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REGULATION OF CYTOCHROME P450 2B1/2 GENES BY DIALLYL SULFONE, DISULFIRAM, AND OTHER ORGANOSULFUR COMPOUNDS IN PRIMARY CULTURES OF RAT HEPATOCYTES

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Abstract—Our previous study demonstrated that diallyl sulfide (DAS), a compound derived from garlic, transcriptionally activated the P450 2B1/2 genes in rat liver. In the present study, rat primary hepatocytes were used to determine the effects of DAS and its metabolite, diallyl sulfone ($DASO_2$), on the expression of the P450 2B1/2 genes. Freshly isolated adult rat hepatocytes were cultured in a serum-free medium on a reconstituted basement membrane matrix "matrigel" that enabled the hepatocytes to maintain expression of numerous liver-specific genes for more than 1 week. After 48-hr of acclimation, 0.1, 0.5, and 2.0 mM concentrations of DAS or $DASO_2$ were added to the culture medium and the cells were harvested at 4, 12, 24, or 36 hr after the treatment for the preparation of microsomes and RNA. Cytotoxicity was not observed by morphological examinations after DAS and $DASO_2$ treatments. In contrast to the *in vivo* results, there was only a slight increase in the levels of P450 2B1/2 mRNA and protein in DAS-treated cells. However, $DASO_2$ treatment (2 mM) resulted in 11-, 21-, and 22-fold increases in P450 2B1/2 mRNA levels at 12, 24, and 36 hr after the treatment, respectively. P450 2B1/2 protein levels were also increased markedly in $DASO_2$-treated cells. Co-incubation of the rat hepatocyte cultures with a physiological concentration of growth hormone significantly blocked the induction of P450 2B1/2 mRNA by $DASO_2$. Northern blot analysis using oligonucleotide probes specific for 2B1 and 2B2 demonstrated that $DASO_2$ induced mRNA levels of both 2B1 and 2B2, with a greater induction of 2B1 mRNA. For comparison, the effects of disulfiram (DSF) and its metabolite, diethyldithiocarbamate (DDTC), on P450 2B1/2 mRNA expression were also examined in the cultured rat hepatocytes. Both DSF and DDTC caused a significant increase in P450 2B1/2 mRNA level with the highest induction at 0.5 mM. Addition of growth hormone to the culture effectively suppressed the P450 2B1/2 mRNA induction by DSF but had little effect on the induction by DDTC. Neither mRNA nor protein levels of P450 2E1 in cultured hepatocytes were affected by all the organosulfur compounds tested. These results suggest that $DASO_2$, DSF and DDTC selectively modulate P450 isozymes in cultured rat primary hepatocytes and that the induction of P450 2B1/2 by DAS in rat liver may be mediated by its metabolite, $DASO_2$.

Cytochromes P450 (P450¶) are enzymes involved in the biotransformation of various endobiotics and xenobiotics, including drugs and environmental carcinogens [1]. Many of the P450 enzymes are inducible by a variety of chemicals, and such an alteration of the composition of this enzyme system may have profound effects on chemical toxicity and carcinogenesis. The P450 gene superfamily consists of 27 distinct gene families, including 10 in mammals. Among P450 enzymes, P450 2B1 and 2B2 are remarkably similar proteins that share 97% amino acid sequence identity, and only 40 nucleotide differences exist between their mRNAs [2]. In addition to their sequence similarity, P450 2B1 and 2B2 genes are usually coordinately regulated in liver. For example, both mRNA levels of P450 2B1 and 2B2 in rat liver are induced dramatically by phenobarbital (PB).

Diallyl sulfide (DAS), a compound derived from garlic, has been shown to inhibit chemical toxicity and tumorigenesis in several animal models [3–6], presumably by acting as an inhibitor of the P450-mediated metabolic activation of the toxic and carcinogenic chemicals. Our previous work demonstrated that DAS causes a selective alteration of P450 enzymes in rat liver, in which P450 2E1 activity is decreased significantly whereas P450 2B1 is induced [5]. The induction of P450 2B1/2 by DAS in rat liver has been demonstrated to be mainly through the transcriptional activation of 2B1/2 genes [7]. However, it is not clear whether the effect on

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¶ Abbreviations: P450, cytochrome(s) P450; DAS, diallyl
)ASO_2, diallyl sulfone; DSF, disulfiram; DDTC,
iocarbamate; PB, phenobarbital; GH, growth
'SC, sodium chloride and sodium citrate; SDS,
cyl sulfate; and DMSO, dimethyl sulfoxide.

the expression of P450 2B1/2 genes is due to DAS itself, mediated by its metabolite diallyl sulfone ($DASO_2$), or caused by other physiological alterations. It is difficult to define such an effect in living animals; the primary hepatocyte culture is of great use for this purpose.

Recently, Schuetz *et al.* [8] described a new system for primary monolayer culture of adult rat hepatocytes. The cells are cultured on a substratum of matrigel, a reconstituted basement membrane prepared from extracts of the Engelbreth–Holm–Swarm sarcoma. Unlike most of the previously used hepatocyte culture systems, cultures on matrigel extend longevity and express numerous differentiated functions characteristic of the adult liver, including PB-responsive increases in P450 2B1/2 mRNA and protein [8]. In the present work, we used the matrigel hepatocyte culture system to study the regulation of the expression of P450 2B1/2 genes by DAS and $DASO_2$ as well as the effect of exogenous growth hormone on this regulation. Disulfiram (DSF), a compound used in avoidance therapy for alcohol abuse, has been found in our laboratory to induce hepatic P450 2B1/2 when given to rats [9]. The effects of DSF and its metabolite, diethyldithiocarbamate (DDTC), on P450 2B1/2 expression were also examined in the cultured hepatocytes.

MATERIALS AND METHODS

Materials. Collegenase (type I) was purchased from the Cooper Biochemical Co. (Malvern, PA). DAS (purity >99%) and DDTC (sodium salt, purity >99%) were purchased from the Aldrich Chemical Co. (Milwaukee, WI). $DASO_2$ was purchased from the Parish Chemical Co. (Orem, VT) and purified by vacuum distillation and column chromatography on silica gel with CH_2Cl_2 as the elution solvent. The purity of the $DASO_2$ was >98% as determined by integration of the proton nuclear magnetic resonance spectrum and by GC analysis. Crystalline disulfiram (bis[diethylthiocarbamoyl]disulfide), human growth hormone (GH), guanidinium thiocyanate, and *N*-lauroylsarcosine were obtained from the Sigma Chemical Co. (St. Louis, MO). [α-^{32}P]dCTP (3000 Ci/mmol) and [γ-^{32}P]ATP (6000 Ci/mmol) were from New England Nuclear (Boston, MA). The sources for the other chemicals including the reagents for electrophoresis and immunoblot analysis were reported previously [10]. Polyclonal antibodies against P450 2B1 also immunoreactive to P450 2B2 were provided by Dr. F. Gonzalez (Laboratory of Molecular Carcinogenesis, National Cancer Institute, Bethesda, MD). Polyclonal antibodies against P450 2E1 were prepared as described [11].

Preparation of hepatocyte cultures on matrigel. Adult male Sprague–Dawley rats (Dominion Laboratories, Dublin, VA), weighing 175–200 g, were maintained in wire-bottomed cages, with free access to animal chow and water, for 2 weeks before use. Rat hepatocytes were prepared by perfusion of the liver *in situ* with a calcium-free buffer followed by a solution of 0.036% collagenase in a standard serum-free culture medium, which is a modification of Waymouth MB-752 medium with insulin (0.15 μM) as the only hormone. The softened liver was excised, and the hepatocytes were separated from connective tissue by filtering through two layers of gauze and from nonparenchymal cells by repeated low speed centrifugation. The freshly isolated hepatocytes (3.5×10^6) in a total volume of 3.0 mL of culture medium were placed into 60-mm culture dishes coated with 120–150 μL of matrigel. Matrigel was prepared from Engelbreth–Holm–Swarm sarcoma propagated in C57BL/6J female mice and was stored at −20°. Shortly prior to its use, matrigel was warmed to 4° and was applied (1 mg/100 μL) evenly to the bottom of the dishes [8]. Cultures were maintained in a humidified incubator at 35°, under an atmosphere of 5% CO_2/95% air and the medium was renewed daily. In all experiments, cells were incubated for 48 hr with medium only and were then treated with testing chemicals at the doses and durations indicated in the text. Chemicals were added to the cultures as concentrated stock solutions (less than 6 μL/dish) in water (for $DASO_2$, DDTC, and PB) or dimethyl sulfoxide (DMSO, for DAS and DSF). If the designed treatment period was longer than 24 hr, the testing chemicals were added again after the change of medium. Detachment of the cells from the bottom of the culture dishes and the loss of clear membrane structure were considered as indications of cytotoxicity.

cDNA and oligonucleotide probes. P450 2B1* and 2E1 cDNAs were provided by Dr. Frank Gonzalez. The cDNAs were radiolabeled to greater than 1×10^8 cpm/μg, using a nick translation kit (BRL, Gaithersburg, MD) and [α-^{32}P]dCTP (3000 Ci/mmol). The oligonucleotide sequences specific to 2B1 and 2B2 described by Omiecinski *et al.* [12] were synthesized from American Synthesis, Inc. (Peassanton, CA). The oligodeoxynucleotides [12] had the following sequences: 3′-d-(AGTGTGGCCGATGGTTGG)-5′ for P450 2B1 and 3′-d(AGAGTGTCCGGTGGTAGG)-5′ for P450 2B2 (differences in the sequences are underlined). The G-C and A-T contents for each oligomer are identical. These sequences are from a hypervariable region of exon 7 of P450 2B1 and 2B2 genes. Oligonucleotides were radiolabeled to greater than 1×10^9 cpm/μg, using a commercial 5′-labeling kit (BRL) and 15 pmol of [γ-^{32}P]ATP (6000 Ci/mmol) with 10 pmol of oligomer. Labeled oligomers were then purified by an Elutip-d minicolumn (Schleicher & Schuell).

RNA preparation and analysis. The cultured cells were scraped, pooled from three dishes, and kept in a solution containing 4 M guanidinium thiocyanate, 0.5% sodium lauryl sarkosinate, 0.1 M β-mercaptoethanol, and 25 mM sodium citrate, pH 7, at −80°. Total RNA was isolated by an acid guanidinium-phenol-chloroform method [13]. The yield of total RNA from cells of three 60-mm dishes was 180–240 μg. Poly (A)$^+$ RNA was prepared by

* The antibodies and the cDNA probe used in this study could not distinguish between P450 2B1 and 2B2 due to the high homology of these two isozymes. The term of P450 2B1/2 refers to the genes previously designated as P450b/e, the major phenobarbital-inducible cytochromes P450 in rat liver.

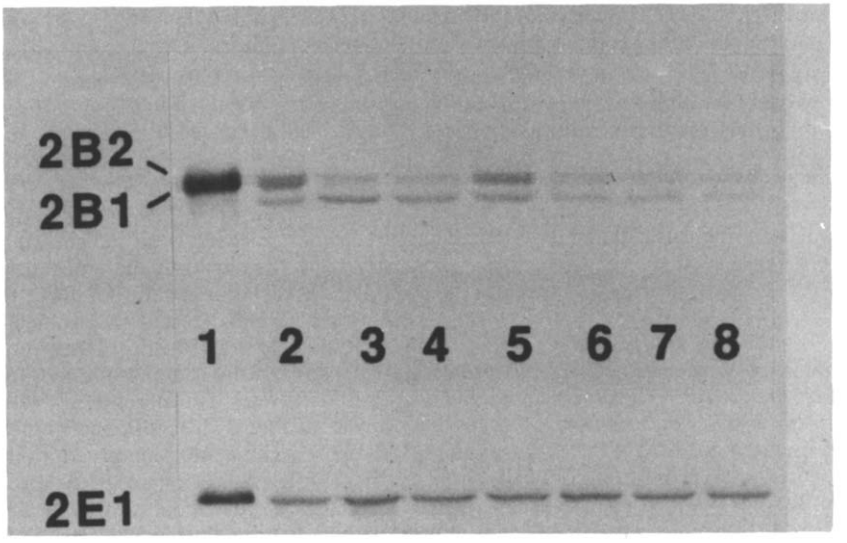

Fig. 1. Immunoblot analysis of P450 2B1/2 protein in cultured rat hepatocytes. Cells were pooled from eight dishes for each group and microsomes were prepared. The amount of microsomal proteins applied to electrophoresis was 20 μg/lane. Lane 1, liver microsomes from PB-treated rats (for 2B1/2) or from acetone-treated rats (for 2E1); Lane 2, hepatocytes treated with 0.1 mM PB for 24 hr; Lanes 3 and 4, untreated hepatocytes cultured for a total of 48 and 72 hr, respectively; Lane 5, hepatocytes treated with 2 mM $DASO_4$ for 24 hr; Lanes 6 and 7, cells treated with 0.5 and 2 mM DSF for 24 hr, respectively; Lane 8, cells treated with 2 mM DDTC for 24 hr.

an mRNA purification kit (Pharmacia, Piscataway, NJ). For the northern and slot blot analyses, RNA was either electrophoretically separated in 1% agarose gel containing 2.2 M formaldehyde and blotted with nitrocellulose filter, or directly applied to the nitrocellulose paper using a slot blot apparatus (Minifold II; Schleicher & Schuell) as described previously [10]. The nitrocellulose filter was baked at 80° for 2 hr *in vacuo*. For hybridization with a cDNA probe, the filter was prehybridized for 3 hr at 42° in 5× Denhardt's solution (1% Ficoll, 1% polyvinylpyrrolidone, 1% bovine serum albumin), 5× sodium chloride and sodium citrate (SSC), 0.1% sodium dodecyl sulfate (SDS), 10 μg/mL of denatured and sheared salmon sperm DNA, and 50% formamide. The filter was then hybridized in the same solution with the ^{32}P-labeled cDNA probe (2×10^6 cpm/mL) overnight. The filter was washed at room temperature twice with 2× SSC–0.1% SDS for 15 min each and then twice with 0.1× SSC–0.1% SDS for 15 min each. Hybridization with the oligonucleotide probes was carried out at 52° in 5× Denhardt's solution, 6× SSPE (3 M sodium chloride, 0.2 M sodium phosphate, 0.02 M EDTA, pH 7.4), 0.1% SDS, and 10 μg salmon sperm DNA/mL. The filter was prehybridized for 3 hr and hybridized overnight with the ^{32}P-labeled oligonucleotide probe (2×10^6 cpm/mL). The filter was washed at 42° for 30 min with 0.1× SSC–0.1% SDS. Autoradiography was carried out by exposing the filters to Kodak XAR-5 film at −80° with a Lightning Plus intensifying screen. The bands on the autoradiographs were quantified by a dual-wavelength thin-layer chromatography scanner (Shimadzu, Columbia, MD) in a transmission mode.

Table 1. Effects of $DASO_2$ and DAS on P450 2B1/2 mRNA level in cultured rat primary hepatocytes

Treatment		P450 2B1/2 mRNA level (arbitrary units) 0 mM	0.1 mM	0.5 mM	2.0 mM
$DASO_2$	12 hr	1.8	2.9	4.3	20.6
	24 hr	1.4	3.0	4.7	29.6
	36 hr	1.2	2.0	3.6	26.2
DAS	12 hr	0.7	2.4	2.0	2.9
	24 hr	0.9	1.7	1.7	2.2
	36 hr	1.0	1.6	1.6	1.9
PB	24 hr	1.4	63		

Freshly isolated hepatocytes were prepared from an untreated male rat and inoculated on dishes precoated with matrigel. Forth-eight hours after inoculation, cells were either treated with DAS (in DMSO), $DASO_2$ or PB (both in H_2O) or with equal volumes of DMSO or H_2O, respectively. Total RNA was isolated from the treated cells at different time points and subjected to slot blot analysis with a P450 2B1 cDNA probe. The intensity of the bands on the autoradiography was quantified by densitometry scanning.

Immunoblot analysis. For each group, cells pooled from eight dishes were disrupted by sonication. Microsomes were prepared from the sonicated cells by differential centrifugation as previously described [10]. Microsomal proteins (20 μg) were resolved on SDS–polyacrylamide (10%) gels and electrophoretically transferred to nitrocellulose filters. The

filters were incubated with 3% non-fat dry milk for 2 hr to block the nonspecific binding and then incubated overnight with anti-P450 2B1 or 2E1 IgG, followed by phosphatase-labeled goat anti-rabbit IgG. For immunostaining, the nitrocellulose sheet was developed with a mixture of 5-bromo-4-chloro-3-indolylphosphate, nitroblue tetrazolium, and 0.1 M Tris buffer solution (1:1:10). Intensities of immunostained bands were measured using the Shimadzu scanner in a reflection mode.

RESULTS

Effects of $DASO_2$ on the level of P450 2B1/2 protein in cultured rat hepatocytes. Primary hepatocytes prepared from male rats were cultured on matrigel in a standard medium for 48 hr. $DASO_2$ was then added to the medium at different concentrations (0.1, 0.5, and 2.0 mM), and the treated cells were harvested 24 hr after the treatment. During the treatment period, the cells were examined periodically under a light microscope. They were found to retain a spherical shape and adhered firmly to the bottom of the dishes. Morphologically, there was no cytotoxicity observed after the treatment. Microsomes were prepared from the cultured hepatocytes and subjected to immunoblot analysis with polyclonal antibodies against P450 2B1/2. In the microsomes from untreated hepatocytes cultured for a total of 48 or 72 hr, P450 2B2, which displayed a slightly slower mobility than 2B1 in the gel electrophoresis, was detected whereas P450 2B1 was not detectable (Lanes 3 and 4, Fig. 1). This is consistent with previous reports that in the liver of untreated rats and in cultured rat hepatocytes, P450 2B1 is not constitutively expressed [7, 14–16]. $DASO_2$ treatment at lower doses (0.1 and 0.5 mM) did not change the level of P450 2B1/2 protein in the cultured cells (data not shown). However, 2 mM $DASO_2$ caused a significant increase in P450 2B1/2 protein content in the microsomes from treated cells (Lane 5, Fig. 1). No alteration in the P450 2B1/2 level was observed in the microsomes from the cells treated with 0.1, 0.5 or 2 mM DAS (data not shown). An induction of P450 2B1/2 protein by DSF and DDTC was not clearly demonstrated (Lanes 6–8 vs Lane 4, Fig. 1) in this experiment. Addition of PB, a known inducer of P450 2B1/2, to the culture medium caused a remarkable increase in P450 2B1/2 content in the hepatocyte microsomes (Lane 2, Fig. 1). In addition to P450 2B1 and 2B2, the anti-P450 2B1 antibodies detected an immunoreactive protein at lower molecular weight in all microsomal samples prepared from the cultured hepatocytes. This result agrees with the observation by Sinclair *et al.* [15] that a monoclonal antibody raised against P450 2B1 also detected a third protein in the microsomes from cultured rat hepatocytes but not in the PB-induced rat liver microsomes. The nature of this protein which is immunoreactive to P450 2B1/2 antibodies is not known. In our experiments, the level of this protein was not altered in the microsomes from PB- or $DASO_2$-treated cells.

To determine the selectivity of $DASO_2$ in modulation of P450 isozymes in the hepatocytes cultured on matrigel, microsomes were analyzed using antibodies against P450 2E1. In comparison to untreated cells, there was no appreciable change in the content of P450 2E1 protein in the $DASO_2$-treated cells (Lane 4 vs Lane 5, Fig. 1). This result suggests that $DASO_2$ selectively modulates P450 isozymes in the cultured primary rat hepatocytes as well as the rat liver [7].

P450 2B1/2 mRNA level in the cultured hepatocytes treated with $DASO_2$ and DAS and the effect of growth hormone. To elucidate the molecular mechanism of P450 2B1/2 induction by $DASO_2$, total RNA was extracted from the cells and subjected to slot blot analysis with a P450 2B1 cDNA probe. Treatment of cells with 0.1 or 0.5 mM $DASO_2$ for various times caused only a slight increase in P450 2B1/2 mRNA levels (Table 1). Treatment of cells with 2 mM $DASO_2$ significantly increased the level of P450 2B1/2 mRNA. Compared to the corresponding controls, the fold increase was 11, 21, and 22 at 12, 24, and 36 hr, respectively, after $DASO_2$ treatment (Table 1). In the same experiment, the mRNA level of P450 2B1/2 in the hepatocytes was increased only slightly by DAS treatment, but was increased 45-fold by PB treatment (Table 1). The effects of $DASO_2$ and DAS on P450 2B1/2 mRNA level in cultured hepatocytes were confirmed by northern blot analysis in a separate experiment and were consistent with their effects on the level of P450 2B1/2 proteins. Significant induction of P450 2B1/2 mRNA level by $DASO_2$ started at 12 hr after the treatment, and increased further at 24 hr (Lanes 6 and 7, Fig. 2). Coadministration of human GH (100 μU/mL) with $DASO_2$ suppressed the induction of P450 2B1/2 mRNA level by $DASO_2$ (Lane 8 vs Lane 7, Fig. 2). This suppressing effect of GH was similar to its effect on the induction of P450 2B1/2 mRNA by PB (Lane 10 vs Lane 9, Fig. 9, Fig. 2) which has been reported previously [17]. Filter prehybridized with the P450 2B1 cDNA sequence was deprobed and then rehybridized with a P450 2E1 cDNA probe to determine whether these treatments also affected P450 2E1. The level of P450 2E1 mRNA decreased rapidly in the cultured hepatocytes (Fig. 2). Compared to the corresponding controls, $DASO_2$ treatment did not induce the P450 2E1 mRNA level in the treated hepatocytes (Fig. 2).

Northern blot analysis using oligonucleotide probes. Since rat P450s 2B1 and 2B2 sequences are rather homologous, the cDNA probe used in the present study was unable to distinguish between the mRNAs for P450s 2B1 and 2B2. To assess the effect of $DASO_2$ on mRNA levels for each of these hemoproteins, we performed northern blot analysis with synthetic oligodeoxynucleotide probes of defined sequence which are either 2B1 or 2B2 specific. Poly(A)$^+$ RNA, instead of total RNA, was used in northern blot analysis to avoid the nonspecific binding of these oligomer probes to ribosomal RNA sequences. Equal amounts of poly(A)$^+$ RNA from each group were applied to electrophoresis and subjected to the hybridization. Starting at 12 hr after the treatment, incubation of the cultured hepatocytes with 2 mM $DASO_2$ resulted in a marked increase in the mRNA levels of both P450s 2B1 and 2B2 (Lanes 3 and 4, Fig. 3). Both induction of P450 2B1 and 2B2 mRNA by $DASO_2$ was suppressed by GH

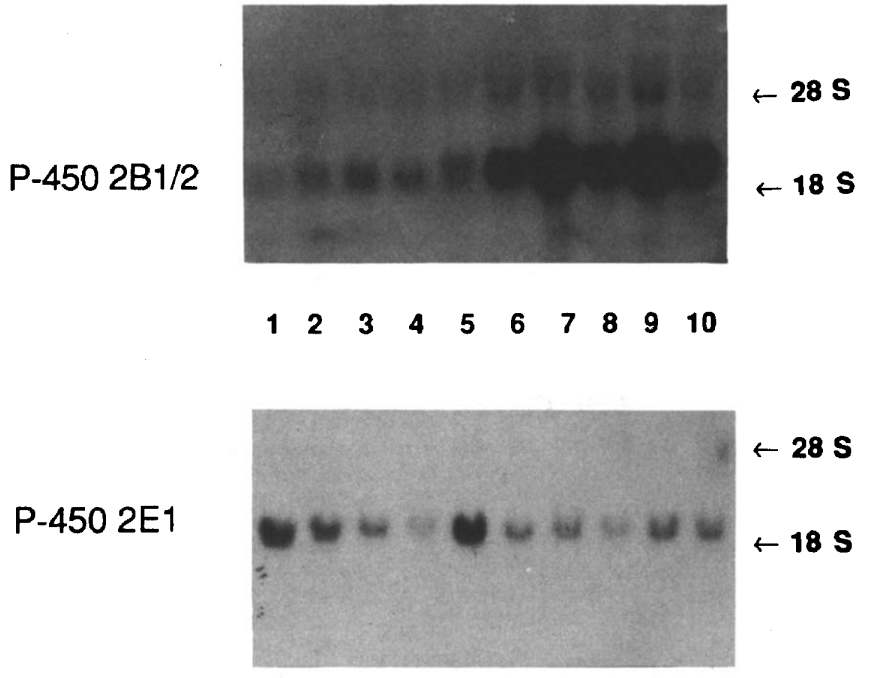

Fig. 2. Expression of cytochromes P450 2B1/2 and 2E1 mRNAs in cultured rat hepatocytes and the effect of growth hormone. Freshly isolated hepatocytes from a male rat were incubated for 48 hr in medium prior to the treatment. Total cellular RNA was extracted from cells pooled from three dishes for each group and subjected to northern blot analysis (15 μg RNA/lane). The filter was first hybridized with a P450 2B1 cDNA probe and then rehybridized with a P450 2E1 cDNA probe after deprobing. Lanes 1–3 are samples from untreated hepatocytes cultured for a total of 52, 60 and 72 hr, respectively; Lane 4, hepatocytes treated with human GH (100 μU/mL) for 24 hr; Lanes 5–7, cells treated with 2 mM $DASO_2$ for 4, 12 and 24 hr, respectively; Lane 8, cells coincubated with 2 mM $DASO_2$ and GH (100 μU/mL) for 24 hr; Lanes 9 and 10, hepatocytes treated with 0.1 mM PB for 24 hr in the absence and presence of GH (100 μU/mL), respectively.

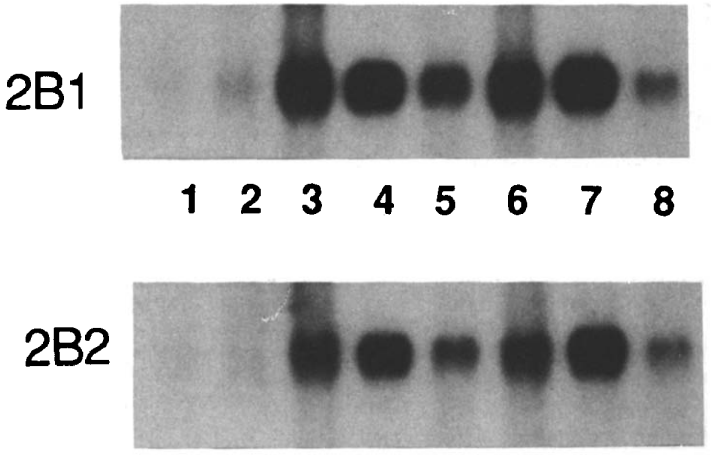

Fig. 3. Northern blot analysis with P450s 2B1 and 2B2 specific oligonucleotide probes. Rat primary hepatocytes were cultured on matrigel for 48 hr prior to treatment. Poly(A)$^+$ RNA was prepared from cells and subjected to northern blot analysis (2.8 μg RNA/lane). The filter was first hybridized with a P450 2B1-specific oligonucleotide probe and then rehybridized with a P450 2B2-specific oligonucleotide probe after deprobing. Lane 1, untreated hepatocytes cultured for a total of 72 hr; Lanes 2–5, cells treated with 2 mM $DASO_2$ for 4, 12, 24, and 24 hr in the presence of GH (100 μU/mL), respectively; Lane 6, cells treated with 0.5 mM DSF for 24 hr; Lanes 7 and 8, cells treated with 0.1 mM PB for 24 hr in the absence and presence of GH (100 μU/mL), respectively.

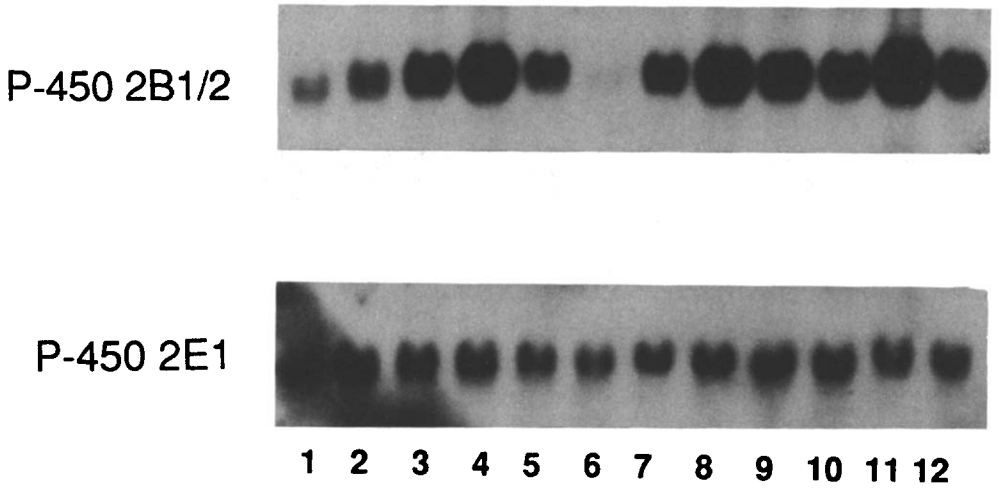

Fig. 4. Effects of DSF and DDTC on the expression of P450 2B1/2 mRNA in cultured rat hepatocytes. Hepatocytes were cultured for 48 hr prior to treatment. Total RNA was extracted from cells and subjected to northern blot analysis. The amount of RNA applied was 15 μg/lane. The filter was first hybridized with a P450 2B1 cDNA probe and then rehybridized with a P450 2E1 cDNA probe after deprobing. Lane 1, untreated cells cultured for a total of 72 hr; Lane 2, cells treated with GH (100 μU/mL) for 24 hr; Lanes 3–6, cells treated with DSF for 24 hr at 0.1, 0.5, 2, and 2 mM in the presence of GH (100 μU/mL), respectively; Lanes 7–10, cells treated with DDTC for 24 hr at 0.1, 0.5, 2, and 2 mM in the presence of GH, respectively; Lanes 11 and 12, cells treated with 0.1 mM PB for 24 hr in the absence and presence of GH, respectively.

(Lane 5 vs Lane 4, Fig. 3). Similarly, the PB-caused coinduction of P450 2B1 and 2B2 mRNA in cultured hepatocytes was also suppressed by GH (Lane 8 vs Lane 7, Fig. 3).

Effect of DSF and DDTC on P4502B1/2 mRNA level in hepatocytes. In addition to $DASO_2$, the effects of DSF and its metabolite, DDTC, on the level of P450 2B1/2 mRNA in the hepatocytes were examined. Northern blot analysis with the 2B1 cDNA probe demonstrated that both DSF and DDTC treatments markedly increased P450 2B1/2 mRNA level in the hepatocytes and the maximal increase appeared to be in the cells treated with 0.5 mM DSF or DDTC (Fig. 4). Slot blot analysis revealed that 24 hr after the treatment with 0.1, 0.5 or 2 mM DSF or DDTC, P450 2B1/2 mRNA level was increased 5-, 19-, and 4-fold for DSF treatment and 4-, 13-, and 11-fold for DDTC treatment, respectively. The decreased induction effect by DSF at 2 mM could be related to the cytotoxicity observed in the cultured hepatocytes. There were no morphological changes in the cells treated with 0.1 or 0.5 mM DSF and with 0.1, 0.5 or 2 mM DDTC. DSF treatment (0.5 mM for 24 hr) caused a coinduction of P450 2B1 and 2B2 mRNA (Lane 6, Fig. 3). The induction of P450 2B1/2 mRNA in hepatocytes by DSF or DDTC was not accompanied by an increase of microsomal 2B1/2 protein content as determined by immunoblot analysis (Lanes 6–8, Fig. 1). Addition of GH to the culture medium during the treatment effectively suppressed the induction of P450 2B1/2 mRNA by DSF but only slightly inhibited the induction by DDTC (Lanes 6 and 10, Fig. 4). The mechanisms involved in this selective suppression by GH are not known. Rehybridization of the deprobed filter with the P450 2E1 cDNA probe demonstrated that the P450 2E1 mRNA level was unaffected by DSF and DDTC treatment (Fig. 4). The result of rehybridization also provided the evidence that all the RNA samples were equally loaded and transferred.

DISCUSSION

The activities of P450 isozymes in rat liver microsomes are known to be selectively modulated by DAS or $DASO_2$, in which the P450 2B1 activity, assayed as pentoxyresorufin dealkylation, was increased remarkably [18]. We have demonstrated recently that the induction of P450 2B1/2 mRNA by DAS in rat liver is mainly mediated by the transcriptional activation of P450 2B1/2 genes [7]. The present study clearly demonstrates that in rat primary hepatocytes cultured on matrigel, DAS had little effect on P450 2B1/2 gene expression, whereas $DASO_2$ significantly increased the protein and mRNA levels of P450 2B1/2. Similar to the effect of DAS on rat liver, $DASO_2$ treatment had no effect on P450 2E1 mRNA expression and caused a coinduction of P450 2B1 and 2B2 mRNAs in the cultured hepatocytes. These results strongly suggest that the induction of P450 2B1/2 by DAS *in vivo* was mediated by its metabolite $DASO_2$. The failure to induce P450 2B1/2 by DAS in cultured hepatocytes could be related to the intracellular level of $DASO_2$. The enzymes responsible for the metabolism of DAS to $DASO_2$ have not been identified. Loss or decrease of activity of such enzymes during preparation and culture of the primary hepatocytes may explain why DAS is a strong P450 2B1/2 inducer *in vivo* but not *in vitro*. The present work also demonstrated that DSF and DDTC are effective inducers of P450 2B1/2 mRNA in cultured hepatocytes. Both the parental

compound (DSF) and its metabolite (DDTC) were about equally effective (19-fold vs 13-fold) in the induction of P450 2B1/2 mRNA at their maximal inducing concentrations (0.5 mM). It is of interest to observe that in the DSF- or DDTC-treated hepatocytes the microsomal P450 2B1/2 protein content was not increased appreciably. We were unable to detect the P450 2B1 activity assayed as pentoxyresorufin dealkylation in the microsomes prepared from the cultured rat hepatocytes. Nevertheless, the differential effects of DSF and DDTC on the expression of P450 2B1/2 mRNA and protein in the cultured rat hepatocytes remain to be studied.

In the present study, the use of the matrigel hepatocyte culture system not only identified $DASO_2$ as the most likely mediator in the induction of P450 2B1/2 by DAS *in vivo* but also clarified the role of hormones in the induction. It has been proposed that pituitary hormones, especially GH, act as suppressors on the constitutive and PB-induced expression of P450 2B1 and 2B2 in rat livers [16]. However, the cultured hepatocytes are not under the control of the endocrine system and pituitary hormones are almost all depleted after the 48-hr acclimation period. The result of the P450 2B1/2 mRNA induction in cultured rat hepatocytes suggests that $DASO_2$ or DSF did not effect the induction by counteracting the action of pituitary hormones.

The induction of P450 2B1/2 mRNA by $DASO_2$ and DSF in the cultured hepatocytes shares many features with induction by PB. These features include the time-response of the induction, coinduction of 2B1 and 2B2, and the susceptibility to the suppressing effect of exogenous GH [17]. It has been demonstrated that induction of P450 2B1/2 by PB *in vivo* and *in vitro* is mainly through transcriptional activation and that exogenous GH inhibits PB-induced P450 2b1/2 gene transcription in cultured hepatocytes [17, 19]. Nuclear run-on assays of $DASO_2$- or DSF-treated hepatocytes are needed to determine the transcriptional activity, and the use of the matrigel culture system should permit more in-depth studies in understanding the regulation of P450 2B1/2 genes.

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