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Pharmacokinetics of Pentoxifylline and Its Metabolites in Healthy Mice and in Mice Infected with *Candida albicans*

KENNETH MILLER,[1]† ARNOLD LOUIE,[2] ALDONA L. BALTCH,[2]* RAYMOND P. SMITH,[2] PATRICK J. DAVIS,[4] AND MORRIS A. GORDON[3]

Albany College of Pharmacy,[1] Stratton Veterans Affairs Medical Center and Albany Medical College,[2] and Mycology Laboratory of the Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York,[3] and College of Pharmacy, University of Texas, Austin, Texas[4]

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Pentoxifylline has immunomodulatory properties and has been shown to decrease organ damage and improve survival in animals with gram-negative sepsis or endotoxemia. This effect is mediated by a reduction in endotoxin-induced production of tumor necrosis factor alpha (TNF-α) by the host. In earlier studies, we observed an unexpected increase in mortality in mice infected with *Candida albicans* that were given pentoxifylline even though concentrations of TNF-α in serum were not affected. The current study was designed to determine whether the pharmacokinetics of pentoxifylline and its metabolites were altered in *C. albicans*-infected mice and, if so, whether these changes could have contributed to the increased mortality. Noninfected mice and mice infected with *C. albicans* were treated with pentoxifylline (60 mg/kg of body weight) intraperitoneally every 8 h. Serum was collected from animals after one (day 0), four (day 1), or seven (day 2) injections of pentoxifylline or saline (controls). The first dose was administered 6 h after *C. albicans* infection. Serum was pooled. Concentrations of pentoxifylline and metabolites I, IV, and V were determined by capillary gas chromatography. Renal function and hepatic profiles were assessed. Pharmacokinetic parameters (maximum concentration of pentoxifylline in serum, half-life, and area under the concentration-time curve from 0 h to infinity [$AUC_{0-\infty}$]) for all noninfected mice were similar and did not differ from those for day 0-infected mice. For day 1-infected mice, values of these three pharmacokinetic parameters for pentoxifylline and metabolite I were increased two- to fourfold over values for noninfected and day 0-infected mice. For metabolites IV and V, the $AUC_{0-\infty}$ was increased approximately eightfold over control values. In addition, day 1-infected mice demonstrated evidence of renal and hepatic dysfunction. In summary, *C. albicans* infection produced marked changes in the pharmacokinetics of pentoxifylline and its metabolites in the mice. The high concentrations of pentoxifylline and its metabolites in serum attained in infected mice may have contributed to the increased mortality of mice with systemic candidiasis.

Pentoxifylline is one of several methylxanthine compounds that has immunomodulatory properties (10). In vitro, pentoxifylline pretreatment can attenuate the production of interleukin 1 and tumor necrosis factor alpha by human mononuclear cells in response to bacterial endotoxin (12). In addition, it can decrease endotoxin-mediated migration, adherence, and production of superoxide radicals by phagocytic cells (13). In vivo, pentoxifylline improves the outcome for animals challenged with high-level inocula of gram-negative bacilli or endotoxin, suggesting that pentoxifylline or related compounds might be of value in treating sepsis (6, 7). These observations have led to its extensive use in animal models of sepsis, burns, and traumatic shock (4).

In a series of experiments to determine the role of cytokine activation in systemic *Candida albicans* infection, we observed decreased survival rates in pentoxifylline-treated mice (5). However, pentoxifylline administration did not affect the production of tumor necrosis factor alpha or interleukin 6 in these infected mice. It was possible that altered pharmacokinetics of pentoxifylline and its metabolites in systemic *C. albicans* infection were responsible for the shortened survival times. However, a review of the literature failed to provide information about the metabolism and clearance of pentoxifylline in infected versus healthy animal models. Therefore, the purpose of this study was to determine the pharmacokinetics, including clearance, of pentoxifylline in a murine model of systemic candidiasis in an attempt to define a possible pharmacologic cause for the unexpected results of our previous studies (5).

MATERIALS AND METHODS

C. albicans. Strain 88-689-6 was isolated from the blood of a neutropenic patient. The microorganism was maintained on Sabouraud dextrose agar (BBL Microbiology Systems, Cockeysville, Md.) at 22°C until use. For each study, two or three colonies of *C. albicans* were subcultured onto potato dextrose agar (BBL) and incubated at 35°C for 48 h. A fungal suspension was prepared with sterile, pyrogen-free, phosphate-buffered saline (PBS; Gibco-BRL Inc., Grand Island, N.Y.) and quantified with a hemocytometer.

Morphologic examination demonstrated that >99% of the organisms were blastoconidia. The viability of the yeast was found to be >95% by trypan blue exclusion and quantitative cultures. The endotoxin concentration in the fungal suspension was found to be <0.05 endotoxin units (EU) per ml by a competitive endotoxin enzyme-linked immunosorbent assay (PyroChek competitive lipopolysaccharide ELISA; ALerCHEK, Portland, Maine).

Mice. Female, 18- to 20-g NYLAR white mice were raised at the Animal Research Facility of the Wadsworth Center for Laboratories and Research (Griffin Laboratories, Guilderland, N.Y.). These outbred Swiss mice were housed in hanging metal cages and received food and water ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committees of the respective institutions.

Pentoxifylline. Pentoxifylline powder was provided by William Novick, Jr. (Hoechst-Roussel, Somerville, N.J.). The potency of the drug was confirmed by the manufacturer just prior to the initiation of this study. Pentoxifylline was dissolved in sterile, pyrogen-free water to produce a stock solution at a concen-

* Corresponding author. Mailing address: Infectious Diseases (111D), Stratton VA Medical Center, 113 Holland Ave., Albany, NY 12208. Phone: (518) 462-3311, ext. 3080. Fax: (518) 462-3350. E-mail: baltch.aldona@albany.va.gov.

† Present address: American Association of Colleges of Pharmacy, Alexandria, VA 22314-2841.

TABLE 1. The linearities, sensitivities, and percentages of coefficient variation at high and low concentrations of pentoxifylline and its metabolites I, IV, and V

Compound	Concn (ng/ml) range[a]	Linearity	Sensitivity limit (ng/ml)	% Coefficient variation at:	
				High concn	Low concn
Pentoxifylline	5–2,000	0.997	5	1.1	3.3
Metabolite I	5–2,000	0.999	5	2.3	4.4
Metabolite IV	5–2,000	0.997	5	4.0	2.8
Metabolite V	25–10,000	0.995	25	3.6	5.6

[a] Range over which standard curve was generated.

tration of 20 mg/ml. The stock solution was passed through a 0.45-μm-pore-size filter (Lida Manufacturing, Kenosha, Wis.). The drug was further diluted to the desired concentrations with sterile, pyrogen-free PBS and was used immediately. The solutions contained less than 0.25 EU of endotoxin per ml, as determined by *Limulus* amebocyte lysate assay (Whittaker M. A. Bioproducts, Walkersville, Md.).

Systemic candidiasis model. Mice were injected intravenously with 10^6 CFU of *C. albicans* or PBS in a 0.2-ml volume. Mice received 60 mg of pentoxifylline per kg of body weight intraperitoneally (i.p.) at 8-h intervals beginning 6 h after the intravenous injection of *C. albicans* or PBS. In a previous study (5), this pentoxifylline regimen resulted in a statistically significant decrease in mean survival of NYLAR mice systemically infected with *C. albicans*. The volume of each i.p. injection was 0.1 ml. Animals were assessed prior to each injection for mortality. Previous studies demonstrated that mice died within 4 h after becoming moribund; therefore, moribund mice were sacrificed.

Measurement of serum levels of pentoxifylline and its metabolites by capillary gas chromatography. The method used in the assay of plasma pentoxifylline and metabolites I, IV, and V was described by Burrows (2). The procedure included a single extraction of all species from plasma into chloroform, derivative formation (the trifluoracetyl derivative in the case of metabolite I and the methyl ester derivatives in the case of metabolites IV and V and their internal standard), and analysis by capillary gas chromatography. An analog of pentoxifylline (Hoechst EH79-0254) was employed as the internal standard for pentoxifylline and metabolite I, while 1-(5′-carboxypentyl)-3,7-dimethylxanthine (Hoechst-Roussel) was used as the internal standard for measuring metabolites IV and V.

Standard curves were generated for pentoxifylline, metabolite I, and metabolite IV in the range of 5 to 2,000 ng/ml, and for metabolite V in the range of 25 to 10,000 ng/ml. Capillary gas chromatography was performed with a Varian model 3700 gas chromatograph, modified for split-injection capillary column analysis and fitted with a thermionic radiation-specific (nitrogen/phosphorus) detector. The separation was conducted with a DB-5 phenylmethyl column (30 by 0.32 [inside diameter] mm; Varian model JW123503-20) fitted with a precolumn glass insert guard column and packed with quartz fiberglass (CapSaver; N-Phase, Inc.). The linearities, sensitivities, and percent coefficients of variation at high and low concentrations of pentoxifylline and metabolites I, IV, and V are given in Table 1.

Pharmacokinetic study. To determine the pharmacokinetics of pentoxifylline and metabolites in noninfected and infected mice, three or four animals in each group were sacrificed by CO_2 asphyxiation at 0, 5, 15, 30, 60, and 90 (infected animals only) min after the first (day 0), fourth (day 1), and seventh (day 2) doses of pentoxifylline. The first dose of drug was administered 6 h after mice were injected with *C. albicans* or PBS. None of the infected mice survived to day 2. Blood was collected by cardiac puncture and allowed to clot at 4°C. At each time point the serum was separated from the clot by centrifugation, pooled, and stored at −70°C until analyzed.

Pharmacokinetic analysis. Pharmacokinetic parameters were determined by standard techniques. The concentration-time data for pentoxifylline and its three metabolites were analyzed separately. The elimination rate constant (k_{el}) was determined by linear regression of the log concentration-time data, and the half-lives ($t_{1/2}$) were calculated as $t_{1/2} = 0.693/k_{el}$. The $t_{1/2}$ of metabolites IV and V could not be determined in any treatment group because concentrations were essentially unchanging over the measurement interval.

The linear-trapezoidal rule was used to calculate the area under the concentration-time curve to the last measured value (AUC_τ), and the residual area, AUC_{res}, was calculated by dividing the last measured concentration (C_τ) by k_{el}. The total area under the curve ($AUC_{0-\infty}$) = AUC_τ + AUC_{res}.

RESULTS

Pharmacokinetics. The maximum plasma concentration (C_{max}) of pentoxifylline occurred at the first sampling time, 5 min after injection. For the noninfected mice on days 0, 1, and 2 and the infected mice on day 0, the C_{max}, $t_{1/2}$, and $AUC_{0-\infty}$ values for pentoxifylline were similar, but the C_{max}, $t_{1/2}$, and $AUC_{0-\infty}$ values were higher on day 1 in infected mice (Table 2). In all treatment groups, pentoxifylline concentrations declined over time in a log-linear fashion (Fig. 1). The pharmacokinetic parameters for noninfected mice on all three treatment days were similar and, thus, were combined to obtain an estimate of the within-treatment variability of pentoxifylline pharmacokinetics. The pharmacokinetic parameters for noninfected mice are reported as the means ± standard deviations.

The C_{max} of metabolite I in all treatment groups also occurred at the first sampling time. The C_{max}, $t_{1/2}$, and $AUC_{0-\infty}$ values for metabolite I on days 0, 1, and 2 for noninfected mice and on day 0 for infected mice were similar, but all parameters were higher for the day 1-infected mice (Table 2). Metabolite I concentrations declined in a log-linear fashion in all treatment groups (Fig. 2). The pharmacokinetic parameters of metabolite I for noninfected mice on all treatment days were

TABLE 2. Pharmacokinetics, including AUC, $t_{1/2}$, and C_{max}, of pentoxifylline and its metabolites for noninfected mice and mice infected with *C. albicans* on days 0, 1, and 2 after fungal infections[a]

Mice	Day(s)	Compound[d]	$AUC_{0-\infty}$ (μg · min/ml)	$t_{1/2}$ min	C_{max} (μg/ml)
Noninfected	0, 1, 2	Pentox	427 ± 78	6.6 ± 1.6	31.8 ± 8.1
Infected	0	Pentox	415	8.6	30.5
Infected	1	Pentox	1,673	13.1	54.5
Noninfected	0, 1, 2	Met I	136 ± 32	6.3 ± 2.8	8.9 ± 1.9
Infected	0	Met I	121	8.3	7.9
Infected	1	Met I	444	14.5	14.5
Noninfected	0, 1, 2	Met IV	6.3 ± 2.3[b]	N/C[c]	0.40 ± 0.2
Infected	0	Met IV	5.42[b]	N/C	0.24
Infected	1	Met IV	51.1[b]	N/C	0.65
Noninfected	0, 1, 2	Met V	766 ± 89	9.4 ± 0.4	31.8 ± 4.8
Infected	0	Met V	759	12.6	26.4
Infected	1	Met V	6,280	N/C	103.8

[a] Results for days 0, 1, and 2 for infected mice were similar; therefore, they were combined (mean ± standard deviation).
[b] Value for AUC_τ, because k_{el} value could not be determined.
[c] N/C, not calculated.
[d] Pentox, pentoxifylline; Met, metabolite.

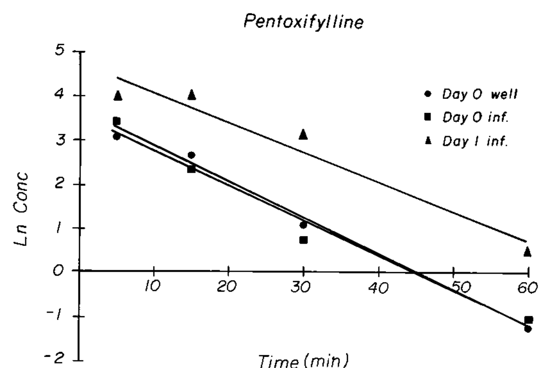

FIG. 1. Natural logarithm of concentrations of pentoxifylline in serum over time after i.p. dosing. Well, uninfected mice; inf., infected mice. All sera for each time point and group were pooled.

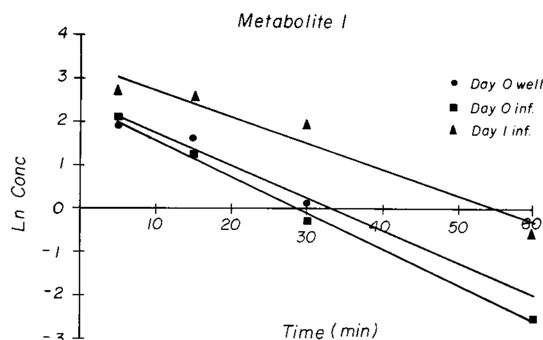

FIG. 2. Natural logarithm of concentrations of pentoxifylline metabolite I in serum over time after i.p. dosing. Well, uninfected mice; inf., infected mice. All sera for each time point and group were pooled.

also combined and reported as the means ± standard deviations.

Metabolite IV (1-[4-carboxybutyl]-3,7-dimethylxanthine), which is important in humans (1, 11), was present in very low concentrations in all treatment groups (Fig. 3). The time to C_{max} (T_{max}) varied in the noninfected mice, occurring at 5 min in two groups and at 30 min in one group. It was not possible to obtain an accurate estimate of the $t_{1/2}$ of metabolite IV in any treatment group. The values of $AUC_{0-\tau}$ of metabolite IV for the day 1-infected group were dramatically higher than those for the noninfected and day 0-infected mice (Table 2).

The T_{max} of metabolite V, the major urinary metabolite of pentoxifylline in humans (3-carboxypropyl), occurred at 5 min in two noninfected-mouse groups and at 15 min in one noninfected-mouse group. For day 0-infected mice, metabolite V concentrations and pharmacokinetic parameters were similar to those for noninfected mice. In day 1-infected mice, metabolite V levels increased throughout the sampling protocol so that C_{max}, $t_{1/2}$, and $AUC_{0-\infty}$ could not be accurately determined (Fig. 4). However, the AUC_{τ} for metabolite V for the day 1-infected mice was almost 10-fold greater than the $AUC_{0-\infty}$ for the noninfected and day 0-infected mice, and the C_{max} for the day 1-infected mice was more than 3-fold higher than those for the noninfected and day 0-infected mice (Table 2).

Organ function. Values of serum creatinine, lactate dehydrogenase, alanine transaminase, aspartate transaminase, and

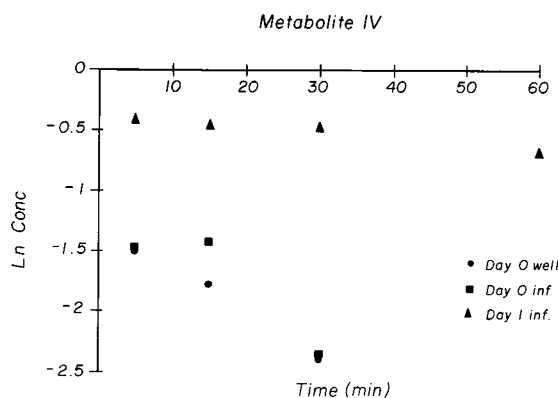

FIG. 3. Natural logarithm of concentrations of pentoxifylline metabolite IV in serum over time after i.p. dosing. Well, uninfected mice; inf., infected mice. All sera for each time point and group were pooled.

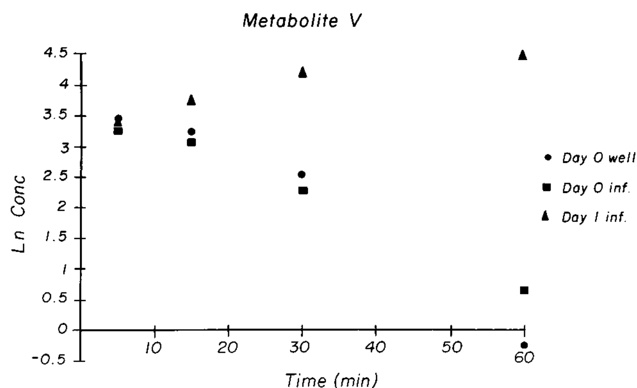

FIG. 4. Natural logarithm of concentrations of pentoxifylline metabolite V in serum over time after i.p. dosing. Well, uninfected mice; inf., infected mice. All sera for each time point and group were pooled.

alkaline phosphatase, measured in aliquots of drug concentration samples, revealed that in day 0-infected mice, renal and hepatic functions were still within normal limits while in day 1-infected mice, renal and hepatic functions were significantly compromised, indicating organ injury (Table 3).

DISCUSSION

Pentoxifylline was eliminated very rapidly after absorption in both the infected and noninfected mice. The $t_{1/2}$ of both pentoxifylline and its metabolites in noninfected mice were within the ranges reported for healthy mice by other investigators (3, 9). There was also an increase in the AUCs for pentoxifylline (fourfold), metabolite I (threefold), and metabolites IV and V (greater than fourfold) in the infected mice (Table 2). These large increases in the AUCs of pentoxifylline and its metabolites in infected mice resulted from decreased hepatic and renal elimination due to the impaired liver and kidney functions, and from increased pentoxifylline absorption following i.p. administration. Raju et al. reported a 29% bioavailability for i.p. administration compared to that for subcutaneous administration for healthy mice (8). These authors speculated that this low i.p. bioavailability was due to extensive "first pass" hepatic metabolism of pentoxifylline. In our study, increased pentoxifylline bioavailability resulting from decreased hepatic elimination during the first pass through the liver would have contributed to an increase in both the AUC and C_{max} of pentoxifylline in the infected mice. Pentoxifylline metabolite formation would also have increased as a result of the increased bioavailability of pentoxifylline in the infected mice.

Murine pentoxifylline pharmacokinetics differ among various mouse strains, resulting in differences in the extent of metabolite formation. This study and that of Honess et al. (3) found measurable concentrations of metabolites I, IV, and V at comparable doses. Conversely, Raju et al. (8) were not able to detect metabolite IV in plasma following i.p. or subcutaneous administration of pentoxifylline. Honess et al. (3) found that metabolite I and IV concentrations were approximately 1/10 that of pentoxifylline, while in the present study metabolite I and IV concentrations were 1/4 and 1/100, respectively, those of pentoxifylline. Raju et al. (8) found that metabolite I levels were about one-fourth those of pentoxifylline, while metabolite V concentrations were somewhat higher than those of pentoxifylline. Metabolite V concentrations were of the same order of magnitude as pentoxifylline concentrations in all three studies. These differences in the metabolite patterns of pen-

TABLE 3. Mean serum creatinine, lactate dehydrogenase, alanine transaminase, aspartate transaminase, and alkaline phosphatase levels in noninfected mice and mice infected with *C. albicans* before and during pentoxifylline administration

Mice	Day	Time (min)	Mean level of[c]:				
			Creatinine (mg/dl)	LD (U/ml)	ALT (U/ml)	AST (U/ml)	AP (U/ml)
Untreated, noninfected mice	N/A[a]	N/A	0.2	812	54	330	222
Noninfected	0	−20[d]	0.2	1,530	64	517	249
		5	0.4	1,022	49	192	260
		15	0.3	891	49	190	276
		30	0.3	1,205	61	297	266
		60	0.1	1,740	71	370	275
Noninfected	1	−20	0.1	1,592	86	631	248
		5	0.3	1,105	40	358	244
		15	0.3	1,360	36	447	255
		30	0.2	1,262	42	393	252
		60	0.2	840	54	319	255
Noninfected	2	−20	0.2	1,762	68	405	251
		5	0.3	1,483	44	364	234
		15	0.2	903	46	232	254
		30	0.2	833	49	225	231
		60	0.2	904	68	441	219
Infected	0	−20	0.2	2,359	52	324	223
		5	0.1	1,659	49	370	190
		15	0.2	1,354	54	402	240
		30	0.2	1,455	48	334	229
		60	0.1	1,510	65	484	209
Infected	1	−20	1.2	>600[b]	296	>1,000[b]	112
		5	1.1	>600[b]	342	>1,000[b]	128
		15	1.2	>600[b]	371	>1,000[b]	100
		30	1.2	9,564	331	5,243	103
		60	1.1	4,835	161	3,089	97
		90	1.1	>600[b]	203	>1,000[b]	>1,000[b]

[a] N/A, not applicable.
[b] Insufficient sample to further quantify value.
[c] LD, lactate dehydrogenase; ALT, alanine transaminase; AST, aspartate transaminase; AP, alkaline phosphatase.
[d] Levels were measured 20 min before drug administration.

toxifylline, particularly pharmacologically active metabolite I, caution against any extrapolation of the physiological effects of pentoxifylline among mouse strains, unless the pharmacokinetics and metabolic profiles are known.

Did the changes in the pharmacokinetics of pentoxifylline and its metabolites caused by the *C. albicans* infection contribute to a reduction in mouse survival time and greater mortality? The magnitudes of the AUCs of pentoxifylline and metabolites I and V for the infected mice would only be achieved for noninfected mice with doses greater than 240 mg/kg, assuming dose-independent pharmacokinetics. Honess et al. reported that single i.p. doses of up to 200 mg/kg were well tolerated by healthy mice but that 400 mg/kg was highly toxic (3). However, in our study, the day 1-infected mice received three doses of pentoxifylline, and as the *C. albicans* infection progressed, liver and kidney functions became progressively impaired. Thus, with each successive dose of pentoxifylline different pharmacokinetics were exhibited, reflective of successively increasing doses. The combination of the infection-induced organ function impairment and the resulting increasing systemic exposure to pentoxifylline and its metabolites likely was responsible for the shorter survival times and greater mortality of the infected animals.

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