by 0.1% and 1% DMSO, respectively. DMSO treatment failed to increase CYP3A or CYP2E1 mRNA levels or CYP2B protein and mRNA levels in the primary cultured hepatocytes (data not shown), paralleling observations *in vivo*. Treatment with 0.1% DMSO for 12 hr also increased CYP3A-mediated testosterone 2β-hydroxylase 2.5-fold in primary cultured rat hepatocytes. Therefore, unlike troleandomycin, DMSO does not inactivate CYP3A protein and does not increase CYP3A mRNA. Overall, these data suggest that the mechanism by which DMSO enhances CYP3A expression may be distinct from that observed with macrolide antibiotics and that the potential for DMSO effects on xenobiotic metabolism in humans as well as experimental animals is significant. These data also demonstrate that CYP3A levels may be elevated by posttranscriptional mechanisms which do not involve catalytic inactivation.

[2] The effects of DMSO on CYP3A expression were observed in two separate cell culture experiments, but because bands were too faint in untreated samples to accurately quantify, we have not provided fold-increases for these data. For the CYP2E1 data, values represent the means of data combined from two cell culture experiments in which two microsomal samples were prepared in each experiment (n=4).

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