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Pharmacokinetics of metronidazole administered intravenously to male rats

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Summary

Metronidazole was selectively extracted from rat plasma (91.4%), prostate gland (82.6%) and seminal vesicles (87.1%) using ethyl acetate as the organic solvent. A reverse-phase high performance liquid chromatographic method was developed allowing the quantitation of metronidazole from these tissues. The pharmacokinetics of metronidazole was studied in male rats following the intravenous administration of metronidazole at a dose of 25 mg/Kg. Higher levels of metronidazole were found in plasma than prostate gland and seminal vesicles during the 8 h time period after i.v. injection. A biphasic phenomenon with a rapid distribution followed by a slower elimination phase was observed from the plasma concentration-time curve. Compartmental analysis based on a two-compartment open model with elimination from the central compartment yielded the following pharmacokinetic parameters: terminal elimination rate constant (β) = 0.0023 min^{-1}; biological half-life ($t_{1/2,\beta}$) = 301 min; apparent volume of distribution in the central compartment (Vc) = 1014 ml/kg; apparent volume of distribution based on the area under plasma concentration-time curve (Vd_β) = 2513 ml/kg; apparent volume of distribution at steady state (Vd_{ss}) = 1769 ml/kg. Statistical moment theory was used to determine noncompartmental pharmacokinetic parameters. The estimates for mean residence time (MRT), clearance (CL) and apparent volume of distribution (Vd_{ss}) were calculated to be 213 min, 5.78 ml min^{-1} kg^{-1}, and 1231 ml/kg, respectively.

Introduction

Metronidazole is a synthetic anti-bacterial agent. It is primarily used in the treatment of anaerobic infections (Dornbusch et al., 1974; Brogden et al., 1978), especially in intra-abdominal infections, skin and skin structure infections, gynecologic infections, bacterial septicemia, bone and joint infections, central nervous system (CNS) infections, lower respiratory tract infections, and endocarditis. It is also used in the prevention of postoperative infection.

Analytical methods for determining metronidazole concentration in biological fluids and tissues have been reported, including gas-liquid chromatography (Mihda et al., 1973) and high performance liquid chromatography (HPLC) (Gulaid et al., 1978; Wheeler et al., 1978; Lanbeck et al., 1979; Kaye et al., 1980; Gattavecchia et al., 1981;

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Nilsson-Ehle et al., 1981; Jensen et al. 1983; Gibson et al., 1984; Adamovics, 1984). No information is available, however, for analytical procedures concerning the extraction and HPLC determination of metronidazole in prostate gland and seminal vesicles.

The penetration of metronidazole across the blood-brain and blood-cerebrospinal fluid barriers in human (Jokipii et al., 1977) and its distribution in human female reproductive organs (Mannisto et al., 1984), male aqueous humor (Mattila et al., 1983), saliva and alveolar bone (Konow et al., 1982), bile (Lykkegaard-Nielsen et al., 1977), placenta (Roe, 1976) and breast milk (Gray et al., 1961) have been reported. Metronidazole elimination in humans was reported by way of metabolism (Stambaugh et al., 1968) and excretion in urine (Kane et al., 1961).

The primary purpose of this investigation was to study the pharmacokinetics of metronidazole in male rat plasma, prostate gland and seminal vesicles.

Materials and Methods

Chemicals and reagents

Metronidazole (lot 03512KT) was purchased from Aldrich (Milwaukee, WI); acetophenetidin (lot 5644) was obtained from Merck (Rahway, NJ); ammonium carbonate (lot KJBN) was from Mallinckrodt (Paris, KY); methanol (lot B26102) was from J.T. Baker (Phillipsburg, NJ); ethyl acetate (lot 872087) was from Fisher Scientific (Fair Lawn, NJ). All materials were used as received.

Sample preparation

Plasma samples were obtained from the supernatant after the whole blood was centrifuged at $2000 \times g$ for 15 min (RC2-B Sorvall superspeed centrifuge, Ivan Sorvall, Norwalk, CT). To each tissue sample was added 1 ml of internal standard solution, containing 8 μg/ml acetophenetidin, and homogenized (Brinkmann Homogenizer, Brinkmann Instruments, Westbury, NY). This homogenization process was undertaken in a temperature-controlled ice-bath with successive mincing cycles of less than 30 s each time. The homogenized samples were centrifuged at $11\,000 \times g$ for 10 min and supernatants were collected. The homogenization and centrifugation processes were repeated twice with 1 ml distilled water. The supernatants from the homogenized samples were combined.

Extraction procedures

At least 2 ml of blood from each mouse after killing was centrifuged to obtain the plasma. To 1 ml of plasma (containing an additional 1 ml of internal standard) or the supernatant from the homogenized tissue mixture (obtained from a specific amount of tissue), 4 ml of ethyl acetate was added and mixed on a mechanical shaker (Model 1105, Adams Nutator, Clay Adams, Parsippany, NJ) for 10 min, followed by centrifugation at $11\,000 \times g$ for 10 min. The organic layer was transferred to a tube and extraction procedures were repeated twice. The organic layer was combined and evaporated to dryness under a stream of nitrogen at $25 \pm 0.2°$ C. The residue was reconstituted with 2 ml distilled water and filtered through a 0.22 μm membrane filter (Type GS, Millipore, Bedford, MA). A 20 μl volume of the filtrate was injected into the high performance liquid chromatograph (HPLC).

Chromatography conditions

A reverse-phase high performance liquid chromatograph equipped with a single piston pump (Waters Model 501, Millipore, Milford, MA), an autosampler (Waters Model 712 WISP, Millipore), a UV absorbance detector (Waters Model 441, Millipore) set at 254 nm, and a μ-Bondapak C_{18} column (3.9 mm $\times$ 30 cm with 10 μm packing), Waters Associates, Milford, MA) was used. The mobile phase consisted of 47% (v/v) methanol and 53% ammonium carbonate (0.1%). The flow rate was maintained at 1.0 ml/min. The absorbance of the drug was recorded using a data station (Waters Model 745 data module, Millipore, Milford, MA) at a chart speed of 0.3 cm/min. A peak area ratio method was used to determine the concentration of metronidazole in reference to the internal standard from the standard curve. The relative retention time for

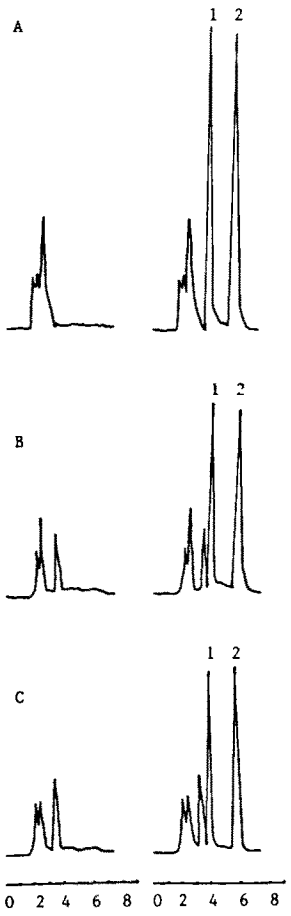

Fig. 1. Chromatograms from extracts of blank and spiked metronidazole (25 μg/ml) with internal standard (8 μg/ml) in rat (A) plasma, (B) prostate gland, and (C) seminal vesicles. (1) Metronidazole; (2) acetophenetidin.

metronidazole and acetophenetidin was found to be 4.8 and 7.0 min, respectively. Representative sets of the chromatograms are shown in Fig. 1. The sensitivity of the assay was 0.1 μg/ml from plasma, prostate gland and seminal vesicles using the described apparatus with 0.1 aufs detector setting and an attenuation of 16 on the recorder. The precision for this HPLC method at the level of 0.1 μg/ml for plasma, prostate gland and seminal vesicles was determined to be ± 4.82, ±5.73 and ±6.21%, respectively.

Standard solutions

A stock solution of 50 μg/ml of metronidazole was prepared by weighing a specific amount of pure material and dissolving in distilled water to volume in a volumetric flask. A series of spiked solutions of metronidazole in plasma, prostate gland and seminal vesicles ranging from 1 to 50 μg/ml were prepared. The linearity of all standard curves was found to be greater than 0.98. The intra- and inter-day assay precision was determined to be ± 2.14% and ±3.41 ($n = 5$), respectively, at a concentration range of 1–50 μg/ml.

Recovery studies for metronidazole in plasma and tissue samples were done by adding specific amounts to simulate 100% recovery of drug to plasma and tissue samples (~ 10 μg/ml) and analyzing the actual quantity of metronidazole recovered using the external standard method. When compared with the actual amount of metronidazole added to the plasma or tissue, the measured absolute recoveries ($n = 5$) were determined to be 91.4 ± 2.2% for plasma, 82.6 ± 2.8% for prostate gland, and 87.1 ± 3.2% for seminal vesicles.

Pharmacokinetic studies

Thirty male rats (Sprague-Dawley strain) in the weight range of 250–300 g (average weight = 292.0 ± 8.6 g, $n = 30$) were used in this study. A loading dose of 25 mg/kg of metronidazole solution (prepared as 50 mg/ml in 0.001 N HCl solution) was administered intravenously via the dorsal vein of the rat tail under light ether anesthesia. Animals were conscious and individually housed in metabolism cages during the study. Food and water were supplied ad libitum. At 15,30,45,60,90,120,180,240,360, and 480 min after i.v. injection, three rats per time period were placed under ether anesthesia and blood samples were collected from the heart in 100 × 13 mm evacuated blood collection tubes (Becton-Dickinson, Rutherford, NJ) containing 10.5 mg disodium edetate. Immediately following collection of the blood sample, the rats were euthanized by exsanguination and the prostate gland and seminal vesicles were obtained by dissection for the measurement of organ weight and determination of metronida-

zole concentration. The epididymal tissue samples were rinsed with 0.9% NaCl solution, blotted, weighed and immediately stored at −20°C until analyzed.

Results and Discussion

Pharmacokinetics

Metronidazole time-course data in rat plasma, prostate gland and seminal vesicles are presented in Table 1. Higher levels of metronidazole were found in plasma, as compared to prostate gland and seminal vesicles. Comparable levels of metronidazole in prostate gland and seminal vesicles with higher concentration in prostate gland was noted. A longer residence time for metronidazole in prostate gland than seminal vesicles at tissue levels > 1 μg/ml was obtained. The concentration-time profile for metronidazole in rat plasma after i.v. injection is shown in Fig. 2.

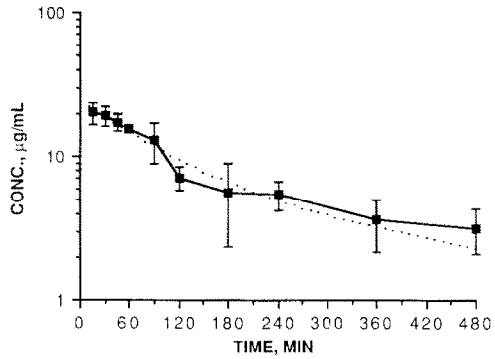

Fig. 2. Observed (■) and simulated (•) metronidazole concentrations in plasma after intravenous administration of metronidazole at 25 mg/kg to male rats. Each data point is the mean ± S.D. of three rats.

Compartmental method

A statistical nonlinear regression program was accessed through an SAS program using the BMDP3R procedure (University of California, Los Angeles, CA) for the kinetic analysis. A two-compartment open model with elimination from the central compartment was proposed and validated through this program to explain the apparent biphasic disposition of metronidazole in plasma after i.v. injection as viewed in Fig. 2. The plasma concentration as a function of time can be described by the following equation:

$$C_p^t = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

Where C_p^t is the plasma concentration of metronidazole at time t; A and B are two pre-exponential constants consisting of the first-order distribution rate constant between central and peripheral compartments, k_{12} and k_{21}, and elimination rate constant k_{10}; α and β are two exponents, representing the distribution and elimination phases, depending solely on k_{12}, k_{21} and k_{10}. The estimates of these pharmacokinetic parameters based on the two-compartment open model are calculated from the best fitting coefficients and exponents of BMDP program and listed in Table 2. C_p^0 was calculated from Eqn. 1 when $t = 0$, and is equal to $A + B$. The apparent volume of distribution in the central compartment immediately after i.v. injection but before distribu-

TABLE 1

Metronidazole concentrations in plasma, prostate gland and seminal vesicles at indicated times after intravenous administration of metronidazole at 25 mg/kg to male rats [a]

	Metronidazole concentrations (μg/ml) at time (min)									
	15	30	45	60	90	120	180	240	360	480
Plasma	20.3 ± 3.4	19.4 ± 2.9	17.4 ± 2.4	15.6 ± 1.2	13.0 ± 4.1	7.1 ± 1.3	5.7 ± 3.3	5.4 ± 1.2	3.6 ± 1.4	3.2 ± 1.1
Prostate gland	– [b]	3.8 ± 0.7	5.2 ± 1.9	7.2 ± 3.1	7.7 ± 2.9	7.5 ± 0.3	1.9 ± 0.0	– [b]	– [b]	– [b]
Seminal vesicles	– [b]	2.3 ± 0.0	3.4 ± 0.7	2.9 ± 0.2	3.3 ± 0.0	– [b]	– [b]	– [b]	– [b]	– [b]

[a] Each value is the mean ± S.D. of 3 rats.
[b] Concentration is not detectable.

TABLE 2

Estimates of pharmacokinetic parameters according to a two-compartment open model with elimination from the central compartment when a dose of 25 mg/kg metronidazole was administered intravenously to male rats

Parameter (units)	Estimate
A (μg/ml)	17.75
B (μg/ml)	6.90
α (min^{-1})	0.012
β (min^{-1})	0.0023
k_{12} (min^{-1})	0.0037
k_{21} (min^{-1})	0.0050
k_{10} (min^{-1})	0.0055
C_p^0 (μg/ml)	24.65
AUC ($\mu g\ min\ ml^{-1}$)	4325
V_c (ml/kg)	1014
Vd_{ss} (ml/kg)	1769
Vd_β (ml/kg)	2513
$t_{1/2,\beta}$ (min)	301

tion or elimination occurred, V_c, was determined based on:

$$V_c = \frac{D}{A + B} \tag{2}$$

where D is the i.v. dose in μg/kg. During the post-distributive phase, the apparent volume of distribution, Vd_β, based on the terminal elimination rate constant and the area under plasma concentration-time curve, was determined by the equation:

$$Vd_\beta = \frac{D}{\beta \cdot AUC} \tag{3}$$

where AUC was obtained by the linear trapezoidal rule (Gibaldi et al., 1982a) with extrapolation to infinity. The apparent volume of distribution at steady state, Vd_{ss}, was estimated when the two-compartment model was characteristic using:

$$VD_{ss} = V_c\left(\frac{k_{21} + k_{12}}{k_{21}}\right) \tag{4}$$

The values of Vd_β (2513 ml) and Vd_{ss} (1769 ml) were significantly larger than V_c (1014 ml), suggesting measurable distribution of metronidazole into a peripheral compartment. When the net transfer of metronidazole between the central and peripheral compartments was equal to zero, a distribution equilibrium was reached. The fraction of total metronidazole in the central compartment remained constant when distribution equilibrium was attained. The fraction (F_c^*) can be determined by the following equation:

$$F_c^* = \frac{\beta}{k_{10}} \tag{5}$$

The F_c^* was found to be 0.42 which indicated 58% of administered metronidazole was distributed into the peripheral compartment when distribution equilibrium was reached. This might be due partly to the lipophilicity of metronidazole (Craig et al., 1976).

The elimination half-life ($t_{1/2,\beta}$) of metronidazole from the central compartment as shown in the terminal phase of the plasma concentration-time curve was determined to be 301 min according to the equation:

$$t_{1/2,\beta} = \frac{0.693}{\beta} \tag{6}$$

Noncompartmental method

Under the assumption of linear pharmacokinetics for metronidazole in this study, a noncompartmental model for the estimation of some pharmacokinetic parameters was possible using the statistical moment theory (Gibaldi et al., 1982b). A list of the estimates is shown in Table 3. The area under the plasma concentration-time curve (AUC) is defined as zero moment. AUMC is the

TABLE 3

Estimates of noncompartmental pharmacokinetic parameters based on statistical moment theory after intravenous administration of metronidazole at the dose of 25 mg/kg to male rats

Parameter (units)	Estimate
AUC ($\mu g\ min\ ml^{-1}$)	4325
AUMC ($\mu g\ min^{-2}\ ml^{-1}$)	920975
MRT (min)	213
CL ($ml\ min^{-1}\ kg^{-1}$)	5.78
Vd_{ss} (ml/kg)	1231

area under the first moment curve which can be determined from the area under the product of drug concentration and time vs time profile using the linear trapezoidal rule with extrapolation to infinity. The mean residence time (MRT), known as the first moment of drug concentration-time curve, was calculated by the ratio of AUMC to AUC. It was found to be 213 min indicating that 63.2% of metronidazole was eliminated at 213 min after intravenous injection to rats. Clearance (CL) was calculated as the ratio of normalized drug dose and AUC. The apparent steady-state volume of distribution (Vd_{ss}) was calculated from the product of MRT and CL. The Vd_{ss} obtained from the statistical moment theory is a little less than the estimate from the two-compartment open model.

Epididymal tissue distribution

The large volume of distribution obtained from the two-compartment open model is predictive of high metronidazole levels in body tissues. However, a lower penetration and shorter residence time of metronidazole in prostate gland and seminal vesicles than plasma was observed. The metronidazole disposition in male rats' prostate gland and seminal vesicles is depicted in Table 1. The availability of metronidazole in these tissues can also be viewed by the information concerning the AUC from the tissue concentration-time curve. Much lower AUC for seminal vesicles (250 μg min/ml^{-1}) and prostate gland (749 μg min ml^{-1}) was found in comparison with plasma (4325 μg min ml^{-1}).

In conclusion, the extraction and chromatographic procedures described in this study allow the quantitation of metronidazole from rat plasma, prostate gland and seminal vesicles. The pharmacokinetic study of metronidazole was characterized by both the two-compartment open model and the noncompartmental model using statistical moment theory. The study shows higher levels of metronidazole found in plasma than prostate gland and seminal vesicles throughout the 8 h study period. From the plasma concentration-time profile, a rapid distribution followed by a slower elimination phase was noted.

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