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Gender does not affect the degree of induction of tirilazad clearance by phenobarbital

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Abstract. *Objective*: Tirilazad mesylate is a membrane lipid peroxidation inhibitor being evaluated for the treatment of patients with subarachnoid haemorrhage (SAH); phenobarbital may be administered to these patients for seizure prophylaxis. Therefore, the effect of phenobarbital on tirilazad mesylate pharmacokinetics was assessed in 15 healthy volunteers (7M, 8F).
Methods: Subjects received 100 mg phenobarbital orally daily for 8 days in one phase of a two-way crossover study. In both phases, 1.5 $mg \cdot kg^{-1}$ tirilazad mesylate was administered (as a 10 minute IV infusion) every 6 hours for 29 doses. Three weeks separated study phases. Tirilazad mesylate and U-89678 (an active metabolite) in plasma were quantified by HPLC.
Results: Phenobarbital had no effect on the first dose pharmacokinetics of tirilazad or U-89678. After the final dose, clearance for tirilazad was increased 25% in males and 29% in females receiving phenobarbital + tirilazad versus tirilazad mesylate alone. These differences were statistically significant, and the degree of induction was not significantly different between genders. AUC_{0-6} for U-89678 after the last tirilazad mesylate dose was reduced 51% in males and 69% in females. The decreases were statistically significant, and there was no gender by treatment interaction.
Conclusion: The results show that phenobarbital induces metabolism of tirilazad and U-89678 similarly in both men and women. Lower levels of tirilazad and U-89678 in SAH patients receiving phenobarbital may adversely impact clinical response.

Key words Tirilazad, Phenobarbital; pharmacokinetics, gender effect, aminosteroid, antioxidant, inducer, cytochrome P-450

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Introduction

Tirilazad mesylate is a membrane lipid peroxidation inhibitor which reduces the damaging effects of lipid peroxidation on the cell membrane, triggered by brief periods of ischaemia. The efficacy of tirilazad has been assessed in animal models for the prevention of neuronal damage due to head trauma, subarachnoid haemorrhage, spinal cord injury, and stroke [1]. Tirilazad is well tolerated during treatment of patients with stroke [2], head injury [3], and subarachnoid haemorrhage [4], and its administration has been associated with reduced mortality in male subarachnoid haemorrhage (SAH) patients [5].

Tirilazad is highly metabolized in humans [6]. One reduced metabolite has been identified which has activity similar to that of tirilazad in a rat model of SAH (U-89678, Figure 1) [7]. This metabolite achieves plasma concentrations on multiple dosing which may be > 50% of those of the parent compound [8]. However, tirilazad is primarily metabolized via the 3A isozymes of cytochrome P-450 (CYP3A) [9]. Coadministration of phenytoin in healthy volunteers reduces plasma concentrations of both tirilazad and U-89678 over a time course consistent with phenytoin's effects on hepatic CYP3A activity [10, 11]. Substantially lower levels of tirilazad and U-89678 in healthy women compared to men have been observed in previous single dose studies [12, 13].

Available pharmacokinetic data thus show that two major factors affect tirilazad pharmacokinetics: gender and phenytoin coadministration. As mentioned above, tirilazad significantly reduced mortality in male SAH patients at a dose of 6.0 mg/kg/day; effects were minimal in women [5]. This suggests that the gender effect on tirilazad pharmacokinetics is clinically relevant. Since the magnitude of the effect of phenytoin administration on tirilazad pharmacokinetics is equal to or greater than that of gender, this drug interaction is also likely to be clinically relevant.

Phenobarbital, an inducer of CYP3A [14], is also administered to SAH patients, so that coadministration of this compound may likewise impact the use of tirilazad in the treatment of SAH. Therefore, the purpose of the present study was to assess the degree of induction of tirilazad clearance by phenobarbital. A 100 mg/day dosage regimen for phenobarbital was chosen because approximately 50% of the patients receiving phenobarbital in the above SAH trial received this daily dose [5] and because 100 mg was likely to be tolerated in healthy males and females.

There are scant data available concerning the effect of gender on the degree of enzyme induction. However, Vesell and Page [15] reported that the degree of enzyme induction due to phenobarbital coadministration is a function of baseline enzyme activity. Therefore, tirilazad metabolism may be differentially induced in males and females, if their baseline metabolic clearance of tirilazad differs. Thus, the second objective of the trial was to assess the effect of gender on the induction of tirilazad clearance by phenobarbital.

Materials and methods

Protocol and subjects

The study was conducted at the Upjohn Research Clinics, Kalamazoo, MI, following approval by the local Institutional Review Board. Each volunteer provided written evidence of informed consent prior to enrollment.

The protocol specified that 16 subjects be enrolled, 8 females and 8 males. Due to recruitment difficulty and time constraints, the study group consisted of 7 males (ages 23–63, mean = 40.1; weights 63.5–83.2 kg, mean = 74.0 kg) and 8 females (ages 23–58, mean = 41.0; weights 45.4–80.3 kg, mean = 61.5 kg). Females were surgically sterile or postmenopausal. Subjects were determined to be in good health by physical examination and standard clinical laboratory tests. Subjects received no known enzyme inducing agents for 30 days prior to the study, no medications during the 7 days prior to study (and during the study period) and no alcohol for 2 days prior to and throughout each study period.

Study design and procedures

Subjects received the following treatments according to a randomized two-way crossover design:

A: 1.5 $mg \cdot kg^{-1}$ tirilazad mesylate sterile solution every 6 hours for 29 doses (7 days) and 1 phenobarbital 100 mg tablet daily for 8 doses (days) starting the evening before tirilazad dosing

B: 1.5$mg \cdot kg$ tirilazad mesylate sterile solution every 6hours for 29 doses (7 days)

Tirilazad mesylate was infused intravenously over 10 minutes at approximately 06:00, 12:00, 18:00, and 24:00 h. Subjects fasted from 22:00 h the night prior to tirilazad dosing until 09:00 h on study Days 1 and 8. Phenobarbital tablets were administered orally with 180 ml water at 22:00 h. At least 3 weeks separated study phases I and II.

Serial measurements of vital signs (supine blood pressure and heart rate) were recorded prior to and through 4 hours following the 06:00 h tirilazad mesylate dose on Days 1 and 8. Blood and urine samples for safety hematology and chemistry assays were obtained before drug administration and again on Day 9. A twelve-lead EKG was also performed prior to the first dose and at 24 h after the last dose of tirilazad mesylate. Volunteers were interviewed during each study period to determine whether they had experienced any medical events.

Sample collection and analytical methods

Venous blood samples (7 ml) for the determination of tirilazad and U-89678 were collected into heparinized vacutainers immediately prior to the first dose on Day 1 and at 12, 15, and 30 min after and again at 1, 1.5, 2, 3, 4, 6, 24, 72, 120, 168, 168.2, 168.25, 168.5, 169, 169.5, 170, 171, 172, 174, 192, 216, 240 and 264 h following the first dose of tirilazad mesylate. Plasma was harvested after centrifugation and frozen at $-70°$ C.

Plasma tirilazad and U-89678 were quantified by a specific high-performance liquid chromatographic (HPLC) method [16]. Calibration standard responses were linear over the calibration range of 6.94 to 6940 $nmol \cdot l^{-1}$ for tirilazad and over the range of 7.98 to 1595 $nmol \cdot l^{-1}$ for U-89678. Inter-day coefficients of variation (CV%) for the quality control pools were $\leq 5.2\%$ and $\leq 4.9\%$ for tirilazad and U-89678, respectively.

Venous blood samples (7 ml) for the determination of phenobarbital were collected into heparinized vacutainers immediately prior to the phenobarbital doses administered on Day-1, Day 1, Day 3, Day 5 and Day 7. Plasma was harvested after centrifugation and frozen until assayed.

Plasma phenobarbital was quantified using a modified HPLC method [17]. Briefly, an acetonitrile solution of phenytoin, the internal standard (IS), was added to plasma (0.2 ml). Each sample was vortexed, centrifuged, and the supernatant transferred to an injection vial. The mobile phase consisted of 10 mM potassium phosphate buffer, pH 6.6:acetonitrile (75:25, v:v). Samples were injected onto a Nucleosil 100A/ODS analytical column (CSC). Peak response was monitored by UV detection at 258 nm. Calibration standard responses were linear over the range of 2.07 to 164 $\mu mol \cdot l^{-1}$. Inter-day CV's for the QC pools were $\leq 4.8\%$.

Urine samples were collected (into plastic containers which contained 4 ml 50% acetic acid) over the interval of 08:00 to 12:00 h on study days -1, 1, 2, 3, 4, 5, 6, 7 and 8. An aliquot was frozen for subsequent analysis of urinary 6β-hydroxycortisol and cortisol.

Urinary 6β-hydroxycortisol (6β-OHC) concentrations were determined by a specific HPLC method (Unpublished method, Mayo Medical Labs, Rochester, MN). The assay was linear over a range of 0.05 to 25 $\mu g/ml$, and the overall precision was 8%. Free cortisol (C) was determined by a similar method (Mayo Medical Labs, Rochester, MN). The assay was linear over a range of 0.01 to 5 $\mu g \cdot ml^{-1}$, and the overall precision was 9%. Brief descriptions of the assay procedures have been presented elsewhere [11].

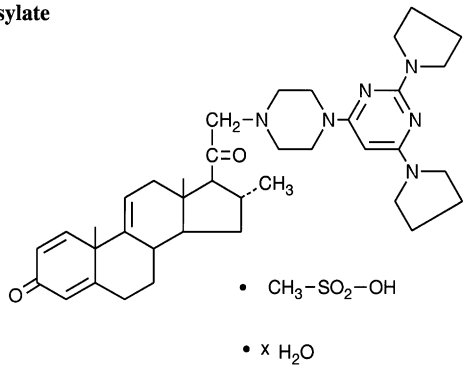
Pharmacokinetic analysis

Pharmacokinetic parameters were determined by noncompartmental techniques [18]. The terminal elimination rate constant (λz) was determined by linear regression of the terminal portion of the log concentration-time profile after the final dose. The terminal half-life ($t_{1/2}$) was calculated as $\ln 2/\lambda z$. Area under the plasma concentration-time curve (AUC_{0-6}) was determined by trapezoidal rule on Days 1 and 8 following the 06:00 dose of tirilazad. Apparent systemic clearance of tirilazad (CL) was calculated from dose 29 data as $Dose/AUC_{0-6}$. This represents an estimate of clearance only, since steady-state may not have been achieved. Volume of distribution (V_z) was calculated as $CL/\lambda z$. For tirilazad, the concentration at the end of the infusion (C_{inf}) was reported. For U-89678, maximal concentrations (C_{max}) and the times at which they occurred (t_{max}) were determined by inspection of the concentration-time profile.

Statistical analysis

Treatment effects on pharmacokinetic parameters within gender were assessed by analysis of variance (ANOVA) with group, treat-

Tirilazad Mesylate



U-89678

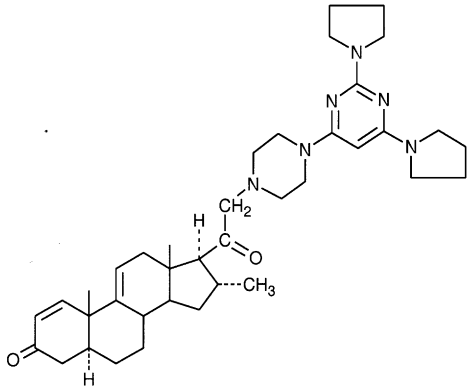


Fig. 1 Structures of tirilazad and U-89678

ment and period as fixed effects and subject within group as a random effect. In addition, an overall analysis of variance including all subjects using the above model was performed. To assess whether the effect of phenobarbital differed between genders, an ANOVA model consisting of treatment, gender, and a treatment by gender interaction term was utilized. Repeated measures ANOVA of the change in 6β-OHC/C ratio from baseline (Day-1) within gender was conducted to assess the effect of phenobarbital on this parameter.

Results

The most frequently reported medical events were local discomfort and/or venous irritation related to tirilazad administration; no substantial differences were apparent between genders. Systemic medical events were infrequently reported, again with no substantial differences between genders. Nervous system effects of phenobarbital (e.g. sedation) were not observed. No clinically important effects of tirilazad or phenobarbital on vital signs, electrocardiogram parameters, or laboratory assays were apparent.

Mean plasma concentration-time profiles for tirilazad after administration of the first and 29th doses of tirilazad mesylate are shown in Fig. 2a. Trough concentrations are depicted in Fig. 3a. Little difference was apparent between treatments in plasma concentrations following the first dose of tirilazad, but plasma concentrations after the 29th dose in the phenobarbital + tirilazad treatment were lower than those in the tirilazad alone treatment. Phenobarbital treatment reduced accumulation of tirilazad on multiple dosing based on trough concentrations.

No significant differences between treatments in C_{inf} or AUC_{0-6} were evident following the first dose of tirilazad (data not shown). Pharmacokinetic parameters for tirilazad following the final dose are summarized in Table 1. On multiple dosing, AUC_{0-6} was reduced 19.7% and 23.6% in men and women, respectively, during phenobarbital coadministration ($P = 0.0056$ and $P = 0.0018$, respectively). Tirilazad clearance was increased 25% in males and 29% in females in the

Table 1 Mean (SD) tirilazad pharmacokinetic parameters in men and women following the 29th dose of 1.5 mg·kg^{-1} tirilazad every 6 h + 100 mg phenobarbital daily or administration of 1.5 mg·kg^{-1} tirilazad every 6 h alone

Parameter	Gender/treatment			
	Males (n = 7)		Females (n = 8)	
	Tirilazad+ Phenobarbital	Tirilazad	Tirilazad+ Phenobarbital	Tirilazad
AUC_{0-6} (nmol·h·l^{-1})	4017* (746)	5007* (902)	3843* (743)	5033* (1033)
CL (l·h^{-1})	40.1* (7.36)	32.1* (5.30)	33.9* (5.08)	26.2* (5.45)
CL (l·h^{-1}·kg^{-1})	0.536* (0.113)	0.426* (0.068)	0.565* (0.146)	0.435* (0.122)
C_{inf} (nmol·l^{-1})	6929 (1153)	6936 (1720)	6595 (1527)	6928 (1254)
V_z (l)	4968* (1584)	3265* (783)	4146 (1727)	3428 (985)
V_z (l·kg^{-1})	66.2* (21.3)	42.9* (8.30)	68.9 (31.6)	56.0 (15.0)
$t_{1/2}$ (h)	90.3 (40.4)	71.3 (15.7)	84.4 (33.2)	90.9 (22.5)

* Significantly different within gender at $P < 0.05$ by least squares means analysis

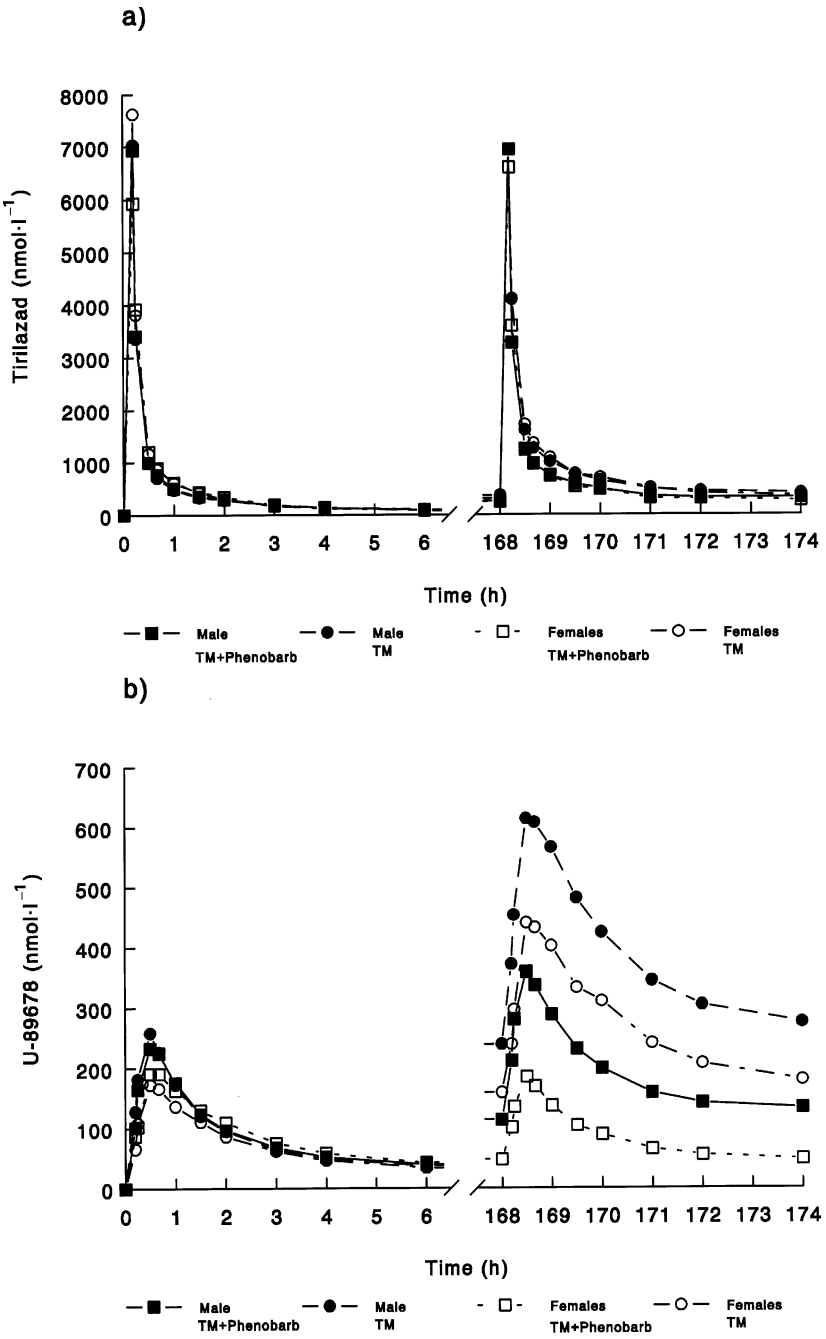


Fig. 2 Mean plasma concentrations of **a** tirilazad and **b** U-89678 after the first and 29th dose of a 6.0 $mg \cdot kg \cdot^{-1} day^{-1}$ tirilazad dosing regimen in the presence and absence of phenobarbital administration

phenobarbital + tirilazad group ($P = 0.0107$ and $P = 0.0002$, respectively). Similar effects were seen for V_z in men ($P = 0.0124$). No significant treatment effects on C_{inf}, $t_{1/2}$ or λ_z were observed in either sex. No treatment by gender interaction was noted in the two-way ANOVA.

Plasma concentration-time profiles for U-89678 after administration of the first and 29th doses of tirilazad mesylate are shown in Figure 2b. Trough concentrations of U-89678 are depicted in Figure 3b. Plasma concentrations of U-89678 after the first dose of tirilazad exhibited no discernible treatment differences. However, plasma concentrations of U-89678 after dose 29 were markedly reduced in the phenobarbital + tirilazad treatment as compared to the tirilazad treatment. When tirilazad mesylate alone was administered, U-89678 accumulated on multiple dosing. However, from 72 h on, trough concentrations of U-89678 decreased slightly when phenobarbital was coadministered. Mean U-89678 pharmacokinetic parameters following the last tirilazad dose are depicted in Table 2. AUC_{0-6} for U-89678 was reduced 51% in males and 69% in females during phenobarbital coadministration as compared to tirilazad alone; these differences were statistically significant ($P = 0.0123$ and

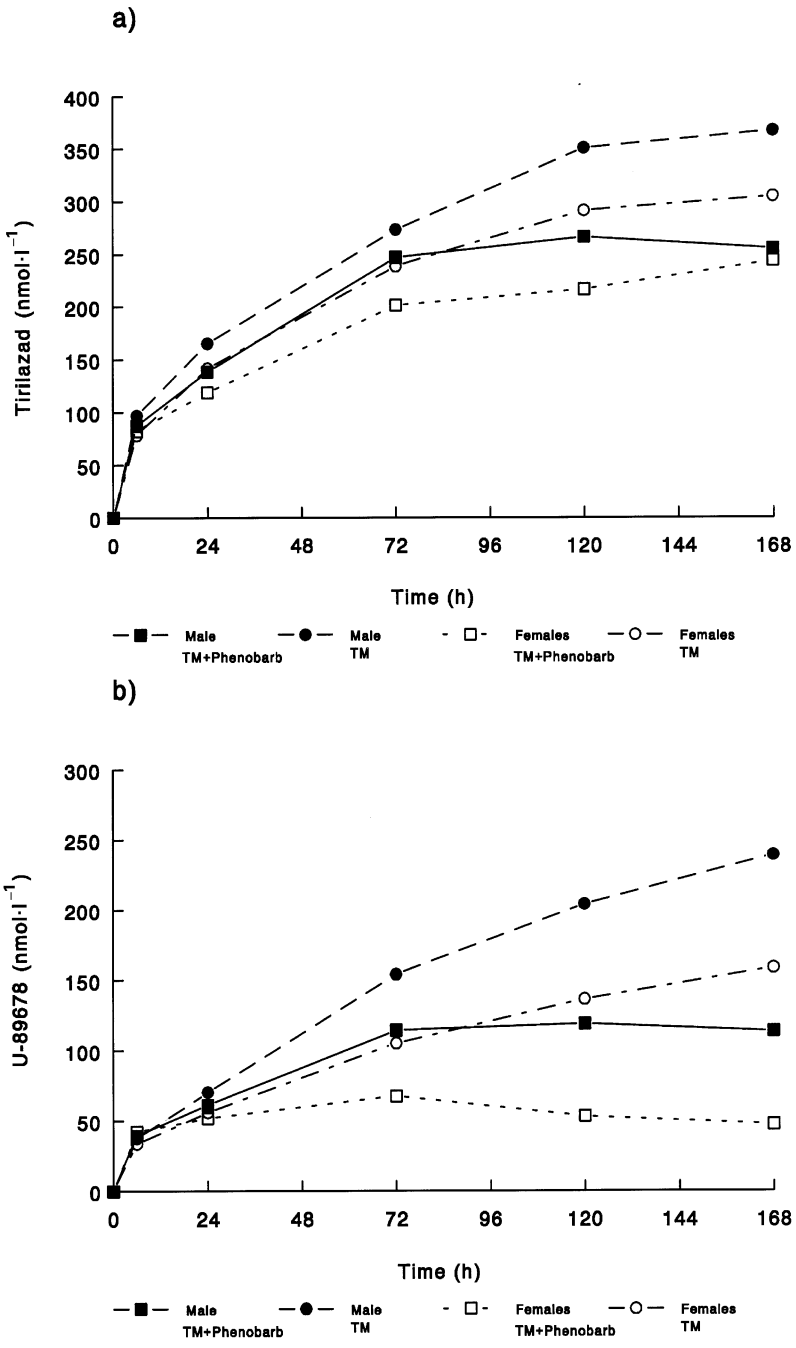


Fig. 3 Mean nadir plasma concentrations of **a** tirilazad and **b** U-89678 during a 6.0 $mg \cdot kg \cdot^{-1} day^{-1}$ tirilazad dosing regimen in the presence and absence of phenobarbital administration

Table 2 Mean (SD) U-89678 pharmacokinetic parameters in men and women following the 29th dose of 1.5 $mg \cdot kg^{-1}$ tirilazad every 6 h + 100 mg phenobarbital daily or administration of 1.5 $mg \cdot kg^{-1}$ tirilazad every 6 h alone

Parameter	Gender/treatment			
	Males (n = 7)		Females (n = 8)	
	Tirilazad+ Phenobarbital	Tirilazad	Tirilazad+ Phenobarbital	Tirilazad
AUC_{0-6} ($nmol \cdot h \cdot l^{-1}$)	1128* (786)	2290* (1464)	490* (292)	1589* (970)
C_{max} ($nmol \cdot l^{-1}$)	359* (244)	635* (394)	185* (117)	455* (254)
$t_{1/2}$ (hr)	83.7 (27.2)	66.1 (16.8)	93.3 (28.0)	85.6 (25.5)

*Significantly different within gender at $P < 0.05$ by least squares means analysis

$P = 0.0060$, respectively). U-89678 C_{max} was reduced by 43% in males ($P = 0.0139$) and 59% in females ($P = 0.0051$) during phenobarbital treatment. No treatment by gender interaction was observed in the two-way ANOVA.

Mean phenobarbital plasma concentrations increased over the course of the study. Mean concentrations prior to administration of the final tirilazad dose (33.5 (6.03) and 35.9 (5.86) $\mu mol \cdot l^{-1}$ in males and females, respectively) were below the usual therapeutic range of 10 to 25 $\mu g \cdot ml^{-1}$ (43–108 $\mu mol \cdot l^{-1}$).

Mean 6β-OHC/C ratios exhibited no clear trend for an increase over the course of the study in the tirilazad + phenobarbital treatment (data not shown). There was no significant effect of treatment and no significant treatment by time interaction for change from baseline data for 6β-OHC/C ratios.

Discussion

Phenobarbital increased mean tirilazad clearance between 25 and 30% in this study, suggesting that phenobarbital, like phenytoin, induces tirilazad metabolism. The effects of phenobarbital on the pharmacokinetics of U-89678 are likewise consistent with induction of the metabolism of this metabolite, but effects on formation clearance cannot be ruled out based on the results of this study alone.

While phenobarbital obviously induced tirilazad clearance, the increase in clearance was less than the 51% increase produced by phenytoin coadministration in males [10]. Likewise, the effects of phenobarbital on U-89678 plasma concentrations were less striking than those seen previously with phenytoin. This may be a function of phenobarbital being a less potent inducer, either in the maximal degree of induction or in the time required for maximal induction, than is phenytoin. In a previous study [14], it appeared that at least 7 days of phenobarbital treatment was necessary for modest, but statistically significant induction (as measured by the ratio of 6β-OHC to 17-hydroxycorticosteroids in urine) to occur; more substantial changes (a doubling in the ratio) were not evident until 11–14 days after dosing. In the present study, we did not observe consistent, statistically significant differences in the 6β-OHC/C ratios over the time course studied. In comparison, phenytoin significantly increases urinary 6β-OHC/C ratios within 3 days and produces a doubling of the ratio from baseline by the 5th day of dosing [11]. The results of the present study are thus consistent with phenobarbital producing only moderate induction of CYP3A and tirilazad clearance over the 7 day period of the trial. Since tirilazad is only administered for 7–10 days, these results reflect conditions during usual therapy for SAH. The clinical impact of phenobarbital coadministration with tirilazad is thus likely to be less than that of phenytoin.

Another factor which may affect the degree of induction by phenobarbital is the dose administered. The dosage regimen in this study reflected that used in a substantial number of subjects in the European/Australasian SAH study [5], and was reasonably tolerated in normal volunteers. This dosage regimen produced plasma phenobarbital concentrations which were, at best, at the low end of the therapeutic range (43–108 $\mu mol \cdot l^{-1}$). It is possible that more aggressive phenobarbital dosing would produce a greater degree of enzyme induction than was observed here.

Phenobarbital coadministration also resulted in an apparent increase in tirilazad volume of distribution, which was statistically significant in the case of males (Table 1). However, it should be noted that the volume term calculated here corresponds to V_{area} or V_{β} rather than the steady state volume of distribution, V_{ss}. Jusko and Gibaldi [19] showed that V_{ss} is unaffected by changes in clearance, whereas V_{area} increases as clearance increases. Therefore, the changes in V_z observed here are probably due to clearance effects rather than true distributional changes.

There are scant data in the literature which address the effect of gender on the induction of cytochrome P-450. However, Vesell and Page [15] found that the degree of shortening of antipyrine half-life by phenobarbital coadministration was directly proportional to the baseline half-life of antipyrine. Thus, subjects with the slowest baseline metabolism would have the greatest degree of induction. In the case of tirilazad then, one might have expected, based on the gender effects on tirilazad clearance observed in single dose studies, that the degree of induction would have been greater in males than females. However, the results of this study indicate that tirilazad clearance is induced to similar degrees in males and females by phenobarbital. The degree of induction of U-89678 metabolism by phenobarbital was likewise similar in males and females.

One other piece of information to gain from this study is an estimate of the effect of gender on the multiple dose pharmacokinetics of tirilazad, since this is the first study completed in which normal female volunteers have received multiple doses of tirilazad. As is evident from Table 1, there was no significant difference between males and females in clearance corrected for body weight following the administration of tirilazad alone. U-89678 plasma concentrations showed a trend for being lower in females than males, but the difference was less than expected based on previous results [12]. These findings were unexpected, as results in single dose studies would have predicted much larger effects. There are several possible explanations for these findings, which are explored below:

a) Under-powered study: Based on the residual error of the two-way ANOVA, which had a coefficient of variation of 19.4% for AUC_{0-6}, the power for detecting a 40% difference in tirilazad AUC_{0-6} at an α level of 0.05 was 94.1%. Thus, the study had sufficient power to detect a gender effect on tirilazad kinetics similar to that observed in the single dose studies.

b) Distributional differences: Area under the curve is a function of clearance only. However, distributional differences may affect the shape of the plasma concentration-time profile, particulary the terminal phase. In the face of limited assay sensitivity after single doses, distributional differences may produce artifactual differences in AUC when no differences in clearance actually exist. One might argue is that this is the case for tirilazad; that distributional differences between males and females result in apparent AUC differences on single dosing which disappear on multiple dosing. However, there are several pieces of data which argue against this hypothesis. One is that the portion of the area accounted for by the terminal phase of the plasma concentration-time profile is generally less than 10% [19]. In addition, if the shape of the plasma concentration-time profile after single doses was different, there would have been significant effects of gender on AUC_{0-6} after the initial dose of tirilazad; this did not occur. Finally, the gender difference in U-89678 AUC_{0-6} is seen in this study, albeit to a more modest extent than in the single dose studies. This supports the hypothesis that there is a gender difference in tirilazad metabolism under multiple dose conditions.

c) Small sample size: In a small study, it is possible that the subjects may not be representative of the population in general; this may affect the comparisons done as part of the study. In this study, the individual values for AUC_{0-6} in males (4058–6710 $nmol \cdot h \cdot l^{-1}$) cluster at the low end of the range of values observed in other studies in healthy males receiving similar multiple dose regimens of tirilazad (3886–10971 $nmol \cdot h \cdot l^{-1}$) [8, 10, 21]. In addition, several women in this study were postmenopausal; we have previously shown that tirilazad clearance in the group approaches that in males [12]. Given these factors, it is reasonable to expect that the difference in tirilazad clearance observed in this study would be less robust than that seen previously.

Therefore, for the reasons outlined above, it is apparent that further work will be necessary to characterize the true effect of gender on tirilazad clearance under multiple dose conditions.

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