Pharmacokinetics of metronidazole in horses after intravenous, rectal and oral administration

A. STEINMAN*
M. GIPS†
E. LAVY*
I. SINAY* &
S. SOBACK†

*Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, PO Box 12, Rehovot 76100, Israel
†National Residue Control Laboratory, Ministry of Agriculture, Kibbutz Veterinary Institute, PO Box 12, Be’er Dagan 50250, Israel


Metronidazole pharmacokinetics in horses was studied after intravenous (i.v.), rectal (p.r.) and oral (p.o.) administration at 20 mg/kg using a triple crossover study design. Metronidazole mean ± SD half-life was 196 ± 39, 212 ± 30 and 240 ± 65 min after i.v., p.r. and p.o. administration, respectively. The metronidazole clearance was 2.8 (mL/min/kg) and the volume of distribution at steady state was 0.68 L/kg. The pharmacokinetic parameters calculated for metronidazole after administration of the drug by the various routes showed that bioavailability (74 ± 18 vs. 30 ± 9%) and maximum serum concentration (22 ± 8 vs. 9 ± 2 μg/mL) were significantly higher after p.o. administration compared with p.r. administration. There were no significant differences in mean absorption time (45 ± 69 vs. 66 ± 18 min) and the time to reach maximum serum concentration (65 ± 36 vs. 58 ± 18 min). The results indicated that p.r. administration of metronidazole to horses, although inferior to p.o. administration in terms of bioavailability, provides an alternative route of administration when p.o. administration cannot be used.

(Paper received 10 April 2000; accepted for publication 28 August 2000)

Amir Steinman, Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, PO Box 12, Rehovot 76100, Israel. E-mail: steinman@agr.hiuji.ac.il

INTRODUCTION

Metronidazole (1-2 hydroxyethyl-2-methyl-5-nitroimidazole) is a bactericidal antimicrobial agent used for treatment of infectious diseases caused by anaerobic bacteria and protozoa. It is active against most obligate anaerobes including Bacteroides spp., Fusobacterium, Veillonella, Clostridium spp., Peptococcus and Peptostreptococcus spp. (Plumb, 1995). Metronidazole pharmacokinetics were characterized by a high volume of distribution and a high concentration in peritoneal fluid (Specht et al., 1992).

Anaerobic bacteria play an important role in equine peritonitis (Hawkins et al., 1993; Hillyer & Wright, 1997) and thus, metronidazole was used successfully for treatment of several infectious diseases including peritonitis in horses (Mair & Yeo, 1987; Sweeney et al., 1991a,b; Hawkins et al., 1993). Peritonitis is a major complication following abdominal surgery in horses (Hawkins et al., 1993). Therefore, metronidazole is used commonly for prophylaxis and therapy of peritonitis associated with celiotomies. In case of postoperative ileus, the oral (p.o.) route for administration of metronidazole is not applicable. Although metronidazole can be administered intravenously (i.v.), it is technically inconvenient and time consuming. Large infusion volumes are needed because of the poor solubility of the drug (Plumb, 1995).

Metronidazole administration per rectum (p.r.) to horses is a potentially useful alternative route of administration. Rectal compared with p.o. administration in general has the benefit that some of the drug absorbed from the rectum will bypass the liver, reducing potential first-pass metabolism (Benet et al., 1996). Successful clinical outcome after p.r. administration of metronidazole has been described in horses (Mair & Yeo, 1987; Sweeney et al., 1991a). Whereas pharmacokinetics of metronidazole administered p.o. (Sweeney et al., 1986, Baggot et al., 1988; Specht et al., 1992) and p.r. (Garber et al., 1993) were described, data are still lacking regarding bioavailability of metronidazole following rectal administration in horses.
The purpose of the present study was to determine the pharmacokinetics of metronidazole in horses after a single dose of 20 mg/kg body weight after i.v., p.r. and p.o. administration.

MATERIALS AND METHODS

Animals

Five healthy local bred geldings and one local bred stallion, age 2–5 years and weighing 350–500 kg, were used. The horses were kept at grass pasture between studies and were housed in a box stall the night before and during each of the different administrations. Physical examination, complete blood counts, and serum biochemical analysis were performed before the beginning of the study. During the entire study period the horses were monitored for adverse reactions. Blood samples for hematological and biochemical tests were collected 24 and 48 h after each drug administration in order to identify potential adverse reactions such as liver damage, drug allergy etc. Feed was withheld from all horses for 12 h before drug administration, and during this time free access to fresh water was provided. Horses were observed for 2 h after rectal drug administration for defecation. Two hours after drug administration the horses were allowed access to feed. The study was approved by the ethics committee of the Koret School of Veterinary Medicine, the Hebrew University of Jerusalem.

Drug administration

Metronidazole (metronidazole injection 500 mg/100 mL: B. Braun Melsungen, Germany) was administered i.v., via an indwelling 14G catheter inserted in the jugular vein, at a dosage of 20 mg/kg body weight by rapid infusion. Approximately 10 min was required for the infusion because of the large volume of the metronidazole solution. For p.o. administration, an aqueous suspension of crushed 250 mg tablets of metronidazole (Metrogi® 250 mg: Teva, Petah-Tikva, Israel) in 40 mL of tap water was administered by nasogastric tube into the stomach at a dosage of 20 mg/kg body weight. For p.r. administration, an identically prepared suspension of crushed tablets was used. After manual evacuation of the rectum, the suspension was injected via a 28F-rubber catheter 30 cm into the rectum at a dose of 20 mg/kg body weight. At least 7 days washout was allowed between studies with the different routes of administration.

Blood samples

Blood samples (10 mL) were collected from the jugular vein via a 14G catheter at 1, 5, 10, 15, 20, 30, 40, 50 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 14, 24 h. In the i.v. administration study, the blood samples were collected via a second 14G catheter inserted in advance in the opposite jugular vein. After collection of the blood samples (10 mL), plasma was separated by centrifugation (2000 × g) and kept at −30 °C until analysed.

Metronidazole assay

Metronidazole was determined by use of a reversed phase HPLC method utilizing a Hewlett-Packard H-P 1100 (Hewlett-Packard, Waldbronn, Germany) liquid chromatography system. The instrument consisted of a low pressure mixing gradient pump, diode-array detector, autosampler, Rheodyne 7750 (Cotati, CA, USA) injection valve, column oven (set at 30 °C) and a H-P DOS-Chemstatment instrument control and data handling station. A C18 column (Luna, 3 μ, 100 × 4.6 mm Phenomenex, Torrance, CA, USA) was used. The mobile phase consisted of 0.05 M sodium acetate (Sigma, St Louis, MO, USA), adjusted to pH 3.0 and acetonitrile (Chromasolv, Riedel & DeHaen, Seelze, Germany) (88:12) at a flow rate of 1.0 mL/min. Detector wavelength was set at 320 nm and the injection volume was 20 μL. The limit of quantification was 0.09 mg/L and the standard curve was linear within the range from 0.09 to 100 mg/L (coefficient of correlation: 0.9999). Precision analysis showed that the inter-day coefficient of variation among four replicates was 5.0% at 0.09 mg/L, 2.0% at 6.25 mg/L and 2.1% at 100 mg/L. The recoveries from plasma samples were more than 75%.

The extraction of metronidazole from plasma was carried out as follows. To each 0.2 mL of plasma 5 mL dichloromethane was added. The tube was vortexed vigorously for 10–15 sec, centrifuged at 3000 × g for 10 min, the supernatant solution was discarded by vacuum aspiration and the sample was evaporated to dryness. The sample was redissolved in 150 μL of mobile phase, centrifuged at 15 000 × g for 10 min, and 100 μL from the clear supernatant solution was collected and used for injection to the HPLC. Metronidazole standards were prepared in antibiotic-free plasma and processed according to the same procedure as the plasma samples. Sample concentrations were determined from the standard curve established by use of peak areas of the standards.

Pharmacokinetic analysis

The pharmacokinetics of metronidazole were determined by use of the non-compartmental approach based on the statistical moment theory (Yamaoka et al., 1978) and utilizing a computer program (Yamaoka, 1986). The linear terminal slope (β) was calculated by a linear, least squares regression analysis, using the last 8–10 ln plasma concentration vs. time points. The terminal half-life ($t_{1/2}$) was calculated according to Equation 1 (Gibaldi & Perrier, 1982)

$$t_{1/2} = \ln 2 / \beta.$$  

(1)

The mean residence time (MRT) was determined according to Equation 2 (Yamaoka et al., 1978) as,

$$MRT = \frac{AUMC0 - \text{int}}{AUC0 - \text{int}}$$  

(2)

where $AUMC$ is area under the first moment curve and $AUC$ is the area under the plasma drug concentration vs. time (zero moment) curve. The $AUMC$ and $AUC$ were calculated by the trapezoidal method and extrapolated to infinity (Gibaldi & Perrier, 1982).

The volume of distribution at steady state was estimated using Equation 3 (Benet & Galeazzi, 1979).

\[ V_{ss} = D \times \frac{AUMC/AUC^2}{CL \times MRT} \]  

(3)

where \( D \) is the dose.

Total body clearance (\( CL \)) was calculated by use of Equation 4 (Gibaldi & Perrier, 1982) as,

\[ CL = \frac{D}{AUC_0 \rightarrow \infty} \]  

(4)

The mean absorption time (\( MAT \)) following p.o. or p.r. administration was determined according to Equation 5 (Riegelman & Collier, 1980)

\[ MAT = MRT_{\text{non-i.v.}} - MRT_{i.v.} \]  

(5)

The absolute bioavailability (\( F \)) was calculated from the \( AUC \) ratio collected following non-i.v. and i.v. administration according to Equation 6 (Gibaldi & Perrier, 1982)

\[ F = \frac{AUC_{\text{non-i.v.}}}{AUC_{i.v.}} \]

(6)

The maximum drug plasma concentration after oral or rectal administration (\( C_{\text{max}} \)) and the time at which \( C_{\text{max}} \) was achieved (\( t_{\text{max}} \)) were determined directly from the concentration vs. time curve.

**Statistical analysis**

Analysis of variance and the Tukey–Kramer test were used to analyse statistical differences (\( P < 0.01 \)) in the determined pharmacokinetic parameters. The Kruskal–Wallis non-parametric ANOVA test with Dunn’s multiple comparisons test was used to analyse the half-life values.

**RESULTS**

Physical and laboratory findings for the six horses were within reference values. Adverse reactions were not observed in any of the six horses during the 3 days following the three drug administrations, although one horse had a mild colic in between studies, which was treated medically. None of the horses delected during the first 2 h after rectal administration of the metronidazole. Figure 1 depicts mean plasma metronidazole concentrations collected following i.v., p.r. and p.o. administrations at 20 mg/kg body weight.

The pharmacokinetic parameters calculated for metronidazole following the various administration protocols are presented in Table 1. The \( AUC \), \( C_{\text{max}} \) and \( F \) were significantly lower following rectal administration compared with p.o. administration.

![Graph](image_url)

**Table 1.** Mean pharmacokinetic parameters of metronidazole determined following i.v., p.r. and p.o. administration of metronidazole at a dose of 20 mg/kg body weight to six healthy horses in a triple crossover study

| Pharmacokinetic parameter | Intravenous | | Oral | | Rectal |
|---------------------------|-------------|--|------|---|--|---|
| Mean (median)             | ± SD        | | Mean (median) | ± SD       | | Mean (median) | ± SD       |
| \( AUC \) (µg/ml \times h) | 126 (115)   | 37 | 88 (88) | 10 | 38 (40) | 7 |
| MRT (min)                 | 243 (244)   | 22 | 288 (261) | 64 | 315 (289) | 69 |
| \( t_{1/2} \) (min)       | 196 (202)   | 39 | 212 (202) | 30 | 240 (213) | 65 |
| \( C_{\text{max}} \) (µg/ml) | 22 (21)    | 8  | 9 (8)    | 2  | 9 (8)    | 2  |
| \( t_{\text{max}} \) (min) | 65 (60)     | 36 | 58 (50)  | 18 | 58 (50)  | 18 |
| \( MAT \) (min)           | 45 (32)     | 69 | 66 (44)  | 81 | 66 (44)  | 81 |
| \( F \) (%)               | 74 (82)     | 18 | 30 (24)  | 9  | 30 (24)  | 9  |
| \( V_{ss} \) (L/Kg)       | 0.68 (0.68) | 0.16 | 68 (3.2) | 1.6 | 68 (3.2) | 1.6 |
| \( CL \) (mL/min.Kg)      | 2.8 (2.9)   | 0.8 | 2.8 (2.9) | 0.8 | 2.8 (2.9) | 0.8 |

DISCUSSION

The present study indicated that metronidazole is rapidly absorbed following rectal administration. The absorption was consistent among the horses. Although mean peak plasma concentration (9.0 mg/L) following rectal administration in the present study was considerably higher than that (4.5 mg/L) reported by Garber et al. (1993), the bioavailability was low (30%). The difference in the mean peak plasma concentration between the two studies, apparently, also resulted from the 33% higher dose used here (20 mg/kg compared with 15 mg/kg). Bioavailability following the p.r. administration of metronidazole in horses was low relative to that (78.3%) reported in humans (Ioanides et al., 1981). The low bioavailability may have resulted from anatomical differences of the equine colon, inactivation or binding of the drug by fecal material, poor drug retention, or the physicochemical properties of the drug as suggested by Steel et al. (1999) in a study on rectal absorption of cefsulodin in horses. Although the rectal bioavailability in our study was low, it was higher and more consistent than that of cefsulodin (Steel et al., 1999). In one horse metronidazole p.r. peaked at 5 min and the Cmax was considerably lower than in the other horses. The reasons for this were not obvious but, theoretically, factors such as inactivation or binding of the drug by fecal material as described above may have contributed to this effect.

Pharmacokinetics of metronidazole after administration by i.v. and p.o. routes were reported earlier by Sweeney et al. (1986) and Baggot et al. (1988). The bioavailability was 85%, mean peak plasma concentration was 12.6 mg/L, and the mean terminal half-life was 174 min after metronidazole administration at 25 mg/kg body weight p.o. (Sweeney et al., 1986). In a study by Baggot et al. (1988) metronidazole was administered p.o. at 20 mg/kg body weight resulting in 74.5% bioavailability, mean peak plasma concentration of 17.6 mg/L, and mean terminal half-life of 360 min. In the present study metronidazole was rapidly absorbed after p.o. administration. The bioavailability was 73%, which was similar to that reported by Baggot et al. (1988), but smaller than reported by Sweeney et al. (1986). However, the mean peak plasma concentration observed in this study (22 mg/L) was higher than described in the previous reports. The mean elimination half-life (212 min) was similar to that reported by Sweeney et al. (1986) but shorter than the 360 min reported by Baggot et al. (1988). The volume of distribution and clearance determined in the present study were similar to those reported earlier by Baggot et al. (1988) and Specht et al. (1992) and much lower than those reported by Sweeney et al. (1986). The differences between the studies may have resulted from different physicochemical properties of drug formulation, biological differences of the horses used in the studies or differences in study protocols. In the present study and that of Baggot et al. (1988), feed was withheld until 2 and 4 h after drug administration, respectively. Access to feed was not restricted in the study of Sweeney et al. (1986) which may have resulted in the lower mean peak plasma concentration despite the higher metronidazole dose used. Therefore, withholding feed for 2 h after p.o. administration of the drug may be therapeutically beneficial.

When the pharmacokinetic properties of metronidazole were compared with those of tinidazole (1-[2-ethylsulphonylethyl]-2-methyl-5-nitro-imidazole), another member of the nitroimidazole group, several differences were seen. Pyorala et al. (1990) administered tinidazole p.o. to horses at 25 mg/kg, bioavailability was 100%, mean peak plasma concentration was 28.5 mg/L, mean elimination half-life was 5.81 h and MRT was 8.5 h. The higher bioavailability, mean peak plasma concentration, mean elimination half-life and mean residence time may have resulted from more lipophilic characteristic of tinidazole as suggested by Mattila et al. (1983).

The in vitro susceptibility, minimum inhibitory concentration for 90% of the strains (MIC90), of gram-negative anaerobes for metronidazole was lower than 2 µg/mL and the respective value for Clostridium species was lower than 4 µg/mL (Prescott & Baggot, 1988). Following p.o. administration of 20 mg/kg body weight of metronidazole, the plasma metronidazole concentration remained above 4 µg/mL for 6 h whereas p.r. administration resulted in such plasma concentrations only for 2.5 h. Because of the large volume of distribution of metronidazole, that exceeds the extracellular fluid volume, it is not clear if this has direct clinical implications.

Adverse reactions to metronidazole were not observed in any of the six horses during the 3 days following the three drug administrations. One horse had a mild colic in between the studies which was not considered to be relative to metronidazole administration.

The results of the present study suggested that the rectal route of administration of metronidazole can be used as an alternative especially when the clinical situation prevents p.o. administration. However, the lower bioavailability of p.r. administered metronidazole, compared with other routes of administration, seemed to warrant dose adjustment. This appears adequate also concerning the time above the MIC90 concentration in plasma of the most relevant anaerobic bacteria following p.r. administration of metronidazole. However, only dose titration studies in clinical situations can clarify this question. Changes in dose should be carried out judiciously, because histological evidence of peripheral neurotoxicity and hepatotoxicity were noted in horses treated with doses as low as 30 mg/kg body weight q 12 h p.o. for 30 days, suggesting narrow margin of safety (White et al., 1996).

ACKNOWLEDGMENTS

We thank Shirly Kachlon for assisting in the technical work.

REFERENCES


