Venous Irritation, Pharmacokinetics, and Tissue Distribution of Tirilazad in Rats Following Intravenous Administration of a Novel Supersaturated Submicron Lipid Emulsion

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Purpose. To compare the venous irritation, pharmacokinetics, and tissue distribution of tirilazad in rats after intravenous administration of a submicron lipid emulsion with that of an aqueous solution.

Methods. Venous irritation was determined by microscopic evaluation of injury to the lateral tail veins of rats. Pharmacokinetic parameters were determined by following plasma concentrations of drug. Tissue distribution of [^{14}C]-tirilazad was determined by quantitative whole body autoradiography.

Results. Single dose injections of tirilazad as an emulsion at doses ranging from 1.52 mg to 13.5 mg were non-irritating whereas the solution was irritating at a dose of 1.3 mg. The pharmacokinetic parameters were not statistically different between the emulsion and the solution ($p > 0.2$) at doses of 6 mg/kg/day and 20 mg/kg/day. However, at 65 mg/kg/day dose, a higher AUC(0,6) (4-fold) and lower V_{ss} (18-fold) and CL(5-fold) were observed for the lipid emulsion as compared to the solution ($p < 0.05$). Tissue distribution showed higher initial concentrations (two fold or more) in most tissues for the solution. These values, however, equilibrated by 4 h and AUC(0,4) differences were less than two fold in most tissues.

Conclusions. Formulating tirilazad in the lipid emulsion significantly reduces the venous irritation without changing the pharmacokinetics and tissue distribution at low doses.

KEY WORDS: submicron lipid emulsion; supersaturation; tirilazad; venous irritation; pharmacokinetics; tissue distribution.

INTRODUCTION

Tirilazad (structure shown in Fig. 1a) is an i.v. administered free radical scavenger that has been investigated for therapeutic intervention in aneurysmal subarachnoid hemorrhage, ischemic stroke, and spinal cord injury (1,2), and currently is investigated for renal cytoprotection. This lipophilic compound has an extremely low aqueous solubility at physiological pH. The current formulation of tirilazad (FREEDOX® IV Solution) employs the mesylate salt of the drug at a concentration of 1.5 mg/mL in a pH 3.0 citrate buffer. This formulation has been associated with pain at the injection site, venous irritation, and occasionally thrombophlebitis (3,4). These side effects may be due to the acidity of the vehicle, the irritant nature of the drug, and possible precipitation of the drug after intravenous administration. To alleviate the local pain and venous irritation, FREEDOX® IV Solution is often diluted four fold before use, resulting in a larger and less convenient injection volume. Hence, there is considerable interest in the development of a more concentrated yet less painful i.v. formulation of tirilazad.

Parenteral lipid emulsions that are formulated using a biocompatible emulsifying agent to disperse an oil in an aqueous phase are used for drug delivery, as well as for parenteral nutrition, oxygen transport, and diagnostic imaging (5). These oil-in-water (o/w) systems based largely on vegetable oils are stabilized by phosphatides and they resemble chylomicrons, the natural fat particles present in the circulation that carry endogenous and exogenous lipophiles. The oil phase of lipid emulsions acts as a solubilizer of lipophiles. Thus, solubility of lipophilic drugs can be significantly enhanced in a lipid emulsion, leading to smaller administration volumes compared to an aqueous solution. Additionally, since lipophilic drugs are incorporated within the innermost oil phase, they are sequestered from direct contact with body fluids and tissues. Thus lipid emulsions can minimize the pain associated with intravenously administered drugs by exposing the tissue to lower concentrations of the drug or avoiding a tissue-irritating vehicle. This has been demonstrated with diazepam (6), methohexital (7), clarithromycin (8) and etomidate (9). Lastly, due to their resemblance to chylomicrons, lipid emulsions are well tolerated and present a lower incidence of side effects as compared to other systems based on organic solvents, pH adjustments, and surface active agents (for example, Cremophor), since there is less chance of drug precipitation upon administration (10). Thus, a lipid emulsion appears to be a viable alternative for the intravenous administration of tirilazad.

In a previous paper (11), we reported the development of a supersaturated submicron lipid emulsion of tirilazad and demonstrated its excellent stability. The purpose of this article is to evaluate the effects of supersaturated tirilazad emulsions on venous irritation, pharmacokinetics, and tissue distribution of tirilazad. The emulsion data are compared to the aqueous solution of tirilazad mesylate (FREEDOX®IV Solution).

MATERIALS AND METHODS

Materials

Tirilazad free base, [^{14}C]-tirilazad free base (specific activity 33.0 μCi/mg), [^{14}C]-tirilazad mesylate (specific activity 33.07 μCi/mg), glycerin (USP grade) and FREEDOX® IV Solution (hereafter the solution) were provided by Pharmacia & Upjohn (Kalamazoo, MI). Miglyol 810 was supplied by Hüs America, Inc. (Piscataway, NJ). Fractionated soybean lecithin and butylated hydroxytoluene (BHT) were purchased from Sigma Chemicals (St. Louis, MO). Organic solvents, all of

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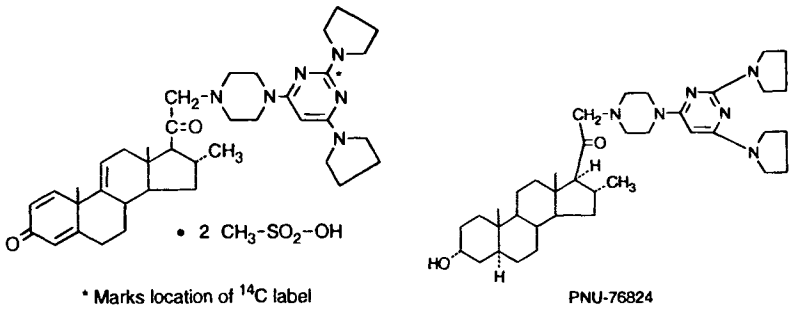
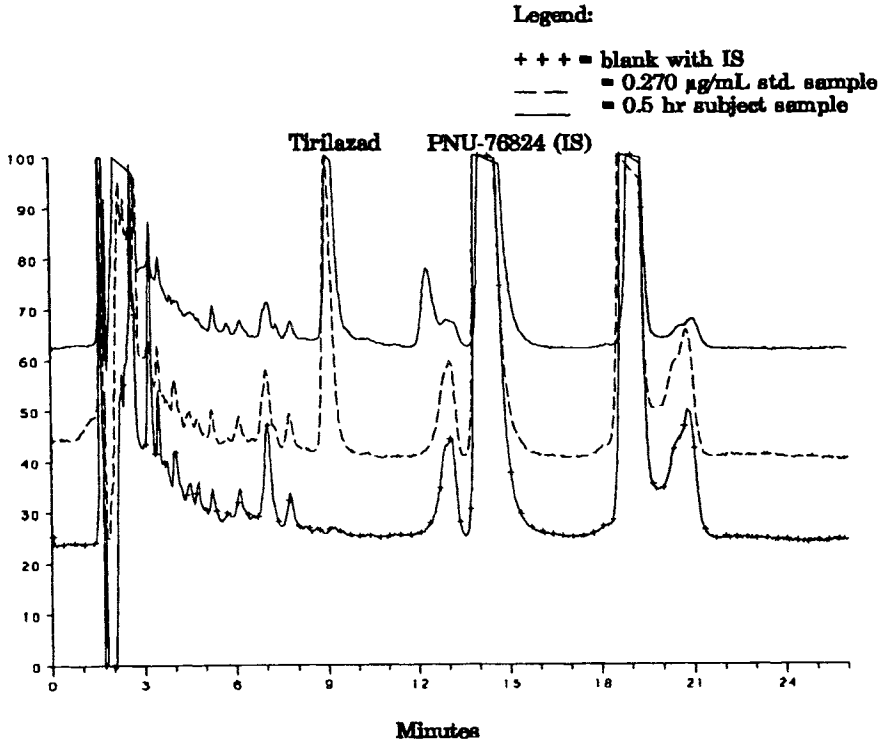


(a) (b)



(c)

Fig. 1. Chemical structures of (a) tirilazad mesylate and (b) internal standard PNU-76824. (c) Representative chromatograms of tirilazad in rat plasma.

HPLC grade, were obtained from Burdick and Jackson (Muskegon, MI).

Drug Formulation

Tirilazad emulsions were formulated with Miglyol 810 (MCT) at levels ranging from 10% to 30%, butylated hydroxy toluene (BHT) was used in amounts relative to MCT at a ratio of 0.1:10, fractionated soybean lecithin and glycerin were used at 1.2% and 2.4% in deionized water, respectively. Preparation steps have already been described in detail (11). The physical and chemical stability of the emulsions were assessed using methods already described (11). The mean particle diameter ranged from about 200 to 300 nm and was independent of drug load (11).

Venous Irritation

Groups of eight male Sprague-Dawley rats (Crl:CD[BR], Charles River Laboratories, Portage, MI) received infusions of 2 mL of 0.75 mg/mL the solution or 1 mL of 1.5 mg/mL the solution (positive control), and 10% MCT emulsions containing 0.76 mg/mL, 1.65 mg/mL, and 3.34 mg/mL tirilazad and a 20% MCT emulsion containing 6.75 mg/mL tirilazad. Also given were 4 mL each of 0.9% sodium chloride for injection USP (negative control), 20% MCT emulsion vehicle, 3.34 mg/mL of 10% MCT emulsion. Rats were infused while in a Broome-type rodent restrainer. Leakage was detected by watching for blebs during the infusion. Rats were killed 24 h after infusion and sections of the tail at 1, 2, 3, and 5 cm proximal to the most cranial injection site were preserved in 10% neutral buffered

Table 1. Experimental Design of the Pharmacokinetics Study

Group #	Formulation	Drug conc. (mg/ml)	Infusion rate (mg/min)	Total daily dose (mg/kg/day)
1	10% lipid emulsion	1.36	0.07	6
2	10% lipid emulsion	1.36	0.36	20
3	10% lipid emulsion	1.36	0.36	65
4	aqueous solution	1.3 (FBE*)	0.07	6
5	aqueous solution	1.3 (FBE*)	0.36	20
6	aqueous solution	1.3 (FBE*)	0.36	65
7	30% lipid emulsion	6.26	0.34	20

* FBE: free base equivalent.

formalin, stained with hematoxylin-phloxine-eosin, and examined light microscopically. A mean irritation index for intimal necrosis was used to compare the relative irritancy of the various formulations. Vascular irritation (intimal necrosis) was graded on the extent of vascular injury. Intimal lesions characterized by a focal (clusters of endothelial cells) to multifocal loss of endothelial cells were graded minimal vascular injury (grade 1). Mild vascular injury (grade 2) was assigned when endothelial cell injury involved most of the intima but with no evidence of medial injury. Moderate vascular injury (grade 3) was designated when either there was a superficial necrosis of the inner aspect of the muscular layer of the vein or there was segmental transmural necrosis of the vein wall. Vascular injury characterized by circumferential and transmural medial necrosis was graded as marked to severe (grade 4–5).

Pharmacokinetics

Experimental Design

Seven groups of six to ten Sprague-Dawley Crl:CD®BR rats (246–383 g, 3–5 per sex) were given a constant-rate i.v. infusion of tirilazad emulsion or the solution every 6 h for five doses (Table 1). Immediately after dosing, the cannula was flushed with 0.05 to 0.1 mL isotonic saline to insure complete delivery of the dose to the systemic circulation. Blood samples (0.3 mL at each time point) were collected with heparinized syringes at −60 (pre-dose), 10 (Groups #1, 2, 4, and 5 only), 15, 30, 45, 60, 90, 180, 270, and 360 min after the beginning of the last infusion. Samples were centrifuged after collection and the resulting plasma was used for HPLC analysis.

HPLC Analysis

To 0.1 mL portions of rat plasma, 0.15 mL of distilled water and 0.3 mL of acetonitrile containing the internal standard PNU-76824 (Fig. 1b) were added. After centrifugation, the samples were extracted using pre-conditioned C_8 Advanced Automated Sample Processor (AASP) cartridges. Aliquots of 0.45 mL of supernatant were applied to the cartridges containing

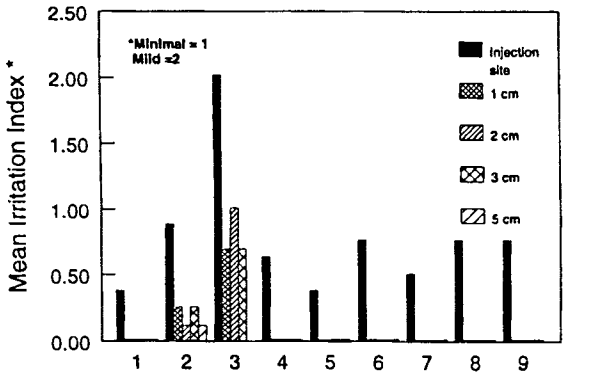


Fig. 2. Mean irritation (intimal necrosis) index in the lateral tail vein of rats infused with FREEDOX® IV Solution and tirilazad emulsions for one day. (1) 4-mL of 0.9% sodium chloride; (2) 2-mL of 0.75 mg/mL FREEDOX® IV Solution (equivalent to 0.65 mg/mL tirilazad free base); (3) 1-mL of 1.5 mg/mL FREEDOX® IV Solution (equivalent to 1.3 mg/mL tirilazad free base); (4) 4-mL of 20% lipid emulsion placebo; (5) 2-mL of 0.76 mg/mL tirilazad emulsion; (6) 2-mL of 1.65 mg/mL tirilazad emulsion; (7) 2-mL of 3.34 mg/mL tirilazad emulsion; (8) 4-mL of 3.34 mg/mL tirilazad emulsion; (9) 2-mL of 6.75 mg/mL tirilazad emulsion.

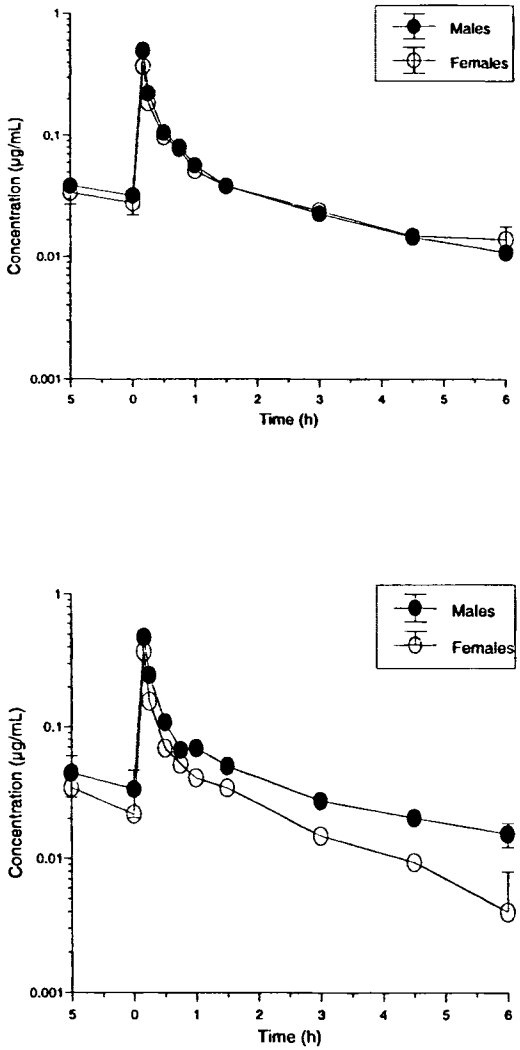
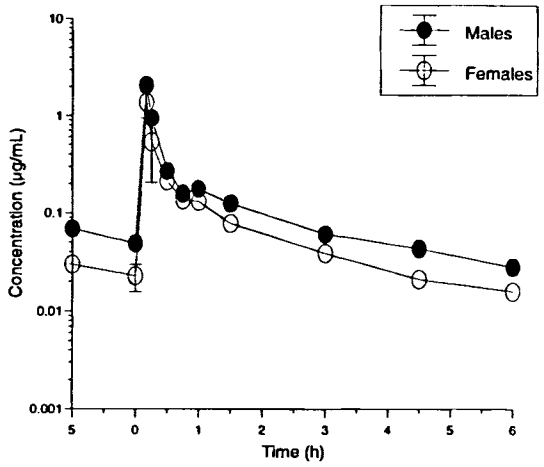


Fig. 3. Average plasma concentrations of tirilazad after i.v. infusion of tirilazad emulsion (group #1, top) and FREEDOX® IV Solution (group #4, bottom) ($R_0 \approx 0.073$ mg/min for 6 min). Error bars represent SE. Concentration at t = 0 was estimated from the previous 5-h (pre-dose) concentration.



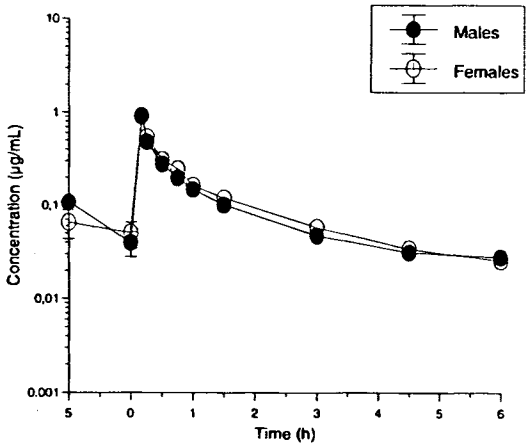
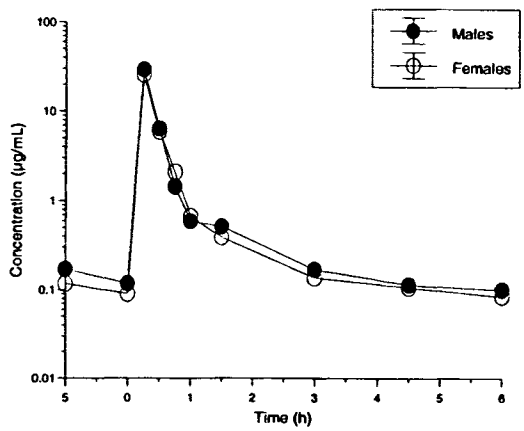


Fig. 4. Average plasma concentrations of tirilazad after i.v. infusion of tirilazad emulsion (group #2, top) and FREEDOX® IV Solution (group #5, bottom) ($R_0 \approx 0.364$ mg/min for 6 min). Error bars represent SE. Concentration at t = 0 was estimated from the previous 5-h (predose) concentration.



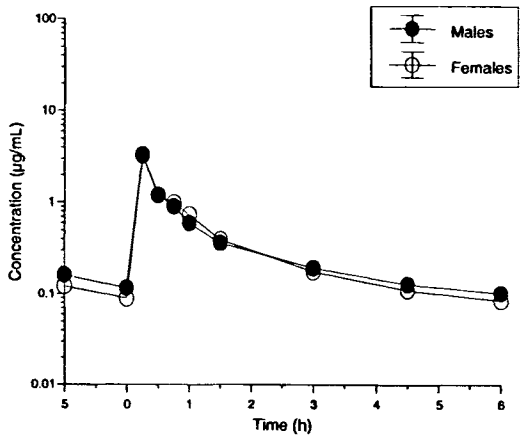


Fig. 5. Average plasma concentrations of tirilazad after i.v. infusion of tirilazad emulsion (group #3, top) and FREEDOX® IV Solution (group #6, bottom) ($R_0 \approx 0.364$ mg/min for 6 min). Error bars represent SE. Concentration at t = 0 wasestimated from the previous 5-h (predose) concentration.

0.5 mL of distilled water. After applying positive pressure, each cartridge was rinsed with 0.5 mL of an aqueous solution of acetonitrile (25%). With the AASP, the adsorbed drugs were eluted directly onto a Brownlee New Guard C18, 7-µm guard column (15 mm × 3.2 mm) using a mobile phase consisting of acetonitrile-water-TEA-acetic acid (80:20:0.1:0.2) at a flow rate of 1.5 mL/min. The guard column was switched out of the analytical stream using the valve reset to provide column clean-up between samples. The eluant was chromatographed on a 5-µm Supelco LC8 column (25 cm × 4.6 mm) and was monitored with an UV detector at λ = 254 nm. Average recoveries by concentration ranged between 92% (at 0.1064 µg/mL) and 105% (at 3.901 µg/mL). No interfering peaks were observed in the chromatograms within the analytes' retention times (Fig. 1c). The lower limit of quantitation was 0.00540 µg/mL.

Pharmacokinetic Analysis

The post-infusion plasma concentration vs. time data fitted a polyexponential equation

$$C_t = \sum_{i=1}^{n} R_i e^{-\lambda_i t}$$

where C_t is the concentration of tirilazad in plasma at time t, and R_i and λ_i are coefficients determined from nonlinear least squares regression of the data (12). Each of the R_i values was corrected to reflect the infusion times (t_{inf}):

$$A_i = \frac{R_i t_{inf} \lambda_i}{1 - e^{-\lambda_i t_{inf}}}$$

Furthermore, to allow calculation of the area under the curve for the dosing interval and clearance of tirilazad, equivalent single-dose coefficients $A_{i,sd}$ were calculated as

$$A_{i,sd} = A_i(1 - e^{-\lambda_i \tau})$$

where τ, the dosing interval, is set to 6 h. Hence, these parameters were computed:

Table 2. Pharmacokinetic Parameters of Tirilazad in Rats

			Lipid emulsion					Freedox®IV Solution				
Dose (mg/kg/day)	Infusion rate (mg/mL)	Gender	Group	AUC(0,6) (μg × h/mL)	λ_1 (h^{-1})	CL (L/h/kg)	V_{ss} (L/kg)	Group	AUC(0,6) (μg × h/mL)	λ_1 (h^{-1})	CL (L/h/kg)	V_{ss} (L/kg)
6	0.07	M	1	0.34	0.32	4.13	8.90	4	0.35	0.18	4.40	10.20
				(0.02)	(0.05)	(0.22)	(1.20)		(0.03)	(0.03)	(0.24)	(2.10)
		F		0.24	0.47	6.60	8.61		0.58	0.26	5.00	10.20
				(0.04)	(0.08)	(1.00)	(0.60)		(0.32)	(0.03)	(1.40)	(3.20)
		Pooled		0.29	0.39	5.36	8.77		0.45	0.22	4.68	10.20
				(0.03)	(0.05)	(0.72)	(0.62)		(0.14)	(0.03)	(0.58)	(1.70)
20	0.36	M	2	1.23	0.35	3.81	4.90	5	1.17	0.26	4.56	6.80
				(0.18)	(0.01)	(0.64)	(1.10)		(0.34)	(0.04)	(0.82)	(1.90)
		F		0.80	0.31	6.50	8.10		0.87	0.29	6.77	11.00
				(0.20)	(0.12)	(1.30)	(1.80)		(0.08)	(0.01)	(0.58)	(1.80)
		Pooled		1.06	0.33	4.89	6.20		1.02	0.28	5.67	8.90
				(0.16)	(0.04)	(0.85)	(1.10)		(0.17)	(0.02)	(0.63)	(1.40)
65	0.36	M	3	12.4	0.38	1.14	0.51	6	2.54	0.34	6.33	11.8
				(1.3)	(0.06)	(0.09)	(0.03)		(0.20)	(0.06)	(0.55)	(1.30)
		F		11.2	0.27	1.45	0.82		2.55	0.33	7.23	13.0
				(1.3)	(0.03)	(0.19)	(0.26)		(0.09)	(0.05)	(0.35)	(1.30)
		Pooled		11.81	0.32	1.29	0.67		2.55	0.34	6.78	12.39
				(0.84)	(0.04)	(0.12)	(0.13)		(0.10)	(0.04)	(0.34)	(0.89)
20	0.34	M	7	1.11	0.32	3.80	8.00					
				(0.39)	(0.02)	(1.20)	(2.70)					
		F		0.82	0.33	6.60	11.90					
				(0.19)	(0.02)	(1.70)	(4.60)					
		Pooled		0.94	0.33	5.50	10.30					
				(0.18)	(0.01)	(1.20)	(2.80)					

Note: The SE values are enclosed in parentheses.

$$AUC(0, 6) = \sum_{i=1}^{n} \frac{A_{i.sd}}{\lambda_i}$$

$$AUMC(0, 6) = \sum_{i=1}^{n} \frac{A_{i.sd}}{\lambda_i^2}$$

$$CL = D/AUC$$

$$V_{ss} = \frac{AUMC(0, 6)}{AUC(0, 6)}$$

where AUC(0, 6) is the area under the plasma concentration vs. time curve of tirilazad during the dosing interval, AUMC(0, 6) is the area under the moment plasma concentration vs. time curve, CL is the clearance, D is the dose of tirilazad, and V_{ss} is the volume of distribution at steady-state (13). The parameter estimates are expressed as the mean ± SE, unless otherwise specified. The effects of formulation, dose, and gender on AUC(0, 6), CL, and V_{ss} of tirilazad in treatment groups #1 to #6 were analyzed by a three-way analysis of variance. Multiple comparisons were performed using least squares means if the F-ratio was significant. All tests employed a significance level of 0.05 (14,15).

Tissue Distribution

Tissue distribution in rats was measured by quantitative whole body autoradiography (WBA). The radiolabel location is shown in Fig. 1a. Specific activities were 33.0 (base) and 33.07 μCi/mg (salt). Sprague-Dawley rats (Crl:CD[BR], 6 per gender per formulation) received i.v. slow push doses via the lateral tail vein. Rats were given 6.64 ± 0.14 mg/kg (249 ± 5.0 μCi/kg) of [^{14}C]-tirilazad emulsion or 6.22 mg/kg (206 μCi/kg) of [^{14}C]-tirilazad mesylate solution. Rats were serially euthanized with CO_2 at 0.25, 4, 24, 72, 168 h after dosing and frozen in dry ice-heptane. Carcasses were embedded in 5% sodium carboxymethycellulose using a dry ice-heptane bath. Sagittal sections (20 μm) were collected, and dried on Scotch tape at −20°C. In a darkroom, sections and calibrated radioactive standards were exposed to a Kodak SB5 Scientific Imaging film (Eastman Kodak, Rochester, NY) for 1 to 8 weeks. The films were developed using Kodak GBX chemicals. Optical density was measured with a Brumagic densitometer (Brumac Industries, Hollywood, CA) with a 1-mm aperture. Tissue section areas less than 1 mm^2 were estimated from a nearby tissue with similar optical density. Optical densities of calibration segment images were fit to a third degree least squares plot of log optical density versus segment number. Tissue optical densities were converted to μg of [^{14}C] tirilazad mesylate-equivalents per gram of tissue. The limit of quantitation was 0.01 to 0.02 μg-equivalents per gram. Mean data were normalized to a 6.64 mg/kg dose. Trapezoidal *AUC*s were calculated, and are estimates, due to the singlicate design. All studies involving rats adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

RESULTS AND DISCUSSION

Vascular Irritation

The mean irritation index in the lateral tail vein of rats infused with tirilazad emulsions and FREEDOX® IV Solution

Table 3. Effect of Gender on the Pharmacokinetic Parameters of Tirilazad Across All Doses in Rats

	Lipid emulsion			Freedox® IV Solution		
	AUC(0,6) (μg × h/mL)	V_{ss} (L/kg)	CL (L/h/kg)	AUC(0,6) (μg × h/mL)	V_{ss} (L/kg)	CL (L/h/kg)
Male	4.02	5.4	3.16	1.37	9.8	5.14
	(1.68)	(1.2)	(0.45)	(0.28)	(1.1)	(0.38)
Female	3.48	7.3	5.17	1.43	11.5	6.41
	(1.56)	(1.7)	(0.88)	(0.28)	(1.2)	(0.51)
	p = 0.3783	p = 0.2567	p = 0.0073	p = 0.8866	p = 0.2763	p = 0.0381

Note: The SE values are enclosed in parentheses.

is shown in Fig. 2. The lipid emulsions, irrespective of drug concentration (0.76 to 6.75 mg/mL corresponding to 1.52 to 13.5 mg tirilazad, respectively) or injection volume (from 2 to 4 mL), were nonirritating in the rat tail vein irritation model as their mean irritation indices were comparable to that of the normal saline control. In contrast, a 2-mL injection of the 0.75 mg/mL solution (equivalent to 0.65 mg/mL tirilazad free base) or a 1-mL injection of the 1.5 mg/mL solution (equivalent to 1.3 mg/mL tirilazad free base) was clearly irritating. The irritation was found to be related to drug concentration: the higher the drug concentration, the more severe the irritation. There was a slight vascular injury in all groups including saline and vehicle treated rats at the injection site related to trauma associated with venipuncture (mechanical effects).

These results clearly demonstrate that the irritation of tirilazad can be eliminated by formulating the drug in lipid emulsion. The reduced vascular irritation of tirilazad is possibly due to reduced direct contact of the drug with vascular tissues as a result of drug encapsulation within the innermost oil phase of the emulsion particles, and/or due to the physiological pH associated with the lipid emulsion as opposed to the acidic pH of FREEDOX® IV Solution.

Pharmacokinetics

The plasma concentration versus time curves for tirilazad, administered either as lipid emulsion or FREEDOX® IV Solution to male and female rats, are shown in Figs. 3–5 and the pharmacokinetic parameters are summarized in Table 2.

There were no gender-related statistical differences in exposure to tirilazad for either formulation: the p-values for AUC (0, 6) across all doses were 0.3783 and 0.8866 for the lipid emulsion and the solution formulation, respectively (Table 3. In addition, there were no gender-related statistical differences in V_{ss} for either formulation, but CL was higher in female rats for the lipid emulsion formulation ($p = 0.0073$, Table 3). Also, no significant differences were found when comparing the plasma concentration-time profiles obtained after infusion of the lipid emulsion with those obtained after the infusion of the solution at 6 mg/kg/day and 20 mg/kg/day dose levels (Figs. 3–4). Statistical analysis of AUC(0, 6), CL, and V_{ss} of tirilazad after infusion of the emulsion indicated no differences from those values obtained after infusion of the solution ($p > 0.2$). However, at 65 mg/kg/day, the emulsion had a four-fold increase in AUC(0, 6) when compared to the solution ($p < 0.05$). Moreover, CL after administration of the emulsion was five times lower than that after the solution ($p < 0.05$). Also at the high dose, the results indicated more extensive distribution: V_{ss} was 20 times higher for the solution than for the emulsion ($p < 0.05$). Similar results have also been reported for propofol (16), palmitoyl-rhizoxin (17) and α-tocopherol (18). In the propofol study (16), it was shown that a lipid-free formulation resulted in a three-fold increase in V_{ss} and a 2-fold increase in CL as compared to the lipid emulsion formulation. In the study of pharmacokinetics of palmitoyl-rhizoxin, it was shown that the AUC of palmitoyl-rhizoxin after i.v. administration of the lipid emulsion was nearly 440-fold higher and CL was 720-fold lower as compared to the lipid-free cosolvent formulation (17). For α-tocopherol, AUC(0, 2) obtained after i.v. administration of the lipid emulsion was approximately 55-fold higher than the value obtained after i.v. administration of the lipid-free cosolvent formulation (18). The higher concentrations seen at the end of the infusion, the increased AUC(0, 6), and the decreased CL and V_{ss} of tirilazad when the drug was given as a lipid emulsion are likely due to prevention of drug precipitation that may have been effected by the emulsion. Because tirilazad is extremely insoluble in water at physiological pH, the drug may precipitate readily upon intravenous administration of the solution at high doses (19). Indeed, in a plasma compability study of the FREEDOX® IV Solution in three models (the static solubility model, the aggregometric model, and the dynamic flow model), it was shown that the precipitation of drug is

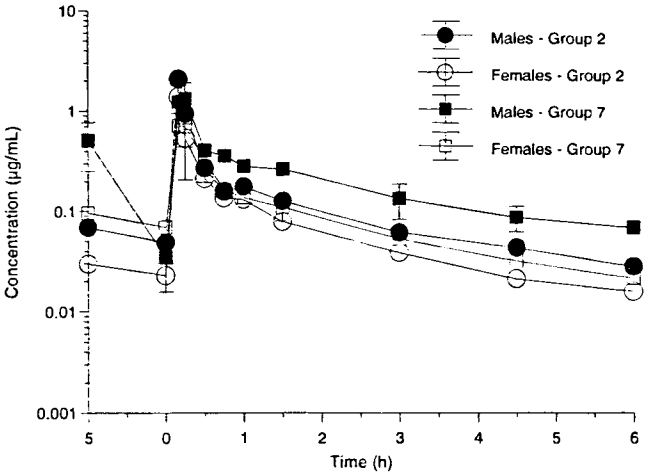


Fig. 6. Average plasma concentrations of tirilazad after i.v. infusion of 30% lipid (group #7) and 10% lipid emulsion of tirilazad (group #2). Error bars represent SE. Concentration at t = 0 was estimated from the previous 5-h (pre-dose) concentration.

Table 4. Concentration and AUC of Drug-Related Radioactivity in Rat Tissues at Various Time Points Following a Single Intravenous 6.64 mg/kg Dose of [^{14}C]-Tirilazad Mesylate (Solution) or [^{14}C]-Tirilazad Free Base (Emulsion)

Pharmacokinetic parameter	Concentration (salt equivalents) (μg-eq/g)												AUC(o-t) (μg-eq. h/g)			
Drug form Tissue/time	Salt 0.25 h	Base 0.25 h	Salt 1 h	Base 1 h	Salt 4 h	Base 4 h	Salt 24 h	Base 24 h	Salt 72 h	Base 72 h	Salt 168 h	Base 168 h	Salt 0.25-4 h	Base 0.25-4 h	Salt 0.25-168 h	Base 0.25-168 h
Adrenal cortex																
Female	123*	26.3*	180*	39.4	33.9	22.1	6.53	4.49	3.32*	1.50	2.38	1.35	435	117	1264*	662*
Male	67.9	37.4*	81.2	32.5	37.2*	17.8	7.28*	2.18*	2.89*	1.58	1.42	1.64*	234	102	1058*	546*
Blood																
Female	2.73	2.08	1.92	1.41	0.47	0.56	0.10	0.09	0.02	0.02	<0.01	<0.02	5	4	14.4	15.3
Male	2.63	2.06	1.56	1.35	0.57	0.33	0.14	0.09	0.03	0.01	<0.01	<0.02	5	4	16.8	11.8
Bone marrow																
Female	13.9	8.52	9.50	10.2	3.12	5.36	0.92	1.10	0.45	0.34	0.32	0.14	28	30	129	153
Male	10.9	7.61	7.50	6.60	4.50	4.43	1.74	0.85	0.29	0.35	0.25	0.15	25	22	151	127
Brain																
Female	2.19	0.28	1.04	<0.09	0.09	0.11	0.04	<0.05	<0.01	<0.02	<0.01	<0.02	3	0	6.07	5.64
Male	2.00	<0.21	0.79	0.31	0.11	<0.10	<0.04	<0.05	<0.02	<0.01	<0.01	<0.02	2	1	8.39	4.16
Brown fat																
Female	135	55.6	218	55.3	72.9	25.5	7.48	7.69	7.20	1.79	0.93	1.05	568	163	1980	858
Male	80.3	45.5	194	>73.3	31.5	23.4	6.02	2.31	1.47	1.26	0.47	0.50	441	190	1020	617
Kidney cortex																
Female	37.8	11.6	14.1	12.9	4.80	6.69	1.11	1.61	0.38	0.30	0.29	0.18	48	39	164	190
Male	26.7	12.0	15.0	12.0	7.73	6.73	1.80	1.28	0.54	0.46	0.21	0.22	50	37	223	192
Liver																
Female	111	42.8	114	33.2	16.1	15.2	2.82	3.26	1.55	1.24	0.94	0.78	280	101	650	490
Male	95.4	41.6	68.6	29.0	20.9	11.9	4.45	3.44	1.64	1.43	0.47	0.61	196	88	653	456
Lung																
Female	21.9	9.00	10.3	5.53	3.71	3.68	0.89	1.08	0.16	0.13	0.26	0.06	33	19	116	105
Male	18.0	8.52	6.73	7.08	6.38	1.93	1.09	0.42	0.26	0.22	0.04	0.05	29	19	141	71.2
Muscle																
Female	17.4	6.59	7.72	7.65	2.46	3.51	0.23	0.50	0.07	0.12	0.04	0.04	25	22	60.6	84.7
Male	17.3	7.20	9.29	5.94	2.55	1.58	0.31	0.20	0.10	0.07	0.02	0.04	28	16	67.2	45.8
Myocardium																
Female	42.8	12.0	11.3	8.14	2.88	3.79	0.62	0.81	0.28	0.20	0.13	0.11	42	25	110	111
Male	29.7	16.8	9.83	7.08	3.95	2.23	0.74	0.47	0.25	0.15	0.07	0.08	36	23	114	75.8
Ovary	8.27	9.90	9.74	10.1	5.66	9.90	2.43	0.89	0.80	0.99	0.04	0.70	30	38	214	272
Testes	1.10	0.41	0.93	0.96	0.77	0.51	0.42	0.36	0.16	0.16	0.09	0.09	3	3	38.2	35.9
Pancreas																
Female	30.0	12.1	23.6	14.4	7.68	7.56	0.78	1.19	0.25	0.21	0.12	0.10	67	43	181	179
Male	14.7	13.6	20.6	12.6	7.95	4.81	1.14	0.91	0.30	0.30	0.07	0.09	56	36	187	141
Pituitary																
Female	68.8*	13.9*	18.7*	11.1*	6.66*	8.57*	1.40*	1.85*	0.48	0.23*	0.28	0.32*	71	39	218*	219*
Male	30.8*	17.6*	14.3	18.7*	15.2*	6.40*	6.67*	2.24*	2.37*	1.26*	1.18*	1.05*	61	51	626*	333*
Spleen																
Female	21.5	12.8	14.0	10.5	7.07	7.43	1.70	3.12	1.13	1.10	1.04	0.56	45	36	285	322
Male	12.5	17.8	13.5	9.44	8.17	8.43	2.46	2.68	0.94	1.64	0.58	0.60	42	37	284	359
Thymus																
Female	4.72	2.21	5.05	3.81	2.94	3.29	0.68	0.92	0.23	0.15	0.10	0.09	16	13	84.1	92.2
Male	3.46	3.41	4.37	4.02	3.62	2.73	1.15	1.13	0.22	0.18	0.06	0.14	15	13	103	98.3
Thyroid																
Female	33.9*	19.2*	16.0*	14.4	28.5*	9.61*	18.9*	10.4	7.33*	6.24	9.98	4.57	85	49	1892*	1165*
Male	23.0*	13.2	17.8	14.4*	19.8	9.65*	6.36*	11.7*	8.13	3.20	2.91	7.12*	72	46	1135*	1113*

Note: AUC(0–4) values highlight tissues differering by approximately 2-fold or greater are in bold. Parameters are expressed in salt equivalents.
* Estimated values.

closely related to the percentage of formulation in plasma (19). When the percent of formulation in plasma was below 40%, the precipitation was minimal. Above 40%, however, the extent of precipitation increased rapidly and reached a maximum at about 60% of formulation in plasma, where 63% of the added drug precipitated (19). In the present study, assuming an average rat body weight of 300 g and an average rat plasma volume of about 7% of the body weight, the percent of formulation in rat plasma is 8%, 22%, and 48% for dose levels of 6, 20, and 65 mg/kg/day, respectively. Thus, at low dose levels, the pharmacokinetics of tirilazad following i.v. dosing with the solution are similar to that after lipid emulsion administration since drug precipitation is minimal. At the high dose, however, the precipitation of the drug upon infusion of the solution is significant, estimated at 50% of the administered dose (19). The precipitated drug can be rapidly removed by macrophages in the liver (and spleen) which will engulf foreign particles (20). As a result, both the concentrations at the end of the infusion and AUC(0, 6) were lower than those seen after dosing with the lipid emulsion (Fig. 5). Moreover, a 45% increase in CL was observed after the high dose administration of the solution ($p < 0.05$, Table 2), further supporting this hypothesis. In contrast, the emulsion caused dose-dependent decreases in CL and V_{ss} ($p < 0.05$ for both parameters, Table 2). This could be attributable to the vehicle, since when the drug is presented in a lipid emulsion formulation, it could have reduced the penetration of drug into the tissues, altering the distribution of the drug. This could have effected a lower V_{ss} of the drug after administration of the emulsion since the ratio of the concentrations of drug in the tissues to that in plasma was reduced (due to a higher concentration that was present in the plasma).

Additionally, in order to study the effect of lipid dose on pharmacokinetics of tirilazad, a 30% MCT emulsion containing 6.26 mg/mL tirilazad was infused to rats (Group #7) at the dose same as that given in Group #2. The total lipid dose for Group #7 was 0.063 mL vs. 0.11 mL for Group #2. The plasma concentration versus time curves for Group #7 and Group #2 were superimposable (Fig. 6). The pharmacokinetic parameter values were essentially the same as that observed with the lower lipid percentage (Table 2), suggesting that slight lipid dose change did not significantly affect the pharmacokinetics of tirilazad at this dose.

Tissue Distribution

Table 4 compares the AUC and temporal changes in the dose-normalized tissue concentrations of radioactivity observed following intravenous doses of FREEDOX® IV Solution and the lipid emulsion of [^{14}C]-tirilazad to rats. Single dose intravenous administration of the emulsion or the solution at a dose of 6 to 7 mg/kg resulted in extensive distribution of radioactivity to most organs and tissues. Liver, brown fat, adrenal cortex, kidney cortex, pituitary, myocardium, and thyroid contained the highest levels of drug-related radioactivity. Relatively low concentrations were observed in brain, blood, testes, and eye. These results are consistent with previous studies which showed that tirilazad mesylate and related materials have high affinity for peripheral tissues (21). Appreciable tissue levels persisted for at least 24 h, while measurable levels were found in most tissues 168 h after dosing. When both concentration and AUC are taken into account, the exposure of tissues or organs to tirilazad-related radioactivity did not differ markedly between genders. Blood concentrations for each formulation were similar at all timepoints. Higher initial concentrations (two fold or more and up to ten fold in brain) were observed in most tissues for the solution. These values, however, equilibrated by 4 h in most tissues and AUC(0, 4) differences were less than two fold, except in the adrenal cortex, brain, brown fat, and liver. In this singlicate, serial sacrifice study design, differences of two fold or less were not considered significant. In accord with the protection against vascular irritation afforded by the emulsion vehicle, we speculate that encapsulation of the tirilazad base within the emulsion particles during the initial distribution phase of the intravenous dose attenuates the initial tissue penetration relative to the aqueous solution formulation. This difference diminishes in the 4 h following the dose, based on similar AUC(0, 4) estimates for most tissues.

CONCLUSIONS

These studies demonstrate that lipid emulsions can significantly reduce the venous irritation without changing the pharmacokinetics and tissue distribution of tirilazad in rats at low doses. At high doses, lipid emulsions may offer improved pharmacokinetics by preventing drug precipitation in blood.

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