Effect of the Immunomodulator Tilorone on Antipyrine Disposition in the Rat

CRAIG K. SVENSSON

Received May 16, 1986, from the *Department of Pharmaceutical Sciences, College of Pharmacy & Allied Health Professions, Wayne State University, Detroit, MI 48202.* Accepted for publication August 11, 1986.

Abstract □ A wide variety of immunomodulators, including the synthetic interferon inducer tilorone (2,7-bis[2-(diethylamino)ethoxy]fluoren-9-one), have been shown to inhibit cytochrome P-450-dependent drug metabolism in vitro. In an effort to determine if tilorone also inhibits drug metabolism in vivo, we examined the effect of this agent on the disposition of antipyrine in the rat. Administration of tilorone hydrochloride (50 $mg \cdot kg^{-1} \cdot d^{-1}$) for 4 d resulted in a 42% reduction in antipyrine clearance and a 71% increase in plasma half-life. Administration of a single dose of tilorone hydrochloride (50 mg/kg) 48 h prior to antipyrine was found to cause a similar alteration in antipyrine clearance and half-life. Both treatment regimens were also found to produce a substantial weight loss in the pretreated animals. Preliminary studies indicated that tilorone treatment reduced food and water consumption in the rat. Therefore, the effect of a 75% decrease in food and water consumption on antipyrine disposition was also examined. This magnitude of restriction had no detectable effect on antipyrine elimination. Thus, tilorone results in a substantial reduction of the in vivo metabolic clearance of antipyrine and appears to be a useful compound for studying the dynamic interaction between the drug metabolizing and immune systems in vivo.

Numerous compounds are known to alter cytochrome P-450-dependent drug metabolism. One of the most recently discovered classes of substances which inhibit P-450-dependent metabolism is the immunomodulators. Among these agents are vaccines, products of microorganisms, viruses, and synthetic compounds.[1,2] The mechanism by which these agents cause a reduction in drug metabolism is not clear. None of these agents has been shown to inhibit drug metabolism when added directly to an in vitro incubation system, suggesting an indirect mechanism of action. The results of several investigations suggest that these agents inhibit drug metabolism by stimulating the release of some component of the immune system (possibly interferon) which in turn either directly or indirectly causes an increase in the catabolism of P-450 cytochromes.[1,2]

Tilorone (2,7-bis[2-(diethylamino)ethoxy]fluoren-9-one), a synthetic interferon inducer which has been shown to cause substantial reductions in microsomal drug metabolism in pretreated animals,[3,4] appears to be a useful agent for studying the mechanism of the interaction between the drug metabolizing and immune systems. To date, however, all studies of the effects of tilorone have examined drug metabolism in vitro. While these investigations have provided much useful information, the isolation of the microsomal system precludes continued interaction with the immune system. In addition, drug metabolizing enzyme activities in vitro vary with the particular isolation and purification procedures and the availability of co-substrates. Thus, results may differ substantially from those obtained in vivo. It would appear, therefore, that assessment of in vivo drug metabolism would prove worthwhile in elucidating the dynamic interaction between these two host defense systems. The purpose of these studies was to examine the effect of tilorone on drug metabolism in vivo.

Experimental Section

Materials—Antipyrine (lot 0504 TK) and phenacetin (lot 42F-0008) were purchased from Aldrich Chemical Company (Milwaukee, WI). Tilorone hydrochloride (2,7-bis[2-diethylamino)ethoxy]fluoren-9-one dihydrochloride) was a gift from Merrell Dow Research Institute (Cincinnati, OH). The melting point of the compound received (236–237 °C) was identical to that reported in the literature for tilorone hydrochloride [lit.[5] mp 235–237 °C] Acetonitrile, perchloric acid, potassium phosphate, and sodium phosphate were purchased from Fisher Chemical Co. (Livonia, MI).

Animal Studies—Male Sprague-Dawley rats weighing 188 to 254 g had an indwelling cannula implanted in the right jugular vein[6] 2 d prior to the administration of antipyrine. Animals were individually housed in plastic metabolism cages and antipyrine (20 mg/kg), dissolved in isotonic saline (10 mg/mL), was infused through the cannula at a rate of 0.34 mL/min. Serial blood samples (0.25 mL) were obtained through the cannula over a 5- or 6-h period. Blood was collected in plastic syringes and transferred to heparinized glass capillary tubes. Plasma was separated by centrifugation and stored at −20 °C until analysis by HPLC. To a 100-μL sample was added 50 μL of 1.5 M perchloric acid containing the internal standard (phenacetin). After mixing and centrifugation, 10 μL of the supernatant was injected onto a Partisil 10 C-8 column (Whatman Inc., Clifton, NJ). A mobile phase of 0.022 M phosphate buffer:acetonitrile (75:25) at a flow rate of 1.5 mL/min was used for the elution. The eluant was monitored at 254 nm using a 441 absorbance detector (Waters Associates, Inc., Milford, MA), and the output was recorded on a 3392A integrator (Hewlett Packard, Avondale, PA). The limit for detection of antipyrine using this method is 0.1 μg/mL. The coefficients of variation at 27.2 and 3.7 μg/mL were 3.7 and 1.8%, respectively (n = 4). Food and water were withheld during the period of blood sampling in all experiments.

In the multiple dose study, animals received isotonic saline or tilorone hydrochloride (50 $mg \cdot kg^{-1} \cdot d^{-1}$) dissolved in isotonic saline (final concentration of 20 mg/mL) by gastric intubation between 8 and 9 a.m. for 4 d. On day 5, antipyrine disposition was determined. In the single dose study, animals received a single dose of isotonic saline or tilorone hydrochloride (50 mg/kg) by gastric intubation 48 h prior to antipyrine administration.

As will be discussed, animals pretreated with tilorone consistently lost weight. Preliminary studies indicated that animals that lost weight during pretreatment with tilorone consumed ~50% less food and water than control animals. Because food and water restriction could influence antipyrine disposition, a group of animals was placed on a restricted diet, designed to provide ~75% less food and water (i.e., 6 g of food pellets and 10 mL of water) than consumed by control animals for 2 days. Control animals had free access to food and water, and food and water consumption were monitored daily.

Data Analysis—The plasma concentration versus time data were analyzed utilizing an unweighted nonlinear least squares regression computer program.[7] The apparent volume of distribution (Vd) was calculated as dose/C_0, where C_0 is the plasma concentration at time 0. Apparent $t_{1/2}$ was determined for the plot of plasma concentration versus time by least squares regression analysis. Total plasma clearance (CL) was calculated as dose/AUC, where AUC is area under the plasma concentration–time curve extrapolated to infinity. Body weights and pharmacokinetic parameters were compared using the t test. A value of $p < 0.05$ was considered statistically significant, and data are presented as mean ± SD.

0022-3549/86/1000-0946$01.00/0

Results and Discussion

Renton and Mannering[3] observed that the administration of tilorone hydrochloride at a dose of 50 mg·kg^{-1}·d^{-1} for 4 d reduced cytochrome P-450 content by 36%. In addition, microsomal ethylmorphine *N*-demethylase, benzo[*a*]pyrene hydroxylase, and aniline hydroxylase activities were reduced to 50, 44, and 22% of control, respectively. We utilized an identical regimen to examine the effect of tilorone on drug metabolism in vivo, using antipyrine as a model substrate. Antipyrine has been widely used as a tool to assess P-450-dependent drug metabolism in both humans and animals in vivo.[8,9] The compound exhibits a low intrinsic hepatic clearance and is not significantly bound to plasma proteins. Changes in its total body clearance, therefore, can be concluded to result from changes in drug metabolizing capability.[10]

Antipyrine plasma concentration–time curves for animals given multiple doses of tilorone and for control animals are shown in Fig. 1. Plasma concentrations declined monoexponentially at this dose. Mean pharmacokinetic parameters for tilorone and saline treated animals are presented in Table I. Pretreatment with tilorone for 4 d resulted in a 42% reduction in antipyrine clearance (from 7.88 ± 1.72 to 4.57 ± 1.00 mL·min^{-1}·kg^{-1}) and a 71% increase in plasma half-life (from 74.8 ± 16.0 to 127.8 ± 25.9 min). Interestingly, the data in Table I indicate that tilorone pretreatment resulted in a significant weight loss in these animals. While control animals averaged a 12-g weight gain over the study period, those that received tilorone lost an average of 10 g. This observation was unexpected considering Renton and Mannering[3] observed no variation in body weight using the same pretreatment regimen. In addition, Leeson et al.[4] found that animals receiving 100 mg/kg of tilorone hydrochloride continued to gain weight, though at a slower rate than control animals. We decided, therefore, to examine the effect of a single dose of tilorone hydrochloride on antipyrine disposition since we anticipated less of a weight loss with a shorter pretreatment period.

Figure 2 illustrates the mean plasma concentration–time curve for the single dose study. The data in Table I indicate that a single dose of tilorone hydrochloride administered 48 h prior to antipyrine administration resulted in a 39% reduction in antipyrine clearance (from 8.08 ± 1.38 to 5.02 ± 0.86 mL·min^{-1}·kg^{-1}) and a 65% increase in terminal half-life (from 67.9 ± 10.0 to 112.0 ± 14.8 min). Table I indicates, however, that these animals lost a similar amount of weight as those receiving multiple doses of the drug.

Preliminary studies[11] indicated that animals which received tilorone and lost weight consumed ~50% less food and water than identically housed, age- and weight-matched control animals. The literature is contradictory

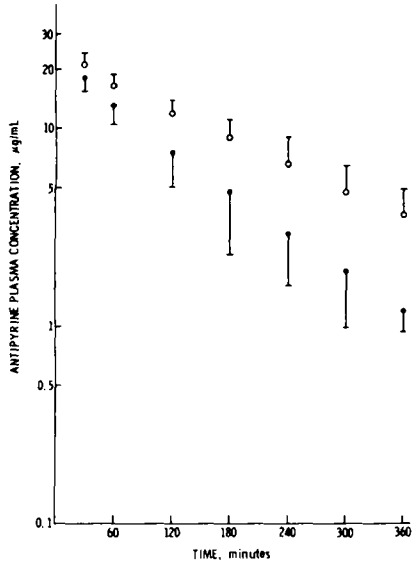

Figure 1—*Mean antipyrine plasma concentration–time profile in control (●) and multiple dose tilorone pretreated (○) rats. Bars represent one SD; n = 6.*

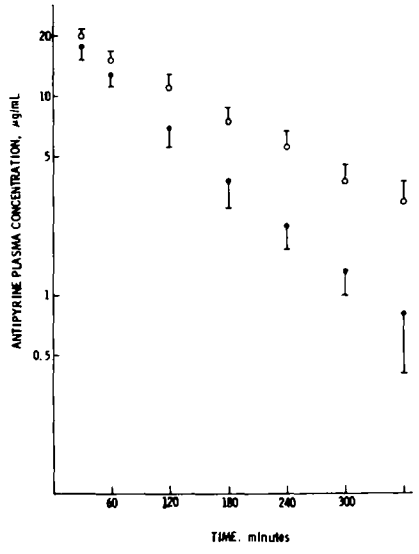

Figure 2—*Mean antipyrine plasma concentration–time profile in control (●) and single dose tilorone pretreated (○) rats. Bars represent one SD; n = 5 for control and n = 6 for tilorone pretreatment groups.*

Table I—Effect of Tilorone Pretreatment on Body Weight and Antipyrine Disposition[a]

Treatment	Body Weight, g[b]		Pharmacokinetic Parameters		
	Day 1	Day 5	*CL*, mL·min^{-1}·kg^{-1}	$t_{1/2}$, min	*Vd*, mL·kg^{-1}
Control	214 (15)	226 (18)	7.88 (1.72)	74.8 (16.0)	817 (45)
Tilorone·HCl, 50 mg·kg^{-1}·d^{-1} for 4 d	205 (17)	195 (15)[c]	4.57 (1.00)[d]	127.9 (25.9)[d]	813 (41)
Control	216 (24)	220 (22)	8.08 (1.38)	67.9 (10.0)	780 (97)
Tilorone·HCl, 50 mg·kg^{-1}· single dose	213 (7)	200 (14)	5.02 (0.86)[e]	112.0 (14.8)[f]	798 (32)

[a] Results are expressed as mean (± SD); n = 6 in all groups except single dose control where n = 5. [b] Pretreatment regimen was begun on day 1 and antipyrine (20 mg·kg^{-1}) was administered on day 3 (single dose) or day 5 (multiple dose). All weights were obtained between 8 and 9 a.m. prior to drug or isotonic saline administration. [c] $p < 0.01$. [d] $p < 0.005$. [e] $p < 0.002$. [f] $p < 0.001$.

Table II—Effect of Short-Term Food and Water Deprivation on Body Weight and Antipyrine Disposition[a]

Treatment[b]	Body Weight, g		Pharmacokinetic Parameters		
	Day 1	Day 3	*CL*, mL·min^{-1}·kg^{-1}	$t_{1/2}$, min	*Vd*, mL·kg^{-1}
Control (n = 7)	217 (20)	222 (20)	7.89 (1.33)	71.0 (13.0)	791 (84)
Restricted (n = 5)	235 (17)	198 (16)	8.17 (2.93)	76.5 (25.3)	818 (44)

[a] Results are expressed as mean (±SD). [b] Control animals were allowed free access to food and water on days 1 and 2. Restricted animals received 75% less food and water than controls for these same two days. Antipyrine (20 mg·kg^{-1}) was administered to both groups on day 3.

regarding the effect of food and water restriction on drug metabolism.[12-16] It appears, however, that the influence of such dietary restriction is both substrate and gender dependent. To determine if this decreased intake of food and water contributed to the results of our studies, we examined what effect similar food and water restriction alone would have on antipyrine disposition. The data in Table II indicate that restriction of food and water intake for 2 d (i.e., a time equivalent to the single dose tilorone study) had no significant effect on the disposition of antipyrine. Antipyrine clearance in control animals was 7.89 ± 1.44 versus 8.17 ± 2.93 mL·min^{-1}·kg^{-1} for food and water restricted animals. The weight loss in the food and water restricted group was greater than that in the tilorone single dose group.

Thus, tilorone causes a substantial reduction in the metabolism of antipyrine in vivo. The magnitude of reduction in antipyrine clearance was similar to the reduction in cytochrome P-450 content noted by Renton and Mannering.[3] The magnitude of the reduction was also similar after both single and multiple dose pretreatments, which suggests that tilorone does not produce its effect by inhibiting the synthesis of cytochrome P-450. Since the turnover of P-450 in the rat has a half-life of 1–2 d,[17,18] chronic dosing would be expected to cause a greater depression of metabolism than single dose administration if the effect of tilorone resulted from inhibition of the synthesis of cytochrome P-450. These results are consistent with those previously reported by el Azhary et al.[19]

The decrease in food and water intake caused by tilorone in these studies neither explains nor contributes significantly to the reduction in antipyrine metabolism. The mechanism by which tilorone causes this reduction in food and water consumption remains to be determined. Animals did not demonstrate overt signs of toxicity (i.e., they did not appear lethargic or ataxic) which might cause this decreased consumption.

References and Notes

1. Mannering, G. J.; Renton, K. W.; el Azhary, R.; Deloria, L. B. *Ann. N.Y. Acad. Sci.* **1980**, *350*, 314–331.
2. Descotes, J. *Drug Metab. Rev.* **1985**, *16*, 175–184.
3. Renton, K. W.; Mannering, G. J. *Drug Metab. Dispos.* **1976**, *4*, 223–231.
4. Leeson, G. A.; Biedenbach, S. A.; Chan, K. Y.; Gibson, J. P.; Wright, G. J. *Drug Metab. Dispos.* **1976**, *4*, 232-238.
5. "The Merck Index", 10th ed.; Windholz, M., Ed.; Merck & Co., Inc.: Rahway, NJ, 1983; p 1353.
6. Weeks, J. R.; Davis, J. D. *J. Appl. Physiol.* **1964**, *19*, 540–541.
7. Statistical Consultants, Inc. *Amer. Statis.* **1986**, *40*, 1.
8. Vesell, E. S. *Clin. Pharmacol. Ther. (St. Louis)* **1979**, *26*, 275–286.
9. Danhof, M.; Krom, D. P.; Breimer, D. D. *Xenobiotica* **1979**, *9*, 695–702.
10. Wilkinson, G. R.; Shand, D. G. *Clin. Pharmacol. Ther. (St. Louis)* **1975**, *18*, 377–390.
11. Svensson, C. K., unpublished results.
12. Baetjer, A. M.; Rubin, R. J. *J. Toxicol. Environ. Health* **1976**, *2*, 131-138.
13. Nakajima, T.; Sato, A. *Toxicol. Appl. Pharmacol.* **1979**, *50*, 549–556.
14. Siegers, C. P.: Strubelt, O.; Dost-Kempf, E. *Toxicol. Lett.* **1982**, *10*, 423–426.
15. Sachan, D. S. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 984–989.
16. Sachan, D. S.; Das, S. K. *J. Nutr.* **1982**, *112*, 2301–2306.
17. Arias, I. M.; DeLeon, A. *Mol. Pharmacol.* **1967**, *3*, 216-218.
18. Levin, W.; Kuntzman, R. *J. Biol. Chem.* **1969**, *244*, 3671–3676.
19. el Azhary, R.; Renton, K. W.; Mannering, G. J. *Mol. Pharmacol.* **1980**, *17*, 395–399.

Acknowledgments

This work was supported in part by a Wayne State University Research Award and by the Roland T. Lakey Education, Research and Development Fund. The technical assistance of Miss Li-Ling Liu is gratefully acknowledged.