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Inductive Effect of Telazol® on Hepatic Expression of Cytochrome P450 2B in Rats

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ABSTRACT. Telazol®, a 1:1 combination of tiletamine HCl and zolazepam HCl, is an anesthetic and immobilizing agent that has been in use in veterinary medicine and animal field studies for more than a decade. No information is available, however, regarding the effects of Telazol®, or its constituents, on hepatic cytochromes P450. The purpose of the present study was to assess the effect of Telazol® on the rat hepatic cytochrome P450 system. Adult male rats were given a single intraperitoneal injection of Telazol® at a dose of 20, 40, 80, or 120 mg/kg body weight (six rats/dose), while control rats received the vehicle only. Animals were killed 24 hr later, and hepatic microsomes were prepared. Treatment with Telazol® resulted in dose-dependent increases in benzyloxyresorufin O-dealkylase and testosterone 16β-hydroxylase activities. Ethoxyresorufin O-deethylase, *p*-nitrophenol hydroxylase, and testosterone 6β- and 7α-hydroxylase activities were essentially unaltered at all doses of the drug. Densitometric quantitation of immunoblots probed with polyclonal antibody against cytochrome P450 2B1 indicated a 17-fold increase in the hepatic level of cytochrome P450 2B1 for rats treated with the highest dose of Telazol®. In contrast, the level of cytochrome P450 2B2 was increased slightly but not significantly. In the presence of 0.5 mg of anti-cytochrome P450 2B1 IgG/nmol P450, benzyloxyresorufin O-dealkylase activity was inhibited by 92% in hepatic microsomes prepared from a rat treated with Telazol® at a dose of 120 mg/kg compared with only 25% inhibition in hepatic microsomes from a control rat. In summary, the results demonstrate that Telazol® specifically induced expression of the cytochrome P450 2B isozymes in rats. BIOCHEM PHARMACOL 52;5:735–742, 1996.

KEY WORDS. Telazol®; cytochrome P450; induction; liver; microsomal monooxygenase; cytochrome P450 2B1

Telazol® is an injectable anesthetic agent currently approved for use in the United States as an anesthetic and immobilizing agent in cats and dogs. It is a 1:1 (w/w) combination of tiletamine hydrochloride (2-[ethylamino]-2-[2-thienyl]-cyclohexanone hydrochloride) and zolazepam hydrochloride (4-[*o*-fluorophenyl]-6,8-dihydro-1,3,8-trimethylpyrazolo[3,4-*e*][1,4]diazepin-7(1*H*)-one-hydrochloride). Tiletamine is a dissociative anesthetic structurally similar to ketamine. When used alone, it produces profound analgesia, normal pharyngeal–laryngeal reflexes, and cataleptoid anesthesia [1]. Zolazepam, on the other hand, is a benzodiazepine derivative with pharmacological properties similar to those of chlordiazepoxide and diazepam [1]. When the two ingredients are combined in a 1:1 ratio, the resulting drug (Telazol®) has a wide margin of safety and other pharmacological advantages, including rapid induction, excellent muscle relaxation, and smooth recovery, that are ideal for use in veterinary diagnostic examinations [2], as well as various dental and minor surgical procedures. It has proven to be an excellent drug for field studies involving immobilization of wild animals such as black bears [3], lions [4], jaguars [5], and polar bears [6]. The drug is absorbed rapidly, produces an effective level of anesthesia, is relatively safe for the handlers, and has a wide tolerance range for the animals [3]. The main disadvantages of Telazol® are a lengthy recovery period and absence of reversibility due to lack of an effective antidote. Despite the fact that it has been used in veterinary medicine and field studies for over a decade, there are no reports in the literature of the effects of Telazol®, or its constituents, on hepatic cytochromes P450.

The hepatic cytochrome P450 system consists of a group of enzymes that catalyze the biotransformation of a wide variety of lipophilic xenobiotic and endogenous compounds in the body. Specific cytochrome P450 isozymes are subject to induction by numerous foreign compounds including various drugs and environmental pollutants such as polychlorinated biphenyls, chlorinated dibenzo-*p*-dioxins, and pesticides. Consequently, induction of cytochromes P450 has been proposed as a biomarker of exposure to environmental contaminants in the food chain [7, 8].

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In a recent study [9], hepatic cytochromes P450 in the polar bear were characterized with respect to both monooxygenase activities and immunochemical relatedness to rat cytochromes P450. In that study, free-ranging polar bears were immobilized with Telazol® prior to their capture. The bears were killed 0.5 to 11 days later, and enzyme activities of hepatic microsomes prepared from individual bears were measured. Elevated levels of cytochrome P450 1A and cytochrome P450 2B were found in polar bear hepatic microsomes, which suggested prior exposure of the animals to polychlorinated biphenyls and other halogenated environmental contaminants in the arctic food chain [9, 10]. However, it was not known if administration of Telazol® was responsible, in part, for induction of any of the cytochrome P450 isozymes. The purpose of the present study was to determine whether treatment with Telazol® was capable of altering hepatic expression of cytochromes P450 using the rat as the experimental model. Cytochrome P450-mediated monooxygenase activities and hepatic levels of individual isozymes were measured in rats treated with increasing doses of Telazol®, and an effort was made to correlate the effect of Telazol® in rats with the relatively high levels of cytochrome P450 1A and 2B observed previously in polar bears.

MATERIALS AND METHODS

Chemicals

Telazol® (A. H. Robins, Richmond, VA) was provided by Fort Dodge Laboratories, Inc. (Fort Dodge, IA). Resorufin was purchased from the Aldrich Chemical Co. Inc. (Milwaukee, WI). Ethoxyresorufin and benzyloxyresorufin were bought from Molecular Probes, Inc. (Eugene, OR). Acrylamide 99.9%, *N,N'*-methylene-bis-acrylamide (BIS), SDS, 2-mercaptoethanol and *N,N,N',N'*-tetramethylethylenediamine (TEMED) were obtained from Bio-Rad Laboratories (Mississauga, Ontario). NADPH was purchased from Boehringer Mannheim Canada Ltd. (Laval, Quebec). Skim milk powder was obtained from Carnation, Inc. (Toronto, Ontario). HPLC-grade dichloromethane, HPLC-grade methanol, bromphenol blue, ascorbic acid, glycine, perchloric acid, sodium chloride, sodium phosphate, and tris-(hydroxymethyl)aminoethane (Tris base) were purchased from Fisher Scientific (Vancouver, British Columbia). Bovine serum albumin (globulin and fatty acid free, fraction V) was bought from ICN Biochemicals Canada Ltd. (St-Laurent, Quebec). Carbon monoxide gas (99.5% purity) was obtained from Medigas Pacific (Vancouver, British Columbia). *p*-Nitro-blue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP), disodium salt, were purchased from Pierce (Rockford, IL) and Xymotech Biosystems (Mt. Royal, Quebec), respectively. Tween 20, 4-nitrophenol, and 1,2-dihydroxy-4-nitrobenzene were bought from the Sigma Chemical Co. (St. Louis, MO). Sodium dithionite was bought from J. T. Baker Inc. (Phillipsburg, NJ). Nitrocellulose membrane (Schleicher & Schuell) was obtained from the Mandel Scientific Co. Ltd. (Edmonton, Alberta). Testosterone, androstenedione and 2α-, 6β-, 7α-, 11β-, 16α-, and 16β-hydroxytestosterone were bought from Steraloids Inc. (Wilton, NH). Affinity-isolated, alkaline-phosphatase conjugated, goat $F(ab')_2$ anti-rabbit IgG* (gamma and light chain specific, human IgG adsorbed) was purchased from TAGO Immunologicals Inc. (Immunocorp, Montreal, Quebec).

Animal Treatment and Preparation of Microsomes

Adult (250–300 g) male Long Evans rats (Charles River Laboratories, Montreal, Quebec) were housed in polycarbonate cages on corncob bedding and maintained on 12-hr light and 12-hr dark cycles. The rats were allowed free access to food (PMI Chow, Richmond, VA) and water. After one week of acclimatization, rats received a single i.p. injection of Telazol® in distilled water at a dose of 20, 40, 80, or 120 mg/kg, while control rats received the vehicle at a dose of 1 mL/kg. Rats were decapitated 24 hr after treatment, and hepatic microsomes were prepared from individual animals as described by Thomas *et al.* [11].

Determination of Cytochrome P450 Content and Protein Concentration

Total cytochrome P450 content was determined from the carbon monoxide reduced difference spectrum using the method of Omura and Sato [12]. Protein concentration was determined by the method of Lowry *et al.* [13].

Enzyme Assays

Hepatic microsomal *p*-nitrophenol hydroxylase activity was measured as described by Koop [14] and Reinke and Moyer [15]. Absorbance was measured at 543 nm using a Shimadzu UV-160 spectrophotometer, and the amount of *p*-nitrocatechol formed was determined from a calibration curve. EROD and BROD activities were measured according to the fluorimetric method of Burke *et al.* [16]. All measurements were made using a Shimadzu RF-540 spectrofluorophotometer interfaced with a Shimadzu DR-3 data recorder. The excitation and emission wavelengths were set at 530 and 580 nm, respectively, with a slit width of 2 nm. The amount of resorufin formed was determined from a standard curve of fluorescence intensity versus resorufin concentration. Microsomal testosterone hydroxylase activities were determined by the method of Sonderfan *et al.* [17] with slight modifications as reported previously [9].

Purification of Cytochrome P450 2B1

Cytochrome P450 2B1 was purified to electrophoretic homogeneity from pooled livers of phenobarbital-treated,

* *Abbreviations:* IgG, immunoglobulin G; EROD, ethoxyresorufin O-deethylase; and BROD, benzyloxyresorufin O-dealkylase.

adult female Long Evans rats by a modification of the method of Guengerich *et al.* [18] as reported previously [19].

Preparation of Antibodies

Polyclonal antibodies against rat cytochrome P450 2B1 was raised in female New Zealand rabbits immunized with purified rat cytochrome P450 2B1. IgG was purified from a pool of heat-inactivated high-titer antisera derived from multiple bleedings from several rabbits using a combination of caprylic acid precipitation followed by ammonium sulfate precipitation and final cleanup on a DEAE-Sephacel column. IgG concentration was determined spectrophotometrically at 280 nm; $E_{1\ cm}$ = 13 for a 1% solution in phosphate-buffered saline, pH 7.4. The specificity of the antibody was assessed using Ouchterlony double-diffusion analysis, noncompetitive enzyme-linked immunosorbent assay, and immunoblots with purified rat cytochrome P450 isozymes and with different rat liver microsomal preparations. Anti-cytochrome P450 2B1 IgG reacted with cytochrome P450 2B1, P450 2B2, and a third, noninducible member of the cytochrome P450 2B subfamily, but not with other cytochrome P450 isozymes.

Gel Electrophoresis

SDS–PAGE was performed according to the method of Laemmli [20] using a Hoefer SE 600 vertical slab gel unit. The discontinuous SDS–polyacrylamide gel consisted of a 3.0% acrylamide stacking gel (0.75 mm thick, 1 cm long) and a 7.5% acrylamide separating gel (0.75 mm thick, 12.5 cm long). Microsomes diluted to 0.5 mg/mL in sample dilution buffer containing 0.062 M Tris–HCl (pH 6.8), 1% (w/v) SDS, 10% glycerol, 0.001% (w/v) bromophenol blue, and 5% (v/v) mercaptoethanol were boiled for 2 min. The denatured microsomal proteins (20 μL/well) were subjected to electrophoresis at a constant current of 0.12 mA/gel through the stacking gel and 0.24 mA/gel through the separating gel.

Immunoblots

Proteins resolved by SDS–PAGE were transferred electrophoretically to nitrocellulose membranes according to the method of Towbin *et al.* [21], using a Hoefer Transphor Apparatus (model TE 52) at a setting of 0.4 A for 2 hr at 4°. The membranes were incubated with anti-rat cytochrome P450 2B1 IgG at a concentration of 2.5 μg IgG/mL at 37° for 2 hr with shaking. After washing with wash buffer, the membranes were incubated with alkaline phosphatase-conjugated goat anti-rabbit secondary antibody (1:3000 dilution) at 37° for 2 hr with shaking. Then the nitrocellulose membranes were washed. Substrate solution (0.01% NBT, 0.005% BCIP, in 0.1 M Tris–HCl, 0.5 mM $MgCl_2$, pH 9.5) was added to the membranes under subdued light. The reaction was stopped by immersing the membranes in distilled water. Assay conditions were optimized to ensure that colour development did not proceed beyond the linear response range of the phosphatase reaction.

Immunoquantitation

Staining intensities of the bands on nitrocellulose membranes were measured by computer image analysis with a VISAGE 110 Bio Image Analyzer (Bio Image, Ann Arbor, MI) consisting of a high resolution camera and a Sun Microsystems Workstation. The amount of immunoreactive protein was determined from the integral of the optical density of the stained band using the Whole Band Analysis software option. Values of integrated intensity were converted into picomole quantities using calibration curves generated by loading various concentrations of purified cytochrome P450 2B1 on gels followed by immunoblotting and densitometric analysis as described above. In addition, a single concentration of purified cytochrome P450 2B1 was included on each blot as an internal standard.

Antibody Inhibition Studies

BROD assay was performed as described above except that hepatic microsomes were incubated with increasing concentrations of rabbit anti-rat cytochrome P450 2B1 polyclonal IgG or control rabbit IgG for 5 min prior to initiation of the reaction by addition of NADPH.

Statistical Analysis

Results were analyzed by a single factor analysis of variance, and the differences between pairs of means were tested by the Student–Newman–Keuls test. Differences with a P value of <0.05 were considered to be statistically significant.

RESULTS

Cytochrome P450 Content and Monooxygenase Activities

The total cytochrome P450 content of hepatic microsomes prepared from control (vehicle-treated) and Telazol®-treated rats is presented in Table 1. No significant difference was found between the groups, indicating that treatment with Telazol® at various doses had no effect on total microsomal cytochrome P450 content. Moreover, there was no spectrally detectable peak at 420 nm in microsomes from rats treated with Telazol® at any of the doses tested.

BROD, EROD and *p*-nitrophenol hydroxylase activities were measured in hepatic microsomes prepared from individual control and Telazol®-treated rats, and mean values for each treatment group are reported in Table 1. The mean BROD activity of hepatic microsomes prepared from rats treated with the lowest dose of Telazol® was approximately 3-fold greater than that of the control group. BROD activity increased with increasing dose of Telazol® up to 80

TABLE 1. Effect of treatment with Telazol® on total microsomal cytochrome P450 content, and BROD, EROD, and *p*-nitrophenol hydroxylase activities*

Dose of Telazol® (mg/kg body wt)	Total cytochrome P450 content (nmol/mg protein)	BROD activity (pmol/min/mg protein)	EROD activity (pmol/min/mg protein)	*p*-Nitrophenol hydroxylase activity (nmol/min/mg protein)
Control	1.7 ± 0.1	539 ± 45	740 ± 27	1.1 ± 0.1
20	1.4 ± 0.1	1473 ± 180	729 ± 101	1.2 ± 0.1
40	1.6 ± 0.1	2243 ± 362†	1015 ± 47†	1.2 ± 0.1
80	1.6 ± 0.1	3831 ± 383†	887 ± 26	1.2 ± 0.1
120	1.5 ± 0.0	3876 ± 540†	1142 ± 48†	1.4 ± 0.1

* Each value is the mean ± SEM for six rats per treatment group. EROD activity was recorded after a 5-min incubation, and BROD activity was recorded after a 2-min incubation.

† Mean value of the treatment group was significantly different ($P < 0.05$) from that of the control group.

mg/kg after which there was no further increase in activity. In contrast, only a minor increase in EROD activity was observed in hepatic microsomes prepared from rats treated with the highest dose of Telazol®. No significant difference was found in *p*-nitrophenol hydroxylase activity between control and treated groups at all doses of Telazol® tested.

The rates of formation of various oxidative metabolites of testosterone have been used routinely as catalytic markers for several cytochrome P450 isozymes. The effect of treatment with Telazol® on hepatic microsomal testosterone hydroxylase activities is shown in Table 2. A significant and dose-dependent increase in testosterone 16β-hydroxylase activity was noted for hepatic microsomes prepared from rats treated with Telazol® as compared with the control group. Treatment with Telazol® at a dose of 120 mg/kg resulted in a mean testosterone 16β-hydroxylase activity that was almost 6-fold greater than that of the control group. In contrast, there was no significant difference in mean testosterone 6β- or 7α-hydroxylase activities and in the rate of androstenedione formation between hepatic microsomes from control and Telazol®-treated rats. However, a significant and dose-dependent reduction in testosterone 2α-hydroxylase activity was observed. Similarly, testosterone 16α-hydroxylase activity was decreased significantly at doses of Telazol® of 80 mg/kg and greater.

Immunoquantitation

To determine if Telazol® was exerting an effect at the level of protein expression, cytochrome P450 2B levels in rats treated with various doses of Telazol® were examined by immunoblot analysis. Figure 1 shows an immunoblot of hepatic microsomes probed with polyclonal antibody to cytochrome P450 2B1. Two prominent immunostained protein bands corresponding to cytochromes P450 2B2 (upper band) and P450 2B1 (lower band) were apparent in the lanes containing microsomes from Telazol®-treated rats. A weakly stained third band corresponding to a related protein, possibly cytochrome P450 2B3, was also detected in all of the microsomal samples. The most obvious effect of treatment with Telazol® was a marked increase in the staining intensity of the immunoreactive band corresponding to cytochrome P450 2B1. This result was confirmed by densitometric quantitation of cytochromes P450 2B1 and P450 2B2. As shown in Table 3, there was a dose-dependent increase in microsomal cytochrome P450 2B1 content after treatment with Telazol®. A 17-fold increase in cytochrome P450 2B1 content was found in hepatic microsomes prepared from rats treated with the highest dose of Telazol®. A modest increase in the level of cytochrome P450 2B2 was also seen, but the increase was not statistically significant.

Antibody Inhibition Studies

The role of cytochromes P450 2B1 and P450 2B2 in O-dealkylation of benzyloxyresorufin was studied using polyclonal antibody to cytochrome P450 2B1, and the results are shown in Fig. 2. In the presence of 0.5 mg of anti-cytochrome P450 2B1 IgG/nmol cytochrome P450, BROD

TABLE 2. Effect of treatment with Telazol® on hepatic microsomal testosterone hydroxylase activities*

Dose of Telazol® (mg/kg body wt)	Testosterone metabolites (pmol metabolite formed/min/mg protein)					
	2α	6β	7α	16α	16β	Androstenedione
Control	3752 ± 165	2174 ± 185	228 ± 13	3465 ± 177	68 ± 6	909 ± 43
20	2899 ± 377†	2219 ± 315	242 ± 77	2890 ± 381	158 ± 14	862 ± 87
40	2902 ± 166†	2634 ± 167	312 ± 33	2942 ± 138	222 ± 30†	1002 ± 133
80	2133 ± 178†	2290 ± 228	301 ± 19	2373 ± 161†	342 ± 36†	845 ± 55
120	2043 ± 171†	2488 ± 219	287 ± 21	2355 ± 241†	388 ± 63†	893 ± 61

* Each value is the mean ± SEM for six rats per treatment group.

† Mean value of the treatment group was significantly different ($P < 0.05$) from that of the control group.

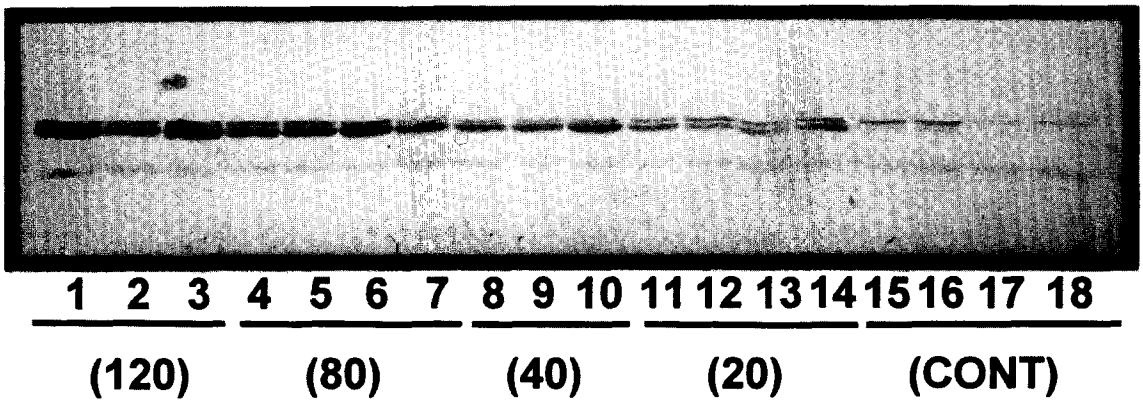

FIG. 1. Immunoblot of liver microsomes from vehicle-treated and Telazol®-treated rats. The blot was probed with polyclonal anti-cytochrome P450 2B1 IgG at a concentration of 2.5 μg IgG/mL as described in Materials and Methods. Microsomal samples were applied to the gel at a final concentration of 10 μg microsomal protein/lane. Lanes 1–14 contain microsomes from individual rats treated with Telazol® at the doses indicated in parentheses, and lanes 15–18 contain microsomes from individual vehicle-treated rats.

activities of hepatic microsomes from a control rat and a rat treated with Telazol® at a dose of 120 mg/kg were inhibited by approximately 25 and 92%, respectively, whereas in the presence of 5 mg anti-cytochrome P450 2B1 IgG/nmol cytochrome P450, BROD activities of hepatic microsomes from the same control and treated rat were inhibited by approximately 55 and greater than 95%, respectively. No inhibition of BROD activity was observed in the presence of control IgG.

DISCUSSION

Telazol® has been used widely as an anesthetic and immobilizing agent in veterinary medicine for over a decade, but little is known about the effect of this drug, or its constituents, on hepatic cytochromes P450. An earlier study reported that treatment of rats with ketamine, a dissociative anesthetic structurally related to tiletamine, produces an inductive effect on cytochromes P450 that is similar to that of phenobarbital [22]. Both treatments significantly enhance total cytochrome P450 content and benzphetamine *N*-demethylase, benzo[*a*]pyrene hydroxylase, and cytochrome *c* reductase activities, but ketamine is much less potent than phenobarbital [22]. No other studies regarding the inductive effects of ketamine, or agents that are structurally similar to tiletamine, were found in the literature. In addition, there are no reports of the effects of zolazepam, a benzodiazepine derivative, on hepatic cytochromes P450. However, in studies involving treatment of rats and mice with better known benzodiazepines including chlordiazepoxide, diazepam, and oxazepam, induction of total cytochrome P450 levels and cytochrome P450-mediated monooxygenase activities such as aniline hydroxylase, aminopyrene *N*-demethylase, and BROD has been observed [23–25]. Thus, the present study was undertaken to examine the effect of treatment with increasing doses of Telazol® on hepatic cytochromes P450 in rats.

Cytochrome P450-mediated enzyme activities and protein levels were measured in hepatic microsomes prepared from rats killed 24 hr after receiving a single i.p. injection of Telazol®. BROD activity, which is highly inducible by treatment with phenobarbital and is catalyzed predominantly by cytochrome P450 2B1 and cytochrome P450 2B2 in the rat, was increased approximately 7-fold in hepatic

TABLE 3. Effect of Telazol® treatment on hepatic levels of cytochrome P450 2B1 and cytochrome P450 2B2*

Dose of Telazol® (mg/kg body wt)	Cytochrome P450 2B1 content (pmol/mg protein)	Cytochrome P450 2B1 as percentage of total cytochrome P450	Cytochrome P450 2B2 content (pmol/mg protein)	Cytochrome P450 2B2 as percentage of total cytochrome P450
Control	2.2 ± 0.8	0.1 ± 0.1	8.2 ± 1.4	0.5 ± 0.1
20	11.3 ± 2.5	0.9 ± 0.2	10.8 ± 2.1	0.8 ± 0.1
40	20.7 ± 3.1†	1.3 ± 0.2†	10.6 ± 0.8	0.7 ± 0.1
80	22.2 ± 3.0†	1.4 ± 0.2†	12.1 ± 1.1	0.8 ± 0.1
120	37.7 ± 6.7†	2.5 ± 0.4†	17.9 ± 5.2	1.2 ± 0.3

* Each value is the mean ± SEM for six rats per treatment group. The cytochrome P450 isozyme contents for individual animals were determined by densitometric quantitation of at least three separate immunoblots.

† Mean value of the treatment group was significantly different ($P < 0.05$) from that of the control group.

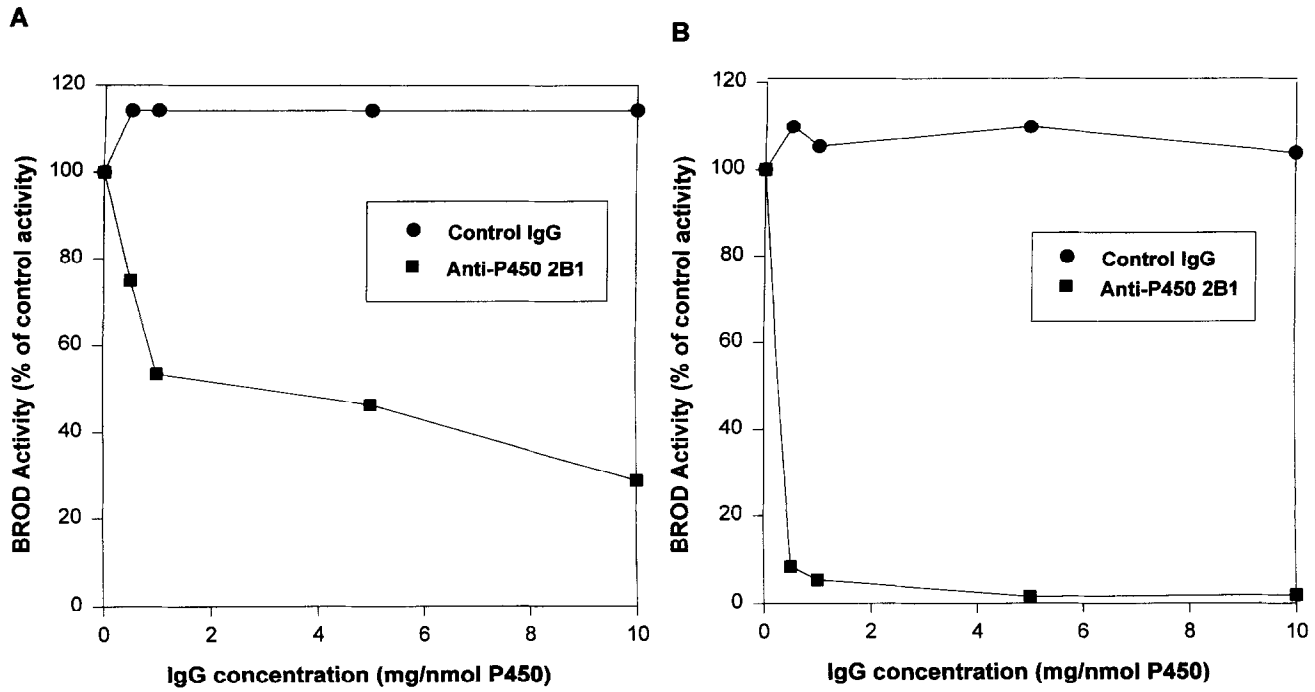

FIG. 2. Effect of polyclonal anti-cytochrome P450 2B1 IgG on BROD activity in hepatic microsomes prepared from (A) an individual vehicle-treated rat and (B) a rat treated with Telazol® at a dose of 120 mg/kg. The reactions were carried out in the presence of various concentrations of control IgG and polyclonal anti-cytochrome P450 2B1 IgG as described in Materials and Methods. Results are expressed as a percent of the activity obtained in the presence of 0 mg IgG. Absolute values of BROD activity determined with hepatic microsomes from the vehicle-treated and Telazol®-treated rats were 484 and 4598 pmol resorufin formed/min/mg, respectively, in the presence of 0 mg IgG; 553 and 4770 pmol resorufin formed/min/mg, respectively, in the presence of 10 mg control IgG/nmol cytochrome P450; and 138 and 86 pmol resorufin formed/min/mg, respectively, in the presence of 10 mg anti-cytochrome P450 2B1 IgG/nmol cytochrome P450.

microsomes from rats treated with Telazol® at doses of 80 mg/kg and greater, whereas EROD activity, which has been used as an indicator for cytochrome P450 1A1, was increased only slightly at the highest dose of Telazol®. The increase in BROD activity was ascribed to induction of the cytochrome P450 2B isozymes. The minor increase in EROD activity could also have been a result of cytochrome P450 2B induction as the majority of this activity has been shown to be catalyzed by cytochromes P450 2B1 and P450 2C6 in rats treated with phenobarbital [26]. Microsomal *p*-nitrophenol hydroxylase activity was not affected by treatment with Telazol®, suggesting that the drug had no effect on expression of cytochrome P450 2E1. In addition, the rates of formation of testosterone metabolites by microsomes from rats treated with Telazol® reflected selective induction of the cytochrome P450 2B isozymes. A dose-related increase in the rate of formation of 16β-hydroxytestosterone, which is a selective probe for cytochrome P450 2B1 activity, was evident in hepatic microsomes prepared from rats treated with Telazol®, while testosterone 6β- and 7α-hydroxylase activities were unaltered, indicating that the drug had no effect on expression of cytochromes P450 3A and 2A1. The observed decrease in testosterone 2α- and 16α-hydroxylase activities was likely a result of suppression of cytochrome P450 2C11 levels, which has been reported to occur in mature male rats after pretreatment with various cytochrome P450 inducers [17, 27]. Testosterone 2α-hydroxylation is known to be catalyzed solely by cytochrome P450 2C11 [17, 27], whereas testosterone 16α-hydroxylation is mediated by cytochromes P450 2C11, P450 2B1, and P450 2B2 [17, 27]. Similarly, formation of androstenedione is catalyzed by cytochromes P450 2B1, P450 2B2, and P450 2C11, and the rate of androstenedione formation was unaltered after treatment with Telazol®. Lack of induction of both testosterone 16α-hydroxylase activity and androstenedione formation may be attributed to decreased hepatic expression of cytochrome P450 2C11 that offset the expected increases in both activities by cytochrome P450 2B1.

Immunoblot analyses confirmed that cytochrome P450 2B1 was preferentially induced by Telazol® in a dose-dependent manner. According to the immunoquantitation data, there was a 17-fold increase in microsomal cytochrome P450 2B1 content for rats treated with Telazol® at a dose of 120 mg/kg. There also appeared to be a dose-related increase in the level of cytochrome P450 2B2, but the increase was not statistically significant. Studies have shown that induction of cytochrome P450 2B1 is accompanied by increased expression of cytochrome P450 2B2 [28–30]. Certain inducers may alter the proportion of cytochrome P450 2B1 to P450 2B2, but selective induction of one of these isozymes without co-induction of the other

form has not been reported [31]. The absence of a significant increase in the hepatic level of cytochrome P450 2B2 in this study suggests that the dose of Telazol® or the duration of treatment was not sufficient to induce this isozyme.

Increases in hepatic levels of cytochrome P450 2B1 were greater than the observed increases in BROD and testosterone 16β-hydroxylase activities at all doses of Telazol® tested. Increases in protein levels are often different from corresponding increases in catalytic activities [32, 33]. A possible explanation for the discrepancy in the present study is that cytochrome P450 2B1 may be involved in the metabolism of either tiletamine or zolazepam and, consequently, cytochrome P450 2B-mediated activities measured with substrates such as benzyloxyresorufin and testosterone did not reflect the full extent of induction. However, there was no evidence of enzyme-metabolite complex formation, nor was there evidence of increased levels of inactive cytochrome P450 (i.e. P420) in microsomes prepared from Telazol®-treated rats.

Antibody inhibition of microsomal BROD activity by polyclonal anti-cytochrome P450 2B1 IgG indicated that BROD activity was catalyzed by cytochrome P450 2B1/2 in hepatic microsomes from a Telazol®-treated rat, whereas no more than 70% of the activity was contributed by these isozymes in hepatic microsomes from a control rat. Our immunoinhibition data are in close agreement with those of a recent study that reported maximal inhibition of BROD activity in microsomes from untreated rats to be approximately 70% in the presence of high concentrations of antibody to cytochrome P450 2B1 [26]. Hence, the results of the catalytic, immunoquantitation, and antibody inhibition assays demonstrate that Telazol® selectively induced expression of the cytochrome P450 2B isozymes in rats and did not increase expression of cytochromes P450 1A, P450 2E1, or P450 3A.

Microsomal BROD and testosterone 16β-hydroxylase activities and cytochrome P450 2B1 levels increased with increasing dose with the greatest effect observed at a dose of 120 mg/kg. However, the doses at which induction occurred were relatively high compared with those normally used to produce anesthesia in rats. In previous studies, the doses given to rats to achieve satisfactory anesthesia and analgesia ranged from 20 to 40 mg/kg [34, 35]. The dose requirement of Telazol® varies with factors such as species, age, gender, and physiological status of the animal. In general, larger species tend to require a lesser dose on a per weight basis than do smaller species [36], because of their slower metabolite rate. Satisfactory pharmacological effects were produced, for instance, in lions at doses from 2.2 to 3 mg/kg intramuscularly [37] and in black leopards, from 5 to 6.25 mg/kg intramuscularly [38]. Similarly, optimal immobilization of polar bears occurred at doses of 5–8 mg/kg [6, 39]. The pharmacological effect of Telazol® in rats can be correlated with that in polar bears by comparing the onset and duration of action of the drug. In rats, a dose of 40 mg/kg produced a mean onset of anesthesia of 2.2 min with an average duration of 47.7 min [34]. In polar bears, an average dose of 5.1 mg/kg had a mean onset of immobilization of 5 min with a duration of approximately 2 hr [6]. In another study, an average dose of 7.9 mg/kg of Telazol® was used and the time to full immobilization was 5.3 min with a mean duration of 54.5 min [39]. Hence, it can be seen that a dose that is pharmacologically equivalent to 120 mg/kg of Telazol® in rats would be too high for use in polar bear and other species of a similar size. Note that a single injection was selected for the present study to approximate the protocol used routinely in the clinic and in the field, which involves a single i.m. or i.v. injection rather than repeated daily injections as in typical induction studies.

In previous studies [9, 10], induction of polar bear hepatic cytochromes P450 had been proposed as an indicator of exposure to environmental contamination in the arctic ecosystem. Cytochrome P450 1A and 2B isozyme levels were measured in polar bear hepatic microsomes and correlated to monooxygenase activities and organochlorine concentrations in liver [10]. Because the bears had been immobilized with Telazol® prior to death, it had been suggested that the drug may have had an inductive effect on the expression of cytochromes P450. The dose of Telazol® administered to the bears in those studies ranged from 5.1 to 14 mg/kg with a mean dose of 8.9 mg/kg. On the basis of the results presented herein, a dose of 8.9 mg/kg is probably too small for induction of cytochrome P450 2B1 to have occurred. Hence, the induction of cytochromes P450 1A and 2B found in polar bears was likely a result of exposure to environmental contaminants in the arctic food chain, rather than an effect produced by Telazol®. A second study is currently underway to investigate the effects of tiletamine and zolazepam on hepatic cytochromes P450 in rat.

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References

1. Lin HC, Thurmon JC, Benson GJ and Tranquilli WJ, Telazol®—A review of its pharmacology and use in veterinary medicine. *J Vet Pharmacol Ther* **16:** 383–418, 1992.
2. Schobert E, Telazol® use in wild and exotic animals. *Vet Med* **82:** 1080–1088, 1987.
3. Gibeau ML and Paquet PC, Evaluation of Telazol® for immobilization of black bears. *Wildl Soc Bull* **19:** 400–402, 1991.
4. Bush M, Custer R, Smeller J, Bush LM, Seal US and Barton R, The acid-base status of lions, *Panthera leo*, immobilized with four drug combinations. *J Wildl Dis* **14:** 102–109, 1978.
5. Boever WJ, Holden J and Kane KK, Use of Telazol® (CI-744) for chemical restraint and anesthesia in wild and exotic carnivores. *Vet Med Sm Anim Clin* **72:** 1722–1725, 1977.
6. Haigh JC, Stirling I and Broughton E, Immobilization of polar bears (*Ursus maritimus* PHIPPS) with a mixture of tiletamine hydrochloride and zolazepam hydrochloride. *J Wildl Dis* **21:** 43–47, 1985.
7. Stegeman JJ, Brouwer M, Di Giulio R, Förlin L, Fowler BA, Sanders BM and Van Veld PA, Molecular responses to environmental contamination: Enzyme and protein systems as in-

dicators of chemical exposure and effect. In: *Biomarkers: Biochemical, Physiological and Histologic Markers of Anthropogenic Stress* (Eds. Huggett RJ, Kimerle RA, Mehrle PM Jr and Bergman HL), pp. 235–335. Lewis Publishers, Chelsea, 1992.
8. Lubet RA, Nims RW, Beebe LE, Fox SD, Issaq HJ and McBee K, Induction of hepatic CYP1A activity as a biomarker for environmental exposure to Aroclor 1254 in feral rodents. *Arch Environ Contam Toxicol* **22:** 339–344, 1992.
9. Bandiera SM, Torok SM, Lin S, Ramsay MA and Norstrom RJ, Catalytic and immunologic characterization of hepatic and lung cytochromes P450 in the polar bear. *Biochem Pharmacol* **49:** 1135–1146, 1995.
10. Letcher RJ, Norstrom RJ, Lin S, Ramsay MA and Bandiera SM, Immunoquantitation and microsomal monooxygenase activities of hepatic cytochromes P450 1A and P450 2B and chlorinated hydrocarbon contaminant levels in polar bear (*Ursus maritimus*). *Toxicol Appl Pharmacol* **137:** 127–140, 1996.
11. Thomas PE, Reik LM, Ryan DE and Levin W, Induction of two immunochemically related rat liver cytochrome P450 isozymes, cytochrome P450c and P450d, by structurally diverse xenobiotics. *J Biol Chem* **258:** 4590–4598, 1983.
12. Omura T and Sato R, The carbon monoxide binding pigment of liver microsomes: I. Evidence for its hemoprotein nature. *J Biol Chem* **239:** 2370–2378, 1964.
13. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
14. Koop DR, Hydroxylation of *p*-nitrophenol by rabbit ethanol-inducible cytochrome P450 isozymes 3a. *Mol Pharmacol* **29:** 399–404, 1986.
15. Reinke LA and Moyer MJ, *p*-Nitrophenol hydroxylation: A microsomal oxidation which is highly inducible by ethanol. *Drug Metab Dispos* **13:** 548–552, 1985.
16. Burke MD, Thompson S, Elcombe CR, Halpert J, Haaparanta T and Mayer RT, Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: A series of substrates to distinguish between different induced cytochromes P450. *Biochem Pharmacol* **34:** 3337–3345, 1985.
17. Sonderfan AJ, Arlotto MP, Dutton DR, McMillen SK and Parkinson A, Regulation of testosterone hydroxylation by rat liver microsomal cytochrome P450. *Arch Biochem Biophys* **255:** 27–41, 1987.
18. Guengerich FP, Dannan GA, Wright ST, Martin MV and Kaminsky LS, Purification and characterization of liver microsomal cytochrome P-450: Electrophoretic, spectral, catalytic, and immunochemical properties and inducibility of eight isozymes isolated from rats treated with phenobarbital or β-naphthoflavone. *Biochemistry* **21:** 6019–6030, 1982.
19. Chang T, Levine M, Bandiera SM and Bellward GD, Selective inhibition of rat hepatic microsomal cytochrome P-450. I. Effect of the *in vivo* administration of cimetidine. *J Pharmacol Exp Ther* **260:** 1441–1449, 1992.
20. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **207:** 680–685, 1970.
21. Towbin H, Staehelin T and Gordon J, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedures and some applications. *Proc Natl Acad Sci USA* **76:** 4350–4354, 1979.
22. Marietta MP, Vore ME, Way WL and Trevor AJ, Characterization of ketamine induction of hepatic microsomal drug metabolism. *Biochem Pharmacol* **26:** 2451–2453, 1977.
23. Jabłońska JK, Knobloch K, Majka J and Wiśniewska-Knypl JM, Stimulatory effects of chlordiazepoxide, diazepam and oxazepam on the drug-metabolizing enzymes in microsomes. *Toxicology* **5:** 103–111, 1975.
24. Diwan BA, Rice JM and Ward JM, Tumor-promoting activity of benzodiazepine tranquilizers, diazepam and oxazepam, in mouse liver. *Carcinogenesis* **7:** 789–794, 1986.
25. Griffin RJ, Burka LT and Cunningham ML, Activity of hepatic drug metabolizing enzymes following oxazepam-dosed feed treatment in B6C3F1 mice. *Toxicol Lett* **76:** 251–256, 1995.
26. Burke MD, Thompson S, Weaver RJ, Wolf CR and Mayer RT, Cytochrome P450 specificities of alkoxyresorufin O-dealkylation in human and rat liver. *Biochem Pharmacol* **48:** 923–936, 1994.
27. Ryan DE and Levin W, Purification and characterization of hepatic microsomal cytochrome P450. *Pharmacol Ther* **45:** 153–239, 1990.
28. Thomas PE, Reik LM, Ryan DE and Levin W, Regulation of three forms of cytochrome P450 and epoxide hydrolase in rat liver microsomes. Effects of age, sex and induction. *J Biol Chem* **256:** 1044–1052, 1981.
29. Parkinson A, Safe SH, Robertson LW, Thomas PE, Ryan DE, Reik LM and Levin W, Immunochemical quantitation of cytochrome P-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rats: A study of structure–activity relationships. *J Biol Chem* **258:** 5967–5976, 1983.
30. Dannan GA, Guengerich FP, Kaminsky LS and Aust SD, Regulation of cytochrome P450: Immunochemical quantitation of eight isozymes in liver microsomes of rats treated with polybrominated biphenyl cogeners. *J Biol Chem* **258:** 1282–1288, 1983.
31. Kelley M, Hantelle P, Safe S, Levin W and Thomas PE, Co-induction of cytochrome P450 isozymes in rat liver by 2,4,5,2′,4′,5′-hexachlorobiphenyl or 3-methoxy-4-aminoazobenzene. *Mol Pharmacol* **32:** 206–211, 1987.
32. Nims RW, Lubet RA, Jones CR, Mellini DW and Thomas PE, Comparative pharmacodynamics of CYP2B induction by phenobarbital in the male and female F344/NCr rat. *Biochem Pharmacol* **45:** 521–526, 1993.
33. Nims RW, McClain RM, Manchand PS, Belica PS, Thomas PE, Mellini DM, Utermahlen WE Jr and Lubet RA, Comparative pharmacodynamics of hepatic cytochrome P450 2B induction by 5,5-diphenyl- and 5,5-diethyl-substituted barbiturates and hydantoins in the male F344/NCr rat. *J Pharmacol Exp Ther* **270:** 348–355, 1994.
34. Silverman J, Huhndorf M, Balk M and Slater G, Evaluation of a combination of tiletamine and zolazepam as an anesthetic for laboratory rodents. *Lab Anim Sci* **33:** 457–460, 1983.
35. Ward GS, Johnsen DO and Roberts CR, The use of CI 744 as an anesthetic for laboratory animals. *Lab Anim Sci* **24:** 737–742, 1974.
36. Eads FE, Tilazol™ (CI-744). A new agent for chemical restraint and anesthesia in nonhuman primates. *Vet Med Sm Anim Clin* **71:** 648–652, 1976.
37. Gray CW, Bush M and Beck CC, Clinical experience using CI-744 in chemical restraint and anesthesia of exotic specimens. *J Zoo Anim Med* **5:** 12–21, 1974.
38. Seidensticker J, Tamang KM and Gray CW, The use of CI-744 to immobilize free-ranging tigers and leopards. *J Zoo Anim Med* **5:** 22–25, 1974.
39. Stirling I, Spencer C and Andriashek D, Immobilization of polar bears (*Ursus maritimus*) with Telazol® in the Canadian arctic. *J Wildl Dis* **25:** 159–168, 1989.