

Pharmacokinetics and Distribution of Metronidazole Administered Intraperitoneally in Mice

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Summary

Female albino Swiss-derived mice were injected intraperitoneally (i.p.) with a single dose of metronidazole at 100 mg/kg body weight. Blood samples, the liver and whole brain were collected from mice (4/each time period) at time 0 (vehicle control) and then at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after the drug administration. Metronidazole concentrations in the plasma and tissues were determined by a UV-spectrophotometric method. The pharmacokinetic parameters of metronidazole were calculated by a non-compartmental analysis. The elimination half-life of metronidazole was 6.98 h with a steady state volume of distribution 1.72 L/kg and total body clearance of 162 ml/h/kg. The mean residence time of the drug was 10.6 h and its area under the plasma concentration-time curve (0-∞) was 617 µg.h/ml. In conclusion, the data show the pharmacokinetic profile of a single i.p. dose of metronidazole in mice and suggest that liver and brain should be considered as potential toxicity targets for the drug as it is well distributed and relatively slowly cleared from the body.

Keywords: metronidazole, pharmacokinetics, antibacterial, antiprotozoal, bioavailability

Introduction

Metronidazole is an antibacterial and antiprotozoal agent widely used in man [1] with some therapeutic uses in veterinary medicine [2]. The drug may cause, especially at high doses liver damage [3,4] and neurotoxic effects characterized by peripheral neuropathy and central nervous effects causing ataxia, incoordination and convulsions [5,6]. Metronidazole is well absorbed orally with a bioavailability of 90-100% [1,7], distributing into different tissues of the body [8-10]. The pharmacokinetic profile of metronidazole has been described in man and many animal species [11-16]. Metronidazole administered intravenously is widely distributed into different tissues of mice [8,10]. Mice are frequently used to evaluate the toxicity potential toxicity of drugs and chemicals including antibacterials. The present study was undertaken to examine the pharmacokinetics and tissue (whole brain and liver) distribution of metronidazole in mice following a single intraperitoneal (i.p.) administration at the

dose rate of 100 mg/kg body weight. This dose of metronidazole was chosen because it represents approximately one fifth of a toxic but non-lethal dose of the drug in mice [17], and the i.p. route is one of the common routes of drug administration in this species.

Materials and methods

Female albino Swiss-derived mice (body weight 20-30 g) were housed at a temperature of 21-25 °C with water and food ad libitum. Metronidazole base (kindly donated by the State Company for Drugs and Medical Appliances, Samarra, Iraq) was dissolved in distilled water at 100 mg/10 ml; the pH of the final solution was 3.4. Mice were randomly divided into 9 groups of 4 animals each. The first group was injected with the vehicle at 10 ml/kg body weight, i.p. (control) and the other groups were treated with metronidazole at 100 mg/10ml/kg body weight, i.p. Mice were anesthetized with ether to collect blood from the heart using heparinized syringes. Blood samples were obtained from the vehicle-treated mice (0 time control) and then from the metronidazole-treated ones (4 mice/each time period) at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after the drug administration. Following the blood sampling, the mice were killed by cervical dislocation to obtain the whole brain and liver. Plasma was separated from erythrocytes by centrifugation of blood samples at 3000 rpm (Centurion, U.K.) for 15 minutes. Plasma and tissues were stored at -18 °C pending analysis within 48 h.

Metronidazole concentration in the plasma was determined using a U-V spectrophotometric method [18]. Plasma sample (0.1 ml) was vortex mixed with 0.2 ml of methylcyanide (BDH, U.K.) and 1 ml of distilled water and the phases were separated by centrifugation at 6000 rpm for 10 minutes. The absorbance of the organic phase containing metronidazole was read at 318 nm using a spectrophotometer (Cecil 1000, Cecil, U.K.). The calculation was based on readings of standard solutions of metronidazole at concentrations ranging between 2.5 to 160 µg/ml. Brain or liver samples were individually homogenized at 100 mg/2 ml buffered physiological saline solution (BPS) at pH 7.2. Then 1 ml of methylcyanide was added and mixed. The tissue homogenate was centrifuged at 6000 rpm for 15 minutes and the absorbance of the organic phase was read at 318 nm with a spectrophotometer. Standards of metronidazole were prepared from 0.1 ml of drug solutions at concentrations of 2.5 to 160 µg/ml added to 1.9 ml PBS and 1 ml of methylcyanide. The means of plasma concentrations of metronidazole at each sampling time (0.25-24 h) were used to calculate the pharmacokinetic parameters by a non-compartmental analysis [19] using a Windows-based computer program [20]. Calculations included the following pharmacokinetic variables: area under plasma concentration-time curve ($AUC_{0-\infty}$) and area under the moment curve ($AUMC_{0-\infty}$) from time zero to infinity, elimination half-life ($t_{1/2\beta}$), elimination rate constant ($k_{el}=0.693/ t_{1/2\beta}$), steady state volume of distribution ($V_{ss}=\text{Dose}\cdot AUMC/ (AUC)^2$), mean residence time ($MRT=AUMC/AUC$) and total clearance ($CL=\text{Dose}/AUC$).

The present study has been approved by the Scientific Committee of the College of Veterinary Medicine at the University of Mosul (Iraq). All experiments complied with regulations addressing animal use, and proper attention and care have been given to the mice used in the study.

Results

Metronidazole appeared in the plasma at mean concentrations of 140.6, 126.3, 98.8, 67, 45.3, 19.5, 15 and 12.3 $\mu\text{g/ml}$ after 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h post injection, respectively (Table 1). At the same time intervals between 0.25 to 24 h, the mean concentrations of metronidazole in the liver were 79, 70, 74, 68, 22, 44, 59 and 37 $\mu\text{g/g}$, respectively, whereas those of the whole brain were 94, 89, 66, 53, 29, 30, 21 and 8 $\mu\text{g/g}$, respectively (Table 1).

The log plasma concentrations vs. time of metronidazole are shown in figure 1 and the related pharmacokinetic variables of the drug are shown in table 2. The $t_{1/2\beta}$ of metronidazole was 6.98 h with a V_{ss} 1.72 L/kg and CL of 162 ml/h/kg. The MRT of the drug was 10.6 h and its AUC $_{(0-\infty)}$ was 617 $\mu\text{g.h/ml}$ (Table 2). Other related pharmacokinetic parameters are also shown in table 2.

Table 1: Metronidazole concentration (Mean \pm SE) in the plasma, liver and whole brain of mice following a single intraperitoneal administration with metronidazole at a dose of 100 mg/kg body weight

Time (h)	Plasma ($\mu\text{g/ml}$)	Liver ($\mu\text{g/g}$)	Whole brain ($\mu\text{g/g}$)
0.25	140.6 \pm 13.0	79 \pm 17	94 \pm 9
0.5	126.3 \pm 9.2	70 \pm 21	89 \pm 14
1	98.8 \pm 5.8	74 \pm 24	66 \pm 3
2	67.0 \pm 8.3	68 \pm 32	53 \pm 8
4	45.3 \pm 4.0	22 \pm 4	29 \pm 7
6	19.5 \pm 4.5	44 \pm 5	30 \pm 5
8	15.0 \pm 4.5	59 \pm 14	21 \pm 3
24	12.3 \pm 4.0	37 \pm 5	8 \pm 1

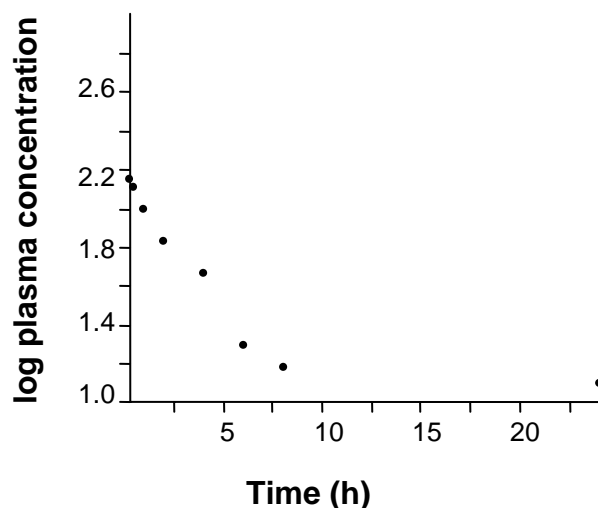
n = 4 mice/each time.

Table 2: Pharmacokinetic parameters of metronidazole in mice following a single intraperitoneal administration with metronidazole at a dose of 100 mg/kg body weight

Parameter*	Unit	Value
Mean residence time (MRT=AUMC/AUC)	h	10.6
Steady state volume of distribution ($V_{ss}=\text{Dose.AUMC}/(\text{AUC})^2$)	L/kg	1.72
Elimination rate constant ($k_{el}=0.693/t_{1/2\beta}$)	h^{-1}	0.0993
Elimination half-life ($t_{1/2\beta}$)	h	6.98
Total clearance ($\text{CL}=\text{Dose}/\text{AUC}$).	ml/h/kg	162
Area under plasma concentration-time curve ($\text{AUC}_{0-\infty}$)	$\mu\text{g.h/ml}$	617
Area under the moment curve ($\text{AUMC}_{0-\infty}$)	$\mu\text{g.h/ml}$	6549

* The means of plasma concentrations of metronidazole at each sampling time (0.25-24 h) were used to calculate the pharmacokinetic parameters by non-compartmental analysis using the Windows-based computer program.

Figure 1: The log plasma concentrations vs. time of metronidazole in mice following a single intraperitoneal administration with metronidazole at a dose of 100 mg/kg body weight



Discussion

The data of the present study suggest that metronidazole is well distributed following i.p. administration in mice, since the V_{ss} of the drug was 1.7 L/kg. V_{ss} is reliable estimate of volume of drug distribution, since it is calculated independent of the k_{el} [19]. Metronidazole appeared in the liver and brain of the mice; these are two vital organs implicated in some of the side effects of the drug [4-6]. In accordance with the results of the present study, metronidazole has been reported to distribute into various tissues of mice following intravenous administration [8]. Metronidazole is not significantly bound to plasma proteins and together with its small molecular size favor its wide distribution into different organs of the body [8,9,11,21]. However, the elimination of metronidazole from the body via the bile and urine appears to be slow which favors prolonged therapeutic or possible toxic actions of the drug [6,9,11,22].

The pharmacokinetic parameters of metronidazole ($t_{1/2\beta}$, V_{ss} or CL) determined in the present study in mice are close to those reported in man [11,12], buffaloes [14], camels and sheep [16] as well as in hens [23], but they are different from those of rabbits [14] and horses [13,15]. These differences could be attributed mainly to variations in the dose rate of metronidazole, route of administration, animal species and the experimental protocol applied. Different pharmacokinetic models (one, two or three compartments) have also been described for metronidazole [11,12,14-16]. In the present study we used a non-compartmental analysis. The long

$t_{1/2\beta}$ of metronidazole in the plasma of the mice favors the drug persistence as long as 24 h after the i.p. administration. However, the CL of metronidazole was not slow in the mice. Therefore, the prolonged $t_{1/2\beta}$ of the drug could be attributed to its wide tissue distribution and possible persistence in the liver [8-10].

Metronidazole concentration in the liver gradually decreased reaching the lowest point 4 h after the drug administration; thereafter it increased between 6-24 h (Table 1). This increment could be attributed to the enterohepatic circulation and intestinal reabsorption of metronidazole. Metronidazole is metabolized in the liver and secreted in the bile [3,9,22]. The elimination of metronidazole from the body also includes the renal system [9,22]. In conclusion, the data show the pharmacokinetic profile of a single i.p. dose of metronidazole in mice and further suggest that liver and brain should be considered as potential toxicity targets for the drug as it is well distributed and relatively slowly cleared from the body.

Acknowledgements

This report represents a portion of a thesis submitted by the first author to the University of Mosul, Iraq as partial fulfillment of the requirements of PhD degree in Veterinary Pharmacology. The study was supported by the College of Veterinary Medicine, University of Mosul, Iraq. The authors have no competing interests or financial relationships to declare.

References

- [1] Roach SS. Introductory clinical pharmacology, 8th ed. Philadelphia: Lippincott, Williams and Wilkins, 2007.
- [2] Plumb DC. Plumb's veterinary drug handbook, 5th ed. Ames: Blackwell Publishing, 2005.
- [3] Moreno SNJ, Docampo R. Mechanism of toxicity of nitro compounds used in the chemotherapy of trichomoniasis. *Environ Health Perspect* 1985; 64: 199-208.
- [4] Bjornsson E, Nordlinder H, Olsson R. Metronidazole as a probable cause of severe liver injury. *Hepatogastroenterology* 2002; 49: 252-254.
- [5] Thomas RJ. Neurotoxicity of antibacterial therapy. *South Med J* 1994; 87: 869-875.
- [6] Kapoor K, Chandra M, Nag D, et al. Evaluation of metronidazole toxicity: a prospective study. *Int J Clin Pharmacol* 1999; 19: 83-88.
- [7] Lau AH, Lam NP, Piscitelli SC, et al. Clinical pharmacokinetics of metronidazole and other nitroimidazole anti-infective. *Clin Pharmacokinet* 1992; 23: 328-364.
- [8] Li T, Qu, S. A comparison of metronidazole distribution following intravenous metronidazole and metronidazole phosphate disodium in mice. *Drug Metabol Drug Interact* 1992; 10: 229-236.
- [9] Liu CX, Wei GL, Xiao SH. Pharmacokinetics of sodium bimetronidazole glycinate in mice and rats. *Yao Xue Xue Bao* 2000; 35: 770-773. (In Chinese).
- [10] Lamp KC. Metronidazole (review). *Clin Pharmacokinet* 1999; 36: 353-373.
- [11] Ralph ED, Clarke JT, Libke RD, et al. Pharmacokinetics of Metronidazole as determined by bioassay. *Antimicrob Agents Chemother* 1974; 6: 691-696.
- [12] Visser AA, Hundt HKL. The pharmacokinetics of a single intravenous dose of metronidazole in pregnant patients. *J Antimicrob Chemotherap* 1984; 13: 279-283.

- [13] Sweeney RW, Sweeney CR, Soma LR, et al. Pharmacokinetics of metronidazole given to horses by intravenous and oral routes. *Am J Vet Res* 1986; 47: 1726-1729.
- [14] Roy RK, Garg BD, Uppal RP. Comparative disposition kinetics of metronidazole in buffalo, sheep and rabbits. *Ind J Vet Res* 1999; 8: 19-25.
- [15] Steinman A, Gips M, Lavy E, et al. Pharmacokinetics of metronidazole in horses after intravenous, rectal and oral administration. *J Vet Pharmacol Therap* 2000; 23: 353-357.
- [16] Ali, B. H., Charles, B. G., Al-Yousif, M. and Bashir, A. K. (2003) Comparative pharmacokinetics of metronidazole in camels, sheep and goats. *Acta Vet. Brno*, **72**, 49-53.
- [17] El-Nahas AF, El-Ashmawy IM. Reproductive and cytogenetic toxicity of metronidazole in male mice. *Pharmacol Toxicol* 2004; 94: 226-231.
- [18] Gratteri P, Cruciani G. Experimental design and multivariate calibration in the development, set-up and validation of a different pulse polarographic and UV spectrophotometric method for the simultaneous plasmatic determination of the therapeutic metronidazole-pefloxacin combination. *Analyst* 1999; 124: 1683-1688.
- [19] Gibaldi M, Perrier D. *Pharmacokinetics*, 2nd ed. New York: Marcel Dekker, 1982.
- [20] Laub PB, Gallo JM. NCOMP-a windows-based computer program for noncompartmental analysis of pharmacokinetic data. *J Pharm Sci* 1996; 85: 393-395.
- [21] Jokipii AMH, Myllaya VV, Hokkanen E, et al. Penetration of the blood brain barrier by metronidazole and tinidazole. *J Antimicrob Chemother* 1977; 3: 239-245.
- [22] Stambaugh JE, Feo LG, Manthei RW. The isolation and identification of the urinary oxidative metabolites of metronidazole in man. *J Pharmacol Exp Therap* 1968; 161: 373-381.
- [23] Cybulski W, Larsson P, Tjalve H, et al. Disposition of metronidazole in hens (*Gallus gallus*) and quails (*Conturnix conturnix japonica*): pharmacokinetics and whole-body autoradiography. *J Vet Pharmacol Therap* 1996; 19: 352-358.