Pharmaceutical Research, Vol. 3, No. 6, 1986

Report

Influence of Malnutrition on the Disposition of Metronidazole in Rats

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Received March 19, 1986; accepted June 20, 1986

The influence of dietary protein deficiency on the disposition of metronidazole and its two major metabolites was examined in male Sprague–Dawley rats fed for 4 weeks on a 23% (control-) or a 5% (low-) protein diet *ad libitum*. Following an intravenous bolus dose of 10 mg/kg metronidazole hydrochloride, blood samples were obtained serially for a period of 24 hr after drug administration. Serum concentration–time data were analyzed by nonlinear least-squares regression, as well as noncompartmental techniques. The average mean residence time (MRT) was significantly prolonged by 48%, while the systemic clearance (Cl) was decreased by 42% in the protein-deficient rats. Since there was no alteration in the apparent steady-state volume of distribution (V_{ss}), the mean harmonic half-life was increased from 2.9 to 5.0 hr in the protein-deficient rats. Although the percentage of metronidazole recovered as total drug in the urine over 24 hr was not significantly different between the two groups of animals, rats on a low-protein diet excreted a significantly smaller percentage of the administered dose as unchanged metronidazole (mean ± SD, 24.6 ± 3.8 vs 36.5 ± 12%) and a larger percentage (16.7 ± 2.6 vs 8.3 ± 1.8%) as the hydroxylated metabolite. No significant difference in the partial metabolic clearance of the hydroxylated metabolite of metronidazole was seen between the two groups of animals; however, there was a significant decrease in the renal clearance of metronidazole (1.45 ± 0.68 vs 0.55 ± 0.06 ml/min/kg) in the rats fed a low-protein diet. We conclude that the decreased clearance of metronidazole in protein deficiency is a result primarily of the decreased glomerular filtration rate, decreased biliary excretion, and/or increased net tubular reabsorption of metronidazole.

KEY WORDS: malnutrition; metronidazole; pharmacokinetics; rats; metabolism.

INTRODUCTION

In recent years, there has been a growing interest in the effects of malnutrition on drug metabolism and pharmacokinetics (1–3). Protein-calorie malnutrition (PCM) is a major public-health problem especially in developing countries. Although malnutrition itself cannot be treated by drug therapy, it can induce situations where drugs are the primary form of treatment. The treatment of infections is the most common and severe drug-related problem associated with PCM (4). A malnourished individual is known to be much more susceptible to infections than the general population. As a result, the most widely used class of drugs in PCM is antibiotics. In fact the proportion of the pharmaceutical budget spent on antibiotics in the Third World is manyfold greater than in developed countries (5). In light of the wide usage of antibiotic drugs in developing countries, it becomes extremely important to determine the effects of malnutrition on the disposition of antibiotics.

Metronidazole has been used for many years as an extremely effective systemic agent currently used for the treatment of amebiasis (6), giardiasis (7), trichomoniasis (8,9), acute ulcerative gingivitis (10), and other infections. Recently, it has been found that metronidazole is effective in the treatment of Crohn's disease (11,12) and in the prevention of cholestasis associated with total parenteral nutrition (13). It is a fairly nontoxic drug in animals and is very well tolerated by the majority of patients receiving it. An investigation was therefore undertaken to determine whether PCM alters the kinetics and metabolism of metronidazole in an animal model.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing between 125 and 150 g at the beginning of the study were purchased from Bio-Labs, Inc., Oak Park, Ill. The rats were randomly assigned to one of two diets containing either 23% (control) or 5% (low) protein. All rats were provided food and water *ad libitum* for 4 weeks as described previously (14). Diets were isocaloric and purchased in a pellet form from Teklad Test Diets (Madison, Wis.). The control diet contained 263.5 g casein/kg, 466.8 sucrose/kg, and 20 g cellulose/kg; the 5% protein diet contained 57 g casein/kg, 651.5 g sucrose/kg, and 39.97 g cellulose/kg. All other dietary constituents (vi-

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0724-8741/86/1200-0352$05.00/0

tamins, minerals, corn oil, and corn starch) were identical. Food intake and body weight of all rats were recorded at least twice a week to assess the influence of the low-protein diet.

Metronidazole hydrochloride was administered to control and protein-deficient rats intravenously (10 mg/kg) as a 5 mg/ml normal saline solution through a jugular vein cannula. Blood samples (0.1 ml) were collected from the jugular vein periodically over 24 hr. Plasma was separated by centrifugation and stored at −20°C until assayed. Urine was collected for 36 hr.

Metronidazole and its two major metabolites, the hydroxy metabolite [1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole] (M1) and the acid metabolite (1-acetic acid 2-methyl nitroimidazole) (M2) in plasma and urine were determined by a high-performance liquid chromatographic procedure similar to that described by Gibson *et al.* (15) modified to use only 25 μl biological fluid. The chromatographic conditions were as follows: a 3.9 × 300-mm column (μ-Bondapak C-18, Waters Associates), a mobile phase of 92% potassium phosphate monobasic adjusted to pH 3 and 8% acetonitrile delivered with a Beckman Model 100A dual-piston pump at a flow rate of 3 ml/min, and a temperature of 23°C. The absorbance was measured at 313 nm with a fixed-wavelength spectrophotometer (Beckman Model 160). The lower detection limit and precision of the assay are 0.1 mg/liter and less than 2.5%, respectively.

Total plasma proteins and albumin concentration were determined with commercially available kits (Sigma Chemical Co., St. Louis, Mo.) using albumin as the standard protein.

Individual plasma metronidazole concentrations were fitted to a first-order, two-compartment open model using a nonlinear regression program, MULTI (16), to obtain initial estimates of the first-order terminal elimination rate constant, k. Using noncompartmental methods (17), the zero and first moments of the plasma concentration vs time data were determined to obtain values for the mean residence time (MRT), total-body clearance (Cl), and apparent volume of distribution at steady state (V_{ss}). All area terms were calculated by a linear trapezoidal rule, with end correction where necessary. Renal clearance of metronidazole (Cl_R) was determined as the product of the total clearance and the fraction of the dose recovered from the urine as unchanged metronidazole. Partial metabolic clearance to the respective metabolites was determined as the product of the total clearance and the fraction of the dose recovered from the urine as either the acid (Cl_{M2}) or the hydroxy metabolite (Cl_{M1}) of metronidazole.

Statistical Methods. Differences in the pharmacokinetic parameters of metronidazole following the two diets were determined by Student's t test for unpaired data. A probability of less than 0.05 was considered to be statistically significant. All results are expressed as mean ± SD.

RESULTS AND DISCUSSION

Animals maintained on a low-protein diet (5%) consumed approximately 50% as much food as those maintained on a control-protein diet (23%). Protein and caloric intakes were significantly higher in the animals fed a control, 23% protein diet *ad libitum* than in the animals on a low-protein diet (Table I). Despite an *ad libitum* supply of food, the rats on a low-protein diet showed a marked decrease in body weight gain over the 4 weeks. A significant reduction in total plasma proteins and albumin was observed in rats on a 5% protein diet (Table I) as previously reported (14,18). Since the protein and caloric intakes were significantly reduced in the animals on the low-protein diet, it is important to realize that any changes or lack thereof in the disposition of metronidazole can be attributed not to protein deficiency but, rather, to PCM.

Table I. General Effects of Dietary Protein Level in Male Rats

Parameter	Protein level[a] 23%	5%
Initial body weight (g)	149 ± 13	153 ± 16
Final body weight (g)	318 ± 9	173 ± 26*
Food intake (g/day/rat)	16.9 ± 1.2	10.8 ± 2.0*
Protein intake (g/day/rat)	4.5 ± 0.3	0.6 ± 0.1*
Caloric intake (kcal/day/rat)	59.5 ± 1.0	31.4 ± 0.7*
Plasma proteins (g/dl)	5.7 ± 0.4	4.9 ± 0.7*
Plasma albumin (g/dl)	3.3 ± 0.4	2.7 ± 0.4*

[a] Each value is the mean ± SD.
* $P < 0.001$.

A semilogarithmic plot of the mean plasma metronidazole concentration as a function of the time following intravenous injection of 10 mg/kg metronidazole is shown in Fig. 1. The disposition of metronidazole was best described by a two-compartment open model. The pharmacokinetic parameters of metronidazole in rats on either a low- or a control-protein diet are given in Table II. The average MRT was significantly prolonged, by 48%, from 4.3 ± 0.9 to 6.4 ± 0.5 hr in the protein-deficient rats. The mean metronidazole harmonic half-life was found to be 2.9 hr in rats on a normal

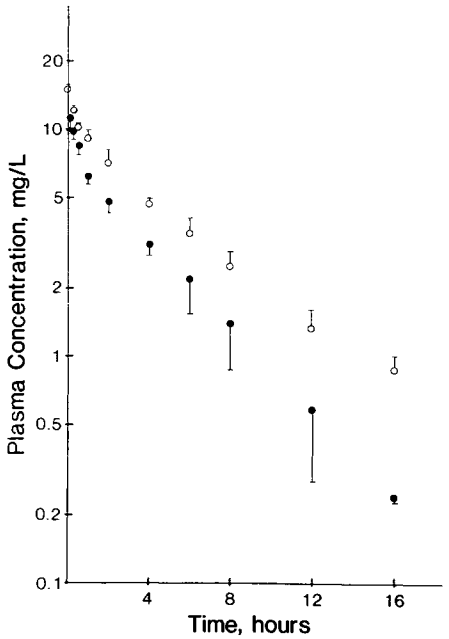

Fig. 1. Plasma metronidazole concentrations after intravenous administration of 10 mg/kg metronidazole hydrochloride in rats fed a 23% (control-) (●) or 5% (low-) (○) protein diet for 4 weeks. Each data point is the mean ± SD for four to six animals.

Table II. Effect of Dietary Protein on the Pharmacokinetics of Metronidazole in Male Rats

Parameter	Protein level[a] 23%	5%
MRT (hr)	4.3 ± 0.9	6.4 ± 0.5*
V_{ss} (liters/kg)	0.96 ± 0.08	0.86 ± 0.07
Cl (ml/min/kg)	3.86 ± 0.66	2.24 ± 0.27*
k (min^{-1})	0.0040 ± 0.0009	0.0023 ± 0.0005**
Cl_R (ml/min/kg)	1.45 ± 0.68	0.55 ± 0.06*
Cl_{M1} (ml/min/kg)	0.31 ± 0.06	0.38 ± 0.09
$t_{1/2}$ (hr)[b]	2.9	5.0

[a] Each value is the mean ± SD.
[b] Harmonic mean.
* $P < 0.005$.
** $P < 0.01$.

Table III. Effect of Dietary Protein on the Composition of Urinary Excretion Products After an Intravenous Dose of 10 mg/kg Metronidazole Hydrochloride to Male Rats

Urinary excretion product	Protein level[a] 23% ($N = 6$)	5% ($N = 4$)
Metronidazole	36.5 ± 12.0	24.6 ± 3.8
M1	8.3 ± 1.8	16.7 ± 2.6*
M2	ND[b]	ND
Total	45.0 ± 7.3	42.6 ± 4.3

[a] Results are percentages of the administered dose collected over 36 hr and are expressed as mean ± SD. A correction factor was made for the difference in molecular weights for metronidazole and M1.
[b] Not detectable.
* $P < 0.05$.

diet and 5.0 hr in rats on a low-protein diet, which was associated with a statistically significant decrease in metronidazole systemic clearance, from 3.86 ± 0.66 to 2.24 ± 0.27 ml/min/kg. However, the apparent steady-state volume of distribution (V_{ss}) between the control and the protein-deficient animals (0.96 ± 0.08 vs 0.86 ± 0.07) was not found to be statistically significant. Although no significant difference was observed in the partial metabolic clearance to M1 (Cl_{M1}), there was a dramatic 62% decrease in the renal clearance (Cl_R) of metronidazole, from 1.45 ± 0.68 to 0.55 ± 0.06 ml/min/kg, in the rats fed a low-protein diet. The significant reduction in the renal clearance of metronidazole appears to be one reason for the increase in the MRT and half-life of metronidazole in protein-deficient rats. The observed decrease in metronidazole renal clearance on a low-protein diet reflects an alteration in renal tubular function associated with protein restriction. Since the renal clearance was found to be much less than the glomerular filtration rate (3.3 ml/min/kg) (19) and since metronidazole is minimally bound to plasma proteins (20), these data suggest that protein deficiency decreases the clearance of metronidazole by decreasing the glomerular filtration rate and/or increasing the net renal tubular reabsorption of metronidazole. The present study is consistent with findings of others that decreased protein intake may lead to decreased clearance of drugs excreted by glomerular filtration (e.g., gentamicin) (21) and increased tubular reabsorption of others (e.g., oxipurinol) (22). In addition, there was a significant reduction in the clearance of metronidazole by routes other than renal excretion and metabolism to the hydroxylated metabolite ($Cl - Cl_R - Cl_{M1}$), from 2.10 ± 0.46 to 1.32 ± 0.21 ml/min/kg, in the protein-deficient rats. Metronidazole undergoes enterohepatic recirculation, with approximately 24% of an intravenous dose eliminated in the bile. Since the reduction in the renal clearance accounted for about half of the difference in the total-body clearance, it is suggested that there is a decreased biliary excretion of metronidazole in rats on a low-protein diet.

The urinary excretion of metronidazole in rats on a normal-protein diet is consistent with the observations by other investigators (23). Previous studies (23,24) reported that approximately 70–80% of an oral or intravenous dose of ^{14}C-metronidazole was excreted in 24 hr, 24% in bile and 47–53% in urine. In the present study, the recovery of total metronidazole excreted in the urine over 24 hr was about 45% and was not significantly different from that in the protein-deficient animals (Table III). However, there was a decreased percentage of metronidazole excreted unchanged in urine associated with a concomitant increase in the urinary excretion of M1, the hydroxylated metabolite (16.7 ± 2.6 vs 8.3 ± 1.8%). No detectable levels of the acetic acid metabolite, M2, was recovered in the urine in either group of rats.

The clinical implications of the present findings are unknown. Generally, metronidazole is considered a safe drug because of its relative high therapeutic index and limited duration of treatment. However, prolonged therapy with metronidazole has been used for treating Crohn's disease patients (11,12), who often tend to be malnourished. Since metronidazole does not stimulate or inhibit its own metabolism following repeated oral administration (25) and its elimination kinetics are linear, the findings presented herein are applicable to the chronic administration of metronidazole. Therefore, the protein-calorie-malnourished population potentially may be predisposed to side effects from short or prolonged metronidazole therapy because of possible decreased elimination and high blood concentrations of metronidazole. Metronidazole not only has been used in developed countries, but also is used in the treatment of protozoan infections such as amebiasis, which is more commonly seen in underdeveloped countries where PCM is prevalent. Thus, the present studies indicate the need for systematic studies on the disposition of metronidazole in the malnourished population so that appropriate dosage guidelines may be developed.

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