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Induction of Hepatic Cytochrome P450 2B and P450 3A Isozymes in Rats by Zolazepam, a Constituent of Telazol®

*Anne Wong and Stelvio M. Bandiera**

Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3

ABSTRACT. Telazol®, a 1:1 combination of tiletamine HCl and zolazepam HCl, is an anesthetic and immobilizing agent that is capable of inducing cytochrome P450 (CYP) 2B isozymes in rats. The primary goal of the present study was to determine the constituent of Telazol® responsible for the enzyme induction. A secondary goal was to compare the effects produced by Telazol® and its constituents with those elicited by sodium phenobarbital (PB) using the same dosing regimen. Adult male Long Evans rats were given a single i.p. injection of tiletamine or zolazepam at a dose of 60 mg/kg, Telazol® at a dose of 120 mg/kg, PB at a dose of 60 and 120 mg/kg, or vehicle at a dose of 1 mL/kg. Animals were killed 24 hr later, and hepatic microsomes were prepared. Treatment with zolazepam and Telazol® increased microsomal benzyloxyresorufin *O*-dealkylase (BROD) activity by approximately 9- and 15-fold, respectively, and increased microsomal testosterone 16β-hydroxylase activity by 5- and 8-fold, respectively. Treatment with tiletamine had a slight, but insignificant, effect on CYP-mediated enzyme activities. In comparison, BROD and testosterone 16β-hydroxylase activities were increased by 22- and 13-fold, respectively, after treatment with PB at a dose of 60 mg/kg. Densitometric quantitation of immunoblots revealed that the hepatic CYP2B content was elevated by approximately 15-, 22-, and 25-fold, and the hepatic CYP3A content was increased by 2-, 2-, and 8-fold after treatment with zolazepam, Telazol®, and PB, respectively. In contrast, levels of CYP1A1 and CYP2E1 were unaltered after treatment. In summary, the results indicate that zolazepam was the constituent primarily responsible for the inductive effect of Telazol®, and the pattern of enzyme induction produced by zolazepam and Telazol® was similar to, but weaker than that elicited by PB at a similar dosing regimen. BIOCHEM PHARMACOL **55**;2:201–207, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. tiletamine; zolazepam; Telazol®; cytochrome P450; induction; rat liver

Telazol® is officially approved for use in the United States as an injectable anesthetic for cats and dogs. It has also proven to be an excellent immobilizing agent for field studies involving large animals such as lions and polar bears [1]. Telazol® is a 1:1 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 belongs to a class of dissociative anesthetics that includes ketamine and phencyclidine [2], whereas zolazepam is a benzodiazepine derivative structurally similar to diazepam [2]. The pharmacological activities of tiletamine include rapid induction, profound analgesia, normal pharyngeal-laryngeal reflexes, and cataleptoid anesthesia [2]. It is a poor muscle relaxant when used alone, and it may induce convulsions at high doses [3]. Zolazepam, on the other hand, is an excellent anticonvulsant, anxiolytic, and muscle relaxant [4]. When used in combination with tiletamine, it prevents tiletamine-induced convulsions, produces optimal muscle relaxation, and provides smooth emergence from anesthesia [2]. The use of tiletamine and zolazepam in a 1:1 ratio (Telazol®) is ideal in enhancing the pharmacological activities of both agents while minimizing adverse effects associated with the use of either agent alone [2].

The hepatic CYP† enzymes constitute part of a large family of ubiquitous hemeprotein enzymes and are involved in the biotransformation of lipophilic endogenous and xenobiotic compounds in the body. In a recent study, we showed that acute treatment with Telazol® increased hepatic levels of CYP2B1 and CYP2B2, as well as CYP2B-catalyzed enzyme activities, namely BROD and testosterone 16β-hydroxylation, in adult male rats in a dose-dependent manner [5]. This was the first report of hepatic microsomal enzyme induction by this drug. However, as no

* Corresponding author: Dr. Stelvio M. Bandiera, Faculty of Pharmaceutical Sciences, University of British Columbia, 2146 East Mall, Vancouver, BC, Canada V6T 1Z3. Tel. (604) 822-3815; FAX (604) 822-3035.
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† *Abbreviations:* BROD, benzyloxyresorufin *O*-dealkylase; CYP, cytochrome P450 (EC 1.14.14.1); EROD, ethoxyresorufin *O*-deethylase; IgG, immunoglobulin; PB, sodium phenobarbital; and PROD, pentoxyresorufin *O*-dependentylase.

information is available in the literature regarding the effect of tiletamine or zolazepam on CYP, the constituent responsible for the inductive effects of Telazol® remains to be determined.

Induction of CYP enzymes by dissociative anesthetics and benzodiazepines that are structurally related to tiletamine and zolazepam has been reported previously. Ketamine, a potent, rapid-acting dissociative anesthetic, was shown to elicit induction of CYP enzyme activities including benzphetamine *N*-demethylase and benzo[*a*]pyrene hydroxylase activities in rats [6]. Similarly, treatment of rats and mice with benzodiazepines such as oxazepam and chlordiazepoxide produces dose-dependent increases in liver weight, hepatic protein content, and total CYP content [7–11]. In particular, high doses of dietary oxazepam elicited increases of 6-, 50-, and 28-fold in BROD, EROD, and PROD activities, respectively, in mice [12].

The purpose of the present study was to examine the effects of tiletamine and zolazepam on hepatic expression of CYP enzymes in rats. In addition, the effects of each constituent will be compared with those produced by PB, a prototypic CYP2B inducer, using a similar dosing regimen.

MATERIALS AND METHODS

Chemicals

Dr. M. A. Ramsay, University of Saskatchewan, provided the Telazol® (A. H. Robins) and Dr. H. A. Semple, University of Saskatchewan, provided the tiletamine and zolazepam used in the study. All other chemicals were obtained from sources described previously [5].

Animal Treatment and Preparation of Microsomes

Adult male Long Evans rats were purchased from Charles River Laboratories. Rats 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) and water. After 1 week of acclimatization, rats (253–306 g) (six per group) received a single i.p. injection of Telazol® at a dose of 120 mg/kg, tiletamine at a dose of 60 mg/kg, or zolazepam at a dose of 60 mg/kg, while control rats received the vehicle (distilled water) at a dose of 1 mL/kg. In another treatment set, rats (253–313 g) (five per group) received a single i.p. injection of PB at a dose of 60 mg/kg, 120 mg/kg, or vehicle (distilled water) at a dose of 1 mL/kg. Animals from both treatment sets were decapitated 24 hr after treatment, and hepatic microsomes were prepared from individual animals as described by Thomas *et al.* [13].

Determination of CYP Content and Protein Concentration

Total CYP content was determined from the carbon monoxide reduced difference spectrum using the method of Omura and Sato [14]. Protein concentration was determined by the method of Lowry *et al.* [15].

Enzyme Assays

Microsomal BROD and EROD activities were measured using a fluorimetric method as described previously [5]. Microsomal testosterone hydroxylase activities were determined by the method of Sonderfan *et al.* [16], with the following modification. Gradient elution of the HPLC column was achieved using a mobile phase consisting of 100% solvent A (methanol:water:acetonitrile, 39:60:1) for the first 10 min, followed by a linear gradient to 45% solvent B (methanol:water:acetonitrile, 80:18:2) from 10 to 28 min, then a second linear gradient to 100% solvent B from 28 to 29 min. Solvent B was held at 100% for 2 min, followed by a return to 100% solvent A at 31 min and re-equilibration with 100% solvent A for a further 2 min. Total flow rate for the analysis was 2 mL/min.

Preparation of Antibodies

Polyclonal antibodies against rat CYP2B1, CYP1A1, and CYP3A1 were raised separately in female New Zealand rabbits immunized with electrophoretically homogenous proteins as described previously [17]. The specificity of each antibody was assessed using Ouchterlony double-diffusion analysis, noncompetitive enzyme-linked immunosorbent assay, and immunoblots with purified rat CYP isozymes and with different rat liver microsomal preparations. The antibodies reacted with the following proteins, but not other CYP isozymes. Anti-CYP2B1 IgG reacted with CYP2B1, CYP2B2, and a third, noninducible member of the CYP2B subfamily. Anti-CYP1A1 IgG reacted with CYP1A1 and CYP1A2. Anti-CYP3A1 IgG was back-absorbed as described [17] and reacted with CYP3A1 predominantly, although it also recognized CYP3A2. Antibody to rat CYP2E1 was provided by Dr. Paul E. Thomas, Rutgers-The State University of New Jersey.

Gel Electrophoresis

SDS–PAGE was performed according to the method of Laemmli [18] as described previously [5].

Immunoblots and Immunoquantitation

Proteins resolved by SDS–PAGE were transferred electrophoretically to nitrocellulose membranes according to the method of Towbin *et al.* [19]. Densitometric quantitation of immunoblots was performed using computer image analysis with a VISAGE 110 Bio Image Analyzer as described previously [5]. Defined amounts of purified CYP2B1 or CYP3A1 were included on each gel for construction of calibration curves.

TABLE 1. Effect of treatment with Telazol®, tiletamine, zolazepam, or PB on total CYP content, and BROD and EROD activities*

Treatment (dose)	Total CYP content (nmol/mg protein)	BROD activity (pmol/min/mg protein)	EROD activity (pmol/min/mg protein)
Control	1.4 ± 0.1 (1.0)	257 ± 24 (1.0)	409 ± 44 (1.0)
Telazol® (120 mg/kg)	1.2 ± 0.0 (0.9)	3933 ± 634† (15.3)	429 ± 70 (1.0)
Tiletamine (60 mg/kg)	1.3 ± 0.1 (0.9)	536 ± 66‡ (2.1)	441 ± 75 (1.1)
Zolazepam (60 mg/kg)	1.2 ± 0.1 (0.9)	2292 ± 201†‡ (8.9)	438 ± 70 (1.1)
Control	1.3 ± 0.1 (1.0)	323 ± 22 (1.0)	529 ± 50 (1.0)
PB (60 mg/kg)	1.8 ± 0.1† (1.4)	6974 ± 118† (21.6)	1035 ± 55† (2.0)
PB (120 mg/kg)	1.9 ± 0.1† (1.5)	7292 ± 56† (22.6)	1037 ± 92† (2.0)

* Each value is the mean ± SEM for five or six rats per treatment group. Numbers in parentheses denote the difference relative to the value of the control group.

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

‡ Mean value of the tiletamine or zolazepam treatment group was significantly different ($P < 0.05$) from that of the Telazol® treatment group.

Statistical Analysis

Results were analyzed by a one-way ANOVA, and the differences between pairs of means were tested by the Student–Newman–Keuls test (SNK). Two-way ANOVA was used to determine the interacting effect between tiletamine and zolazepam. Mean differences that had a P value of < 0.05 were considered to be statistically significant.

RESULTS

CYP Content and Monooxygenase Activities

The total CYP content of hepatic microsomes prepared from rats treated with Telazol®, tiletamine, zolazepam, and PB is reported in Table 1. It can be seen that none of the treatments, except PB, had a significant effect on total hepatic CYP content. The total CYP content of hepatic microsomes prepared from rats treated with PB at doses of 60 and 120 mg/kg was increased significantly as compared with the respective vehicle-treated group.

Microsomal BROD and EROD activities are also presented in Table 1. Treatment with zolazepam and Telazol® resulted in significant increases in BROD activity of 9- and 15-fold, respectively, whereas treatment with tiletamine produced a small and insignificant increase in BROD activity. All three agents had no effect on EROD activity. In comparison, BROD activity was increased by 22- and 23-fold, and EROD activity was increased by 2-fold, after treatment with PB at doses of 60 and 120 mg/kg.

The effect of treatment with Telazol®, tiletamine, zolazepam, and PB on hepatic microsomal testosterone hydroxylase activities is shown in Table 2. Treatment with Telazol® and zolazepam resulted in increases in testosterone 16β-hydroxylase activity of 8- and 5-fold, respectively, relative to the control group. In contrast, testosterone 2α- and 16α-hydroxylase activities were decreased significantly after treatment with Telazol®, tiletamine, and zolazepam, but there was no significant difference in the rates of formation of 6β-hydroxytestosterone, 7α-hydroxytestosterone, and androstenedione between control and treatment groups. In comparison, treatment with PB at doses of 60 and 120 mg/kg resulted in increases of 13- and 17-fold for testosterone 16β-hydroxylase activity, respectively, increases of 1.6- and 1.7-fold for testosterone 6β-hydroxylase activity, respectively, and a 2-fold increase in the rate of formation of androstenedione. Testosterone 16α-hydroxylase activity was unaltered in hepatic microsomes prepared from rats treated with either dose of PB, but a significant decrease in testosterone 2α-hydroxylase activity was observed with the higher dose.

Immunoquantitation

The enzyme activity data suggested that zolazepam, like Telazol®, had an effect on CYP2B-mediated activities, but not on activities catalyzed by other isozymes such as CYP1A or CYP3A. To confirm that CYP2B protein levels were induced by treatment with zolazepam, and to determine if hepatic levels of other isozymes might be affected without an apparent change in enzyme activities, blots containing microsomal proteins from control and treatment groups were prepared and probed with polyclonal antibodies against CYP2B1, CYP3A, CYP2E1, and CYP1A1. Figure 1A shows an immunoblot of hepatic microsomes from rats treated with Telazol®, tiletamine, zolazepam, or vehicle and probed with anti-CYP2B1 IgG. Three immunostained bands corresponding to CYP2B2 (upper band) and CYP2B1 (middle band), as well as a third related protein, possibly CYP2B3 (lower band), were visible in

TABLE 2. Effect of treatment with Telazol®, tiletamine, zolazepam, or PB on microsomal testosterone hydroxylase activities*

Treatment (dose)	Testosterone metabolites (pmol metabolite formed/min/mg protein)					
	2α	6β	7α	16α	16β	Androstenedione
Control	2628 ± 316 (1.0)	2521 ± 242 (1.0)	204 ± 21 (1.0)	3553 ± 426 (1.0)	192 ± 15 (1.0)	2354 ± 322 (1.0)
Telazol® (120 mg/kg)	903 ± 150† (0.3)	2534 ± 212 (1.0)	241 ± 34 (1.2)	2067 ± 198† (0.6)	1522 ± 271† (7.9)	2486 ± 299 (1.1)
Tiletamine (60 mg/kg)	1753 ± 215†‡ (0.7)	2278 ± 226 (0.9)	218 ± 18 (1.1)	2475 ± 308† (0.7)	284 ± 32‡ (1.5)	1847 ± 194 (0.8)
Zolazepam (60 mg/kg)	1315 ± 100† (0.5)	2698 ± 248 (1.1)	256 ± 18 (1.3)	2317 ± 208† (0.7)	895 ± 58†‡ (4.7)	1996 ± 194 (0.8)
Control	2017 ± 161 (1.0)	2246 ± 278 (1.0)	261 ± 35 (1.0)	2820 ± 237 (1.0)	179 ± 8 (1.0)	1547 ± 63 (1.0)
PB (60 mg/kg)	1743 ± 195 (0.9)	3512 ± 233† (1.6)	260 ± 28 (1.0)	2829 ± 203 (1.0)	2398 ± 125† (13.4)	2604 ± 318† (1.7)
PB (120 mg/kg)	1154 ± 113† (0.6)	3748 ± 433† (1.7)	339 ± 23 (1.3)	3161 ± 125 (1.1)	3123 ± 52† (17.4)	2673 ± 49† (1.7)

* Each value is the mean ± SEM for five or six rats per treatment group. Numbers in parentheses denote the difference relative to the value of the control group.
† Mean value of the treatment group was significantly different ($P < 0.05$) from that of the control group.
‡ Mean value of the tiletamine or zolazepam treatment group was significantly different ($P < 0.05$) from that of the Telazol® treatment group.

almost all lanes containing hepatic microsomes. Although the middle band and, to some extent, the upper band were extremely faint in lanes containing microsomes from vehicle-treated (control) rats, there was a progressive increase in the staining intensities of these two bands when lanes containing microsomes from vehicle-treated rats were compared with lanes containing microsomes from rats treated with tiletamine, zolazepam, and Telazol®. A similar immunoblot of hepatic microsomes prepared from rats treated with PB is presented in Fig. 1B. A dramatic increase in the staining intensities of immunoreactive bands corresponding to CYP2B1 and CYP2B2 was apparent in microsomal samples from rats treated with PB at doses of 60 and 120 mg/kg.

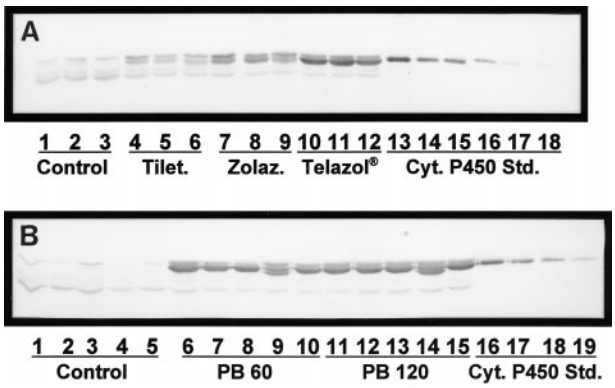

FIG. 1. Immunoblots of rat hepatic microsomes probed with anti-CYP2B1 IgG. The blots were incubated with polyclonal antibody against rat CYP2B1 at a concentration of 2 μg IgG/mL as described in "Materials and Methods." Microsomal samples were applied to the gel at a final concentration of 10 μg microsomal protein/lane. In panel A, lanes 1–3 contained microsomes from individual vehicle-treated rats, lanes 4–6 contained microsomes from individual rats treated with tiletamine at a dose of 60 mg/kg, lanes 7–9 contained microsomes from rats treated with zolazepam at a dose of 60 mg/kg, lanes 10–12 contained microsomes from rats treated with Telazol® at a dose of 120 mg/kg, and lanes 13–18 contained purified rat CYP2B1 at concentrations of 1.0, 0.5, 0.5, 0.25, 0.1, and 0.05 pmol/lane, respectively. In panel B, lanes 1–5 contained microsomes from individual vehicle-treated rats, lanes 6–15 contained microsomes from individual rats treated with PB at a dose of 60 mg/kg (lanes 6–10), or 120 mg/kg (lanes 11–15), and lanes 16–19 contained purified rat CYP2B1 at concentrations of 1.0, 0.5, 0.25, and 0.1 pmol/lane, respectively.

Hepatic levels of CYP2B1 together with CYP2B2 were measured by densitometric quantitation of immunoblots. As shown in Table 3, treatment with zolazepam and Telazol® resulted in increases in the mean hepatic content of CYP2B isozymes of 14- and 22-fold, respectively, relative to the vehicle-treated group. Treatment with tiletamine caused a slight but insignificant increase in the hepatic content of the isozymes. In comparison, the hepatic content of CYP2B isozymes was increased by approximately 25-fold after treatment with PB at both doses.

An immunoblot of hepatic microsomes from rats treated with Telazol®, tiletamine, zolazepam, or vehicle, and probed with anti-CYP3A IgG is displayed in Fig. 2A. A single band representing both CYP3A1 and CYP3A2 was detected in all of the lanes. The staining intensity of the band in lanes containing hepatic microsomes from rats treated with zolazepam and Telazol® appeared to be slightly greater than in lanes containing hepatic microsomes from rats treated with vehicle or tiletamine. A similar immunoblot of hepatic microsomes from rats treated with PB is shown in Fig. 2B. Again, the staining intensity of the band was greater with hepatic microsomes prepared from rats treated with PB than with vehicle, but the amount of staining appeared to be similar between the two doses of PB.

These observations were confirmed by densitometric quantitation of the immunoblots (Table 3). Hepatic levels of CYP3A (CYP3A1 plus CYP3A2) were increased by approximately 2-fold after treatment with Telazol® or zolazepam. In contrast, treatment with tiletamine had no

TABLE 3. Effect of treatment with Telazol®, tiletamine, zolazepam, or PB on hepatic levels of CYP2B and CYP3A isozymes*

Treatment (dose)	CYP2B content (pmol/mg protein)	CYP2B as percentage of total CYP	CYP3A content (pmol/mg protein)	CYP3A as percentage of total CYP
Control	6.5 ± 2.1 (1.0)	0.5 ± 0.1 (1.0)	40.0 ± 4.4 (1.0)	2.9 ± 0.2 (1.0)
Telazol® (120 mg/kg)	141.9 ± 19.3† (21.8)	11.6 ± 1.5† (23.2)	92.1 ± 13.3† (2.3)	7.5 ± 1.0† (2.6)
Tiletamine (60 mg/kg)	26.1 ± 4.9‡ (4.0)	2.0 ± 0.3‡ (4.0)	35.4 ± 3.0‡ (0.9)	2.7 ± 0.2‡ (0.9)
Zolazepam (60 mg/kg)	94.1 ± 6.3†‡ (14.5)	7.6 ± 0.5†‡ (15.2)	72.0 ± 8.0† (1.8)	5.9 ± 0.8† (2.0)
Control	7.9 ± 2.3 (1.0)	0.6 ± 0.2 (1.0)	18.1 ± 2.2 (1.0)	1.4 ± 0.1 (1.0)
PB (60 mg/kg)	195.7 ± 10.7† (24.8)	11.0 ± 0.7† (18.3)	137.0 ± 1.2† (7.6)	7.8 ± 0.5† (5.5)
PB (120 mg/kg)	196.9 ± 23.7† (24.9)	10.2 ± 0.9† (17.0)	162.0 ± 9.1† (8.9)	8.5 ± 0.3† (6.0)

* Each value is the mean ± SEM for five or six rats per treatment group and includes both CYP2B1 and CYP2B2 in the level of CYP2B, and both CYP3A1 and CYP3A2 in the level of CYP3A. Numbers in parentheses denote the difference relative to the value of the control group.

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

‡ Mean value of the tiletamine or zolazepam treatment group was significantly different ($P < 0.05$) from that of the Telazol® treatment group.

significant effect on CYP3A protein levels. Treatment with PB at doses of 60 and 120 mg/kg increased the mean hepatic content of CYP3A isozymes by 8- and 9-fold, respectively.

Additional blots containing hepatic microsomes from rats treated with Telazol®, tiletamine, zolazepam, and vehicle were probed separately with antibody to CYP1A1 and antibody to CYP2E1. Results (not shown) indicated that there was no difference in the staining intensity of bands between lanes containing liver microsomes prepared from rats treated with Telazol®, tiletamine, or zolazepam, and lanes containing liver microsomes from vehicle-treated animals, suggesting that there was no induction of either CYP1A1 or CYP2E1 by any of the three drugs.

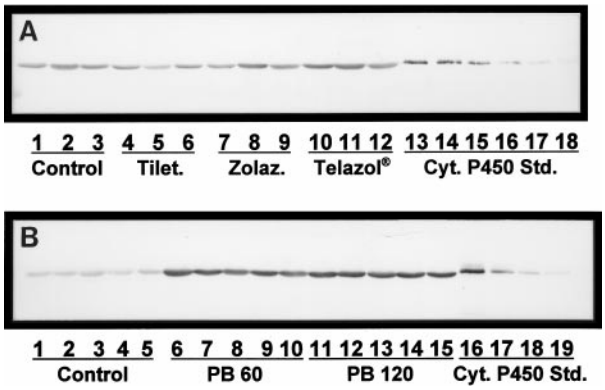

FIG. 2. Immunoblots of rat hepatic microsomes probed with anti-CYP3A IgG. The blots were incubated with polyclonal antibody against rat CYP3A1 at a concentration of 50 μg/mL as described in "Materials and Methods." Microsomal samples were applied to the gel at a final concentration of 10 μg microsomal protein/lane. In panel A, lanes 1–3 contained microsomes from individual vehicle-treated rats, lanes 4–6 contained microsomes from individual rats treated with tiletamine at a dose of 60 mg/kg, lanes 7–9 contained microsomes from rats treated with zolazepam at a dose of 60 mg/kg, lanes 10–12 contained microsomes from rats treated with Telazol® at a dose of 120 mg/kg, and lanes 13–18 contained purified rat CYP3A1 at concentrations of 0.5, 0.375, 0.25, 0.125, 0.0625, and 0.0312 pmol/lane, respectively. In panel B, lanes 1–5 contained microsomes from individual vehicle-treated rats, lanes 6–15 contained microsomes from individual rats treated with phenobarbital at a dose of 60 mg/kg (lanes 6–10), or 120 mg/kg (lanes 11–15), and lanes 16–19 contained purified rat CYP3A1 at concentrations of 0.5, 0.25, 0.125 and 0.0625 pmol/lane, respectively.

DISCUSSION

We reported recently that treatment with Telazol® induced BROD and testosterone 16β-hydroxylase activities, as well as CYP2B isozyme levels, in rats in a dose-dependent manner [5]. Results of the present study confirm the inductive effect of Telazol® toward CYP2B and clearly show that the benzodiazepine derivative, zolazepam, is the constituent primarily responsible for the effects elicited by Telazol®. The profile of microsomal monooxygenase activities and CYP isozymes induced by zolazepam is identical to that induced by Telazol®, although the increases resulting from administration of zolazepam were not as great as those observed after Telazol® treatment. Treatment with zolazepam and Telazol® resulted in a marked increase in CYP2B protein levels and associated activities, a modest increase in CYP3A isozyme levels, and no effect on CYP1A1 or CYP2E1. In contrast, tiletamine proved to be a marginal inducer of cytochrome P450 at the dose used. Because Telazol® consists of equal parts of zolazepam and tiletamine, we expected the combined effects of treatment with the constituents, each administered at a dose of 60 mg/kg, to equal those of Telazol®, when administered at a dose of 120 mg/kg. However, the sum of the values of the tiletamine- and zolazepam-treated groups for BROD and testosterone

16β-hydroxylase activities and hepatic CYP2B content approached, but were always less than those of the Telazol®-treated group. This result led us to consider the possibility that the use of tiletamine and zolazepam in combination had a synergistic inductive effect on CYP expression. Two-way ANOVA indicated there was no significant interaction between tiletamine and zolazepam. However, a larger sample size is necessary to draw a more reliable conclusion from the statistical analysis.

In that the pattern of induction produced by zolazepam and Telazol® closely resembles that elicited by PB, treatment with PB using the same dosing regimen was included in the present study for purposes of comparison. A single treatment with PB at a dose of 60 or 120 mg/kg had a greater inductive effect on BROD and testosterone 16β-hydroxylase activities than treatment with either zolazepam or Telazol®. Moreover, PB treatment induced additional activities, including EROD, testosterone 6β-hydroxylase, and androstenedione formation, which were not affected by treatment with zolazepam, tiletamine, or Telazol®. The increase in EROD activity was likely a result of CYP2B induction, as most of this activity has been shown previously to be catalyzed by CYP2B1 and CYP2C6 in rats treated with PB [20]. Likewise, the increase in the rate of formation of androstenedione was probably due to induction of CYP2B also, as CYP2B and CYP2C11 isozymes catalyze androstenedione formation [16]. The increase in testosterone 6β-hydroxylase activity can be ascribed to induction of CYP3A because CYP3A isozymes are mainly responsible for testosterone 6β-hydroxylation [16] and hepatic CYP3A levels were induced to a greater extent by PB than by Telazol® or zolazepam. Taken together, the results indicate that PB is a more potent inducer of CYP2B and CYP3A isozymes, on a weight and molar basis, than Telazol® or its constituents.

On the other hand, treatment with Telazol®, tiletamine, and zolazepam caused significant decreases in testosterone 2α- and 16α-hydroxylase activities. Testosterone 16α-hydroxylation, like androstenedione formation, is catalyzed predominantly by CYP2B1, CYP2B2, and CYP2C11, whereas CYP2C11 is the sole catalyst for testosterone 2α-hydroxylation [16]. Treatment with PB at the higher dose resulted in a significant decrease in testosterone 2α-hydroxylase activity, but not in testosterone 16α-hydroxylase activity. The results suggest that Telazol® and its constituents elicited a greater suppression of CYP2C11 expression than PB at the same doses. The mechanism of this suppression is unknown, but suppression of CYP2C11 has been reported to occur after pretreatment with various xenobiotics that are inducers of CYP [16, 17]. The combined suppressive and inductive effects of Telazol®, tiletamine, and zolazepam on CYP2B and CYP2C11, respectively, help explain the absence of change in the rate of androstenedione formation in hepatic microsomes prepared from rats treated with these agents.

There are numerous reports of the effects of repeated treatment with PB, at doses ranging from 1 to 80 mg/kg, on CYP, yet there are surprisingly few studies in the literature of the effects of a single injection of PB. A novel finding from the present study is that 24 hr after a single injection of PB at doses of 60 and 120 mg/kg, CYP2B protein levels were induced markedly from approximately 8 to almost 200 pmol/mg microsomal protein. The hepatic content of the CYP2B proteins was considerably less than values reported previously for rats treated with similar doses of PB but for several days [17, 21]. Treatment with PB also increased hepatic levels of CYP3A isozymes, in agreement with the enzyme activity data, which showed that drug treatment elevated CYP3A-mediated testosterone 6β-hydroxylase activity. Repeated administration of PB has been shown previously to induce the CYP3A subfamily [17, 22], hence the results herein indicate that induction also occurs after a single treatment. The inductive effects of PB appear to be similar between the two doses examined, as the lower dose of PB was as effective as the larger dose at inducing CYP2B and CYP3A isozymes and their associated activities.

In summary, results of the enzymatic assays and immunoblot analysis indicate that zolazepam was the constituent that contributed mostly to the inductive effect of Telazol® and that zolazepam and Telazol® both act as "PB-type" inducers. However, Telazol® was a weaker enzyme inducer than PB. Finally, a single treatment with PB at a dose of 60 or 120 mg/kg was equally effective at inducing CYP expression.

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