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COMPARATIVE PHARMACOKINETICS OF METRONIDAZOLE IN HEALTHY VOLUNTEERS AND IN PATIENTS SUFFERING FROM AMOEBIASIS

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ABSTRACT

Disease condition is one of the major factors which can affect the dose of a drug. This study was designed to compare the pharmacokinetic parameters of metronidazole in healthy volunteers and in patients suffering from amoebiasis and to recommend any modification of dose if required. The healthy volunteers were considered as Group A and the patients as Group B. Subjects from both groups were treated with metronidazole 400mg tablet orally. Blood sample of 5ml was collected at 0, 0.25, 0.5, 1.0, 2.0, 3.0, 6.0, 12.0, 24.0 and 36.0 hours from each individual and patient. Plasma was separated by centrifugation and stored at -80ºC until assayed. The drug concentration in plasma was measured by High Performance Liquid Chromatographic (HPLC) method. The pharmacokinetic parameters were calculated using plasma concentration-time data employing software APO pharmacological analysis MW/PHARM (Version 3.02). The bioavailability of metronidazole after oral administration was considered as 1. Pharmacokinetic data of metronidazole in patients and in healthy volunteers were compared using unpaired t test. The data were presented as mean, standard deviation, standard error. No significant difference in pharmacokinetic parameters of metronidazole in amoebiasis patients and in healthy volunteers was noted after oral administration. Thus, there is no need for dose adjustment of metronidazole dose in amoebiasis patients.

Keywords: Pharmacokinetics, Metronidazole, Amoebiasis, HPLC

INTRODUCTION

Metronidazole [1-(hydroxyethyl)-2-methyl-5-nitroimidazole] is a drug used for the treatment of infections caused by protozoa and anaerobic microorganisms. The systemic metronidazole is indicated for treatment of bacterial vaginosis due to *Trichomonasvaginalis* in both symptomatic patients as well as their asymptomatic sexual contacts, infections due to *Gardnerella* or *Mycoplasma hominis* in symptomatic patients, and pelvic inflammatory disease in conjunction with other antibiotics such as ofloxacin or ceftriaxone. Amoebiasis is an infection of large intestine caused by *Entamoebahistolytica*, a single celled-protozoan parasite. Trophozoites of *E. histolytica*can invade colonic epithelium, causing amoebic colitis. Most preferred choice of drugs for intestinal amoebiasis is metronidazole and tinidazole (Tracy *et al.*, 1996).Traditional recommended dosage for IV metronidazole is either 400mg given every 8 h or 7.5 mg/kg of body weight given every 6 h (daily dose of 30 mg/kg) (Reynolds, 1993). However, the dosage regimen is usually 400mg every 6 or 8 h.

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Such a dosage regimen may not appropriate for the different patient populations. Limited data on hospitalized or critically ill patients with normal hepatic and renal functions indicate that the disposition of metronidazole may be different from that in healthy volunteers (Reynolds, 1993; Lau, 1986). However, Information on the metronidazole pharmacokinetics and centration in serum/plasma during amoebiasis is scant. The pharmacokinetic studies that led to current dosage of metronidazole had several advantages, as plasma or total tissue concentrations were measured instead of target-tissue concentrations and were also conducted on healthy volunteers Plaisance *et al.,* 1988; Karjagin *et al.,* 2004). The aim of the study was to assess pharmacokinetic parameters of metronidazole in patients suffering from amoebiasis, to recommend dose adjustment and to compare the drug pharmacokinetics in patients suffering from amoebiasis and in healthy volunteers.

SUBJECTS, PATIENTS AND MATERIALS AND METHODS

This study was conducted in 6 patients suffering from amoebiasis and 6 healthy human volunteers. A written consent was taken from all the human volunteers and patients.

Healthy human volunteers

Six healthy normal volunteers were selected from community. Inclusion criteria for volunteers were age between 25-35 years, disease-free and not taking any medication before starting the study.

Patients

In this study, 6 male patients suffering from amoebiasis (confirmed after stool tests) were selected from medical ward of Services Institute of Medical Sciences, Lahore. Selection of patients was according to the following criteria: patients with confirmed amoebiasis and with positive *E. histolytica* test, age range 25-35 years, patients not on any other medication and without any other disease. Weight of all patients was recorded. A written consent was taken from each patient or from patient's attendant. All the selected patients were informed about the objective of study, frequency of blood sampling and the possible side effects of the drug which they might face during the study period.

Materials

Metronidazole standard was kindly provided by Sanofi-Aventis Pakistan Limited, Karachi. Tinidazole standard was gifted by Pfizer Laboratories Limited, Karachi. Potassium dihydrogen phosphate and HPLC grade acetonitrile (E. Merk, Darmstadt, Germany) were purchased from the local market). Metronidazole Tablets 400mg, Sanofi-Aventis Pakistan Limited, Karachi) were purchased from market.

Administration of drug:

Volunteers were kept empty stomach overnight before the drug administration and after 2 hours post administration. One tablet of metronidazole 400mg was administered orally to each individual with 200ml of water.

Blood sampling:

Blood sample of 5ml was collected at 0, 0.25, 0.5, 1.0, 2.0, 3.0, 6.0, 12.0, 24.0 and 36.0 hours from each individual. Plasma was separated by centrifugation at 5000 RPM for 5 minutes and stored at -80ºC until assayed.

Extraction of drug from plasma

In a 5 ml glass tube, a plasma sample (1 ml) was mixed with 50 µl of the aqueous tinidazole internal standard (100 µg ml^{-1}). Acetonitrile (1.5 ml) was added to sample, vortex-mixed for 5 min and centrifuged at 1600 *g* for 5 min. Processed samples were capped and kept frozen at -20°C for 20 min. An aliquot (1 ml) of the supernatant was transferred to a 5 ml glass tube and the solvent was evaporated to dryness using a Speed–Vac concentrator.

Determination of drug in plasma

Chromatographic System

The chromatography was carried out using previous described method (Galmier *et al.,* 1998) on an HP 1100 instrument (Agilent, France) equipped with a power supplier, an auto sampler and a UV spectrophotometric detector connected to a data collection system. The analytical column was C18, (0.5µm particle size, 150×4.6mm). Acetonitrile and 0.01 *M* phosphate solution (KH_2PO_4), pH 4.7, 15:85, v/v, was used as the mobile phase. The mobile phase was degassed and filtered through a 0.45 mm filter (Millipore, Saint-Quentin, Yvelines, France). The flow-rate was 1 ml min. The detection wavelength was 318 nm. The sample was reconstituted in 0.5 ml of mobile phase before injection onto the HPLC system. The injection volume of the sample was 20 µl and the run time was 6 min.

Calibration standards

A stock solution of metronidazole was prepared by dissolving its 100 mg in 100 ml of deionized water. This solution was used to prepare working standard solutions daily for different concentrations between 0.005µg/ml and 0.1µg/ml by dilution in deionized water. Calibration samples of metronidazole were prepared in blank plasma. The plasma (1 ml) was supplemented with working standard solutions of metronidazole at 0.05, 0.1, 0.5, 1.0, 5.0 and 10.0 µg ml^{-1}.

Pharmacokinetic data analysis

The plasma level time data were used to compute pharmacokinetic parameters on computer software, APO pharmacological analysis MW/PHARM (Ver 3.02) using one and two compartment open models with lag time. Based on the goodness of fit statistics, the compartment model was selected. Thus, one compartment open model was selected to explain and compare the pharmacokinetics parameters of metronidazole in healthy volunteers and in amoebiasis.

RESULTS AND DISCUSSION

The results for calibration curve of metronidazole are given in Table I and Figure 1. The calibration curve was obtained with regression equation, y=0.138X+0.019 and R^2 value 0.999. The concentration of drug in plasma of amoebiasis patients and in healthy volunteers at different time intervals was measured.

Table I: AUC of the metronidazole with different concentrations (μgml^{-1})

Concentration of Metronidazole (μgml^{-1})	Ratio Metronidazole/Tinidazole (MaU)
10	1.39542
5	0.72142
1	0.15424
0.5	0.10254
0.1	0.02658
0.05	0.01654

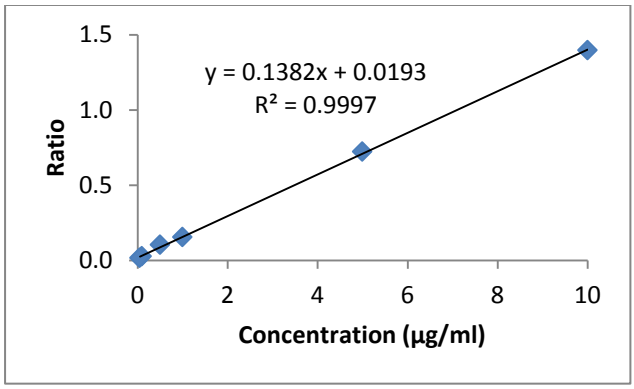

Figure 1: Calibration curve for metronidazole

Compartment model

The plasma concentration-time data was analyzed by one compartmental open model and the values of different pharmacokinetic parameter were determined in amoebiasis patients and in healthy volunteers.

Plasma concentration

Table II and Figure 2 show the plasma concentration of metronidazole in patients at different time intervals. The Table III and Figure 3 show the metronidazole concentration in normal healthy volunteers. Following oral administration of metronidazole, the plasma concentration in healthy volunteers at 0.25 hr was 0.1284 ± 0.01 µg/ml and plasma concentration in patients at 0.25 hr was 0.1256±0.04 µg/ml. It showed that after drug administration, the absorption of drug has started in the blood. The concentration increased with time showing a C_{max} of 8.041±0.41 µg/ml in healthy volunteers as compared to 7.034±0.28 µg/ml in patients. This statistically significant difference is probably due to the decreased absorption of metronidazole from GIT due to the effect of disease on intestinal epithelium or due to increased GIT motility in amoebiasis. C_{max} observed in healthy volunteers was approximately similar to the C_{max} 7.8 µg/ml observed previously (Galmier *et al.,* 1998; Bergan and Arnold, 1980; Mustofa *et al.,* 1991).

Table II: Plasma concentration (µg/ml) of metronidazole at different time intervals following oral administration of 400mg to patients (Mean±SD, n=6).

Time (Hours)	Concentration (µg/ml)
0.25	0.1256±0.04
0.5	3.2038±0.38
1	6.5979±0.29
2	7.2481±0.17
3	6.364±0.29
6	4.5209±0.49
12	3.0652±0.28
24	0.9909±0.2
36	0.2955±0.03

Elimination half life:

The mean half life of metronidazole recorded in healthy volunteers after 400mg of single dose was 7.782 ± 0.78 h which was non-significantly different from 7.451 ± 0.28 h in patients indicating lack of effect of amoebiasis on the drug elimination. The observed elimination half-life was similar to the studies demonstrated by previous studies (Galmier *et al.,* 1998; Bergan *et al.,* 1981; Bergan *et al.,* 1984; Mustofa *et al.,* 1991; Gatchev *et al.,* 2006).

Apparent volume of distribution:

The volume of distribution observed in healthy volunteers was 0.8544 ± 0.09 L as compared to 0.8016 ± 0.02 L in amoebiasis. This difference was statistically non significant. The decrease in volume of distribution in

disease state is probably due to decreased absorption. Volume of distribution observed in volunteers was comparable to the reported values of volume of distribution in different studies (Lau, 1986; Teow-Yee *et al., 1996;* Lamp *et al.*, 1999; Steinman *et al.*, 2000).

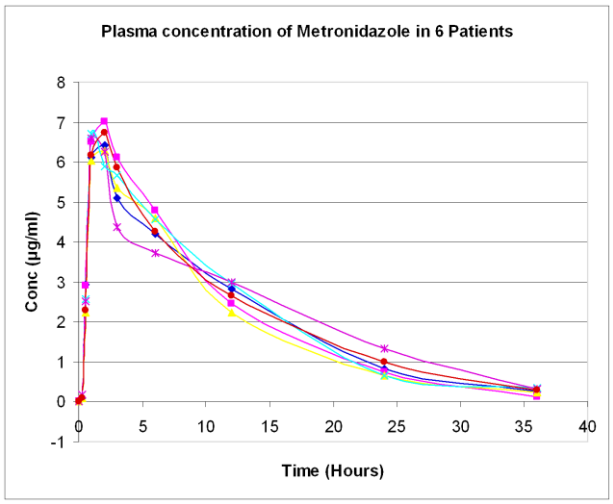

Figure 2: Plasma concentration of metronidazole (µg/ml) at different time intervals following oral administration of 400mg in 6 patients.

Table III: Plasma concentration (µg/ml) of metronidazole at different time intervals following oral administration of 400mg to healthy volunteers. (Mean ± SD, n=6)

Time (Hours)	Concentration (µg/ml)
0.25	0.1284±0.01
0.5	3.7472±0.25
1	7.6274±0.28
2	8.2331±0.66
3	6.9858±0.5
6	5.656±0.76
12	3.5055±0.21
24	1.1369±0.01
36	0.4089±0.12

Total body clearance:

Total body clearance is the sum of all processes which contributes towards elimination of drug from the body. The total body clearance of metronidazole was 0.07624 ±0.008 L/hr.kg in healthy volunteers and 0.07469±0.003 in patients which was however, higher but statistically non significant. Dose should not be adjusted as metronidazole is eliminated through kidney and reduced renal function does not affect the pharmacokinetic parameters of metronidazole (Houghton *et al.*, 1985). Clearance observed was close to 0.048 +/- 0.024 as reported by Lau (1986).

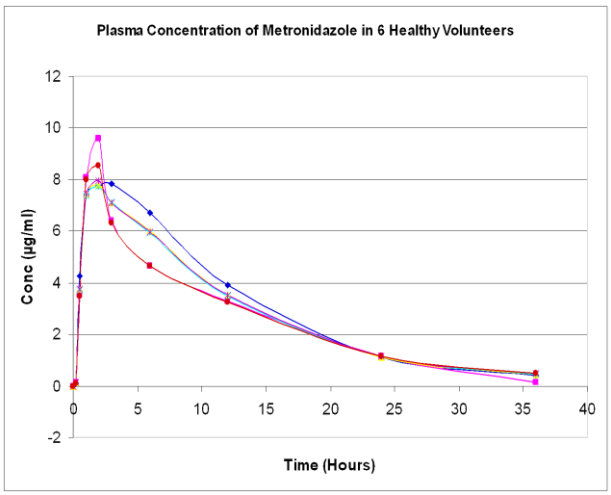

Figure 3: Plasma concentration of metronidazole (µg/ml) at different time intervals following oral administration of 400mg in 6 healthy volunteers.

Area under the concentration curve (AUC)

$AUC_{0-\infty}$ is the total area under plasma concentration curve from t_0 to t_∞. $AUC_{0-\infty}$ of metronidazole after 400mg of oral dose was found to be 103.17 ± 6.25 mg.h/L in healthy volunteers versus 87.37± 2.56 mg.h/l in patients. The difference was statistically significant, may be due to the diminished absorption of drug after oral administration in amoebiasis patients secondary to the disease led changes in intestinal epithelium or increased GIT motility. The present $AUC_{0-\infty}$ value in healthy volunteers was similar to literature cited values (Bergan *et al.*, 1984; Guay *et al.*, 1983).

Time to peak concentration T_{max} (hour)

It is the time at which maximum concentration of drug is attained. The T_{max} of metronidazole after 400mg of oral dose was found to be 1.774 ± 0.06 h in healthy volunteers which was not different from 1.807 ± 0.07 h found in patients. It shows that in patients, the drug took slightly more time to reach to its peak because of slow absorption from the walls of intestine. The peak concentration was similar to 1.5-2.0 h as observed (Bergan *et al.*, 1981; Bergan *et al.*, 1984).

The comparison of average values of pharmacokinetic parameters in healthy volunteers and in amoebiasis patients is shown in Table IV and Figure 4. Difference between pharmacokinetic parameters of metronidazole in healthy human volunteers and amoebiasis patients was calculated by using un-paired T-test. The P value for AUC, Cl, Vd, $T_{1/2}$, C_{max} and T_{max} were calculated as 0.00040, 0.70, 0.27, 0.12, 0.4 and 0.001 respectively which shows no difference of pharmacokinetics of

metronidazole in healthy volunteers and amoebiasis patients except for AUC and C_{max} (Table IV).

Table IV: Pharmacokinetic parameters in healthy volunteers and in patients with amoebiasis. (Mean ± SD, n=6)

Parameter	Healthy volunteers	Patients	P value
AUC(h.mg/l)	103.17±6.25	87.37±2.56	0.00*
Vd(L/kg)	0.8544±0.09	0.8016±0.02	0.27ns
T½ (h)	7.782±0.78	7.451±0.32	0.4ns
Cl (L/h.kg)	0.0762±0.008	0.0746±0.003	0.7ns
T_{max} (h)	1.774±0.06	1.807±0.07	0.46 ns
C_{max}(µg/ml)	8.041±0.41	7.034±0.28	0.00*

*=Significant
ns=Non-Significant P > 0.05

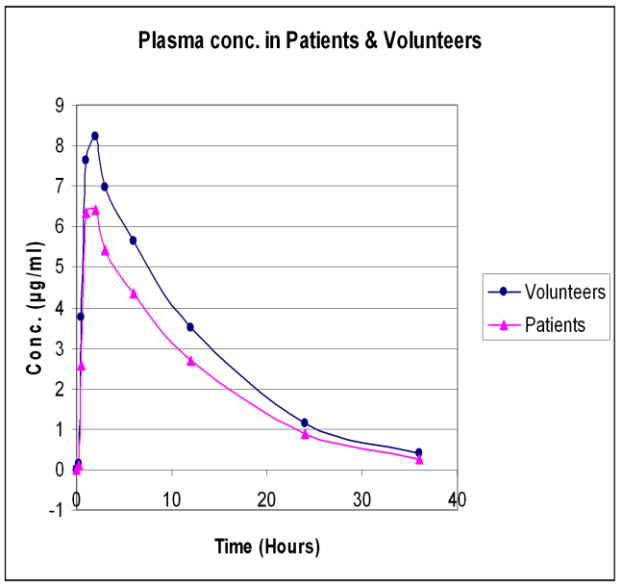

Figure 4: Plasma Concentration of metronidazole in 6 patients with amoebiasis and 6 healthy volunteers

DISCUSSION

In some studies, the pharmacokinetic parameters of metronidazole in disease states were within the range reported for the healthy volunteers (Bergan and Arnold, 1980; Bergan *et al.*, 1984; Lau *et al.*, 1991). Other studies demonstrated that mean pharmacokinetic values of metronidazole were non-significantly lower in the critically ill patients as compared to the healthy human volunteers. Because critically ill patients often exhibit changes in drug clearance, elimination half-life, and volume of distribution, previous studies revealed that concentration of metronidazole tends to change in different disease conditions. The ileostomy patients had a higher area under the curve (260.5 µg/ml) than that found in the healthy volunteers (108.1 µg/ml) and the serum half-life in patients were also higher (Bodenham *et al.*, 1988; Power *et al.*, 1998; Bergan *et al.*, 1981).In patients undergoing continuous ambulatory peritoneal dialysis, the dialysis caused insignificant changes in the apparent volume of distribution, elimination half-life, and total body clearance of metronidazole (Guay *et al.*, 1983; Houghton *et al.*, 1985). The renal clearance of metronidazole was significantly greater in healthy volunteers compared to renally insufficient patients, but accounted for <10% of the total metronidazole clearance The half-life, distribution and total body clearance of metronidazole was not influenced in patients with reduced renal function (Bergan and Thorsteinsson, 1986).

The pharmacokinetics of metronidazole in hospitalized patients were not observed substantially different from those obtained in the normal subjects.[3] A study on metronidazole concentrations in plasma, saliva and periodontal pockets in patients with periodontitis revealed that metronidazole concentrations in crevice fluid were about equal to the protein unbound drug concentrations (Pahkla *et al.* 2005).

CONCLUSION

Statistical significance difference was not found between pharmacokinetic parameters of metronidazole in healthy volunteers and in patients suffering from amoebiasis except C_{max} and AUC. Little elevation of total body clearance cannot be considered for dose adjustment as the drug is eliminated through kidney and amoebiasis do not affect kidney. Thus, it can be concluded that amoebiasis has no effect on pharmacokinetic parameters of metronidazole after oral administration. Therefore, there is no need of dose adjustment for metronidazole while administering to patients suffering from amoebiasis.

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