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Chronopharmacokinetics of metronidazole in healthy human volunteers

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Abstract

Metronidazole is a synthetic antimicrobial agent widely used in different clinical conditions. Changes in pharmacokinetic profile of drugs caused by differences in dosing time has been known to have led to toxicities, treatment failures and in some cases more beneficial effects, depending on the parameters affected. This study was designed to investigate the circadian changes in the pharmacokinetics of metronidazole at three different dosing times; 0700 h, 1300 h, and 1900 h. Six healthy male volunteers, age 26.50 ± 2.66 years, weighing 58.33± 5.61 kg took part in the study. They were administered with 400mg oral metronidazole at the three circadian times, after a fasting period of not less than 4 h for each phase of the study. Saliva samples [2mL] were collected at 0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 12.0 h. Spectrophotometric method was used to determine the metronidazole concentration in saliva, and the pharmacokinetic parameters (Ka, $t_{1/2a}$, C_{max}, T_{max}, AUC, Ke and $t_{1/2e}$) calculated. The values obtained, though varied, were found to be in similar ranges with previously reported values. There were significant differences in Ka and $t_{1/2a}$ values ($P<0.05$) with respect to the time of administration while the other parameters were not significantly altered.

Keywords: Chronopharmacokinetics; metronidazole; human volunteers

Introduction

Metronidazole [2-(2-methyl-5-nitro imidazole-1-yl) ethanol], is a synthetic antimicrobial agent with activity against obligate anaerobic bacteria and protozoa. It is widely used in different clinical conditions or as a prophylactic agent in pre and post surgical operations, bacterial vaginosis, ulcerative gingivitis e.t.c. either as single dose or multiple doses (Goodman and Gilman, 1995). It is well absorbed after oral administration and widely distributed in body tissues including saliva, breast milk and cerebrospinal fluid in concentrations equivalent to those in plasma or serum (http://www.Nlist.com). Metronidazole in body fluids had been assayed by various methods, including titrimetry, spectro photometry, and chromatography (GLC and HPLC) (Wood 1973; Gibson *et al.*, 1984; Jenson *et al.*, 1983; Liu *et al.*, 1986).

Bioavailability of orally administered drugs depends on a number of factors such as gastric motility and/or emptying rate, hepatic blood flow, metabolism etc. All of these factors are affected by the body clock. Endogenous circadian rhythms in gastrointestinal pH, intestinal motility,

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digestive secretions and intestinal blood flow are among other factors responsible for the variation in the pharmacokinetics of many drugs (Markiewicz and Semenowicz 1979, Valli *et al.*, 1980; Kabasakalian *et al.*, 1970; Naranjo *et al.*, 1980; Kolawole *et al.*, 2002; White *et al.*, 1995; Rebuelto *et al.*, 2003). Also plasma proteins (albumin and acid α-1-glycopotein) which are highly involved in active drug transport exhibit circadian rhythmicity in their production by the liver (Naranjo *et al.*, 1980). This was shown to affect the circadian changes in plasma protein binding profile of diazepam, valproic acid and carbamazepine thereby reaching their respective crest in the early morning (Patel *et al.*, 1982; Narajo *et al.*, 1980). In rats and mice the enzymatic activity of aminopyrine–N-demethylase, 4-dimethyl aminobenzene reductase and *p*-nitroanisole-*o*-demethylase is greatest during the second half of the animal activity span and lowest during the second half of the rest span (Radzialower and Bonsquet 1968). Circadian variation in hepatic blood flow is an important factor since hepatic blood flow also determines the clearance of drugs from systemic circulation and in the body.

Endogenous circadian rhythms affect both the pharmacokinetics and pharmaco dynamic properties of drugs. Also the severity of some disease conditions is time and season influenced. For instance asthma is most commonly a nighttime problem with most attacks occurring between 4.00am 6.00am, while blood pressure in an active person rises sharply in the morning after nighttime sleep and peaks in the afternoon or early evening. The concept of chronophysiology is usually ignored in clinical trials. The objective of chronopharmacokinetics in chronotherapy is to apply chronobiologic principles in the treatment of human diseases, (Halberg *et al.*, 1980). Chronopharmacokinetic of metronidazole has not been reported in any literature to our knowledge. The objective of this paper is to report the effect of time of administration on the pharmacokinetics of orally administered metronidazole tablet.

Experimental

Materials. Pure metronidazole powder used was donated by the Department of Pharmaceutical Chemistry, University of Jos. Metronidazole tablets (200mg flagyl tablet, Lot No. 269 by M&B, Plc Dagenhan-England.) were purchased from a pharmacy shop in Jos- Nigeria. UV-Visible Spectrophotometer (Jenway, Italy) was used for the analysis. All chemicals were of analytical grade, and obtained from the department of Pharmaceutical Chemistry.

Sample assay. The method of Liu, *et al.* (1986) was adopted and slightly modified to suit saliva samples. Saliva samples were centrifuged at 3g for 10minutes, and the supernatants collected. A 1.0 mL portion of the supernatant was mixed with 3.0 mL of Borate buffer solution (pH 9) and vortex for a few seconds. The resulting solution was extracted with 5.0 mL x 2 chloroform. The combined chloroform extract was transferred into a separating funnel and 4mL of 0.1M HCl was added. The aqueous phase was carefully taken and the absorbance measured at 277nm against a blank obtained by treating drug free pooled saliva the same way as above.

Calibration. Calibration curve of concentration of metronidazole in human saliva based on absorbance was prepared by spiking drug-free (blank) saliva with standard stock solution of metronidazole to give a concentration range of 0 to 12.5 mcg/mL. The samples were analysed using the modified method of Liu *et al.* (1986) as stated above. The above processes were repeated on five (5) different days using the same concentrations. The absorbances for each concentration was averaged and the corresponding concentration obtained for the construction of the final calibration curve.

Validation of analytical method.

Recovery. Desired concentration of the stock solution (2.5, 7.5, and 12.5mcg/mL) were spiked into 2mL of saliva and the samples analysed using the analytical method. From the absorbance obtained, the corresponding concentrations were obtained after which the percentage recoveries were then calculated.

Precision and Accuracy. Saliva (2mL) was spiked with the following concentrations; 2.5, 5.0, 7.5, 10.0, and 12.5mcg/mL of metronidazole from the stock solution and the samples analysed using the analytical method. From the absorbances obtained, the corresponding concentrations were obtained, and the percentage recovery and percentage relative standard deviation were calculated.
The validation procedure was carried out for five different times on the same day and the average data per concentration used for the statistical analysis.

Subject treatment. Six (6) healthy male volunteer, ages 26.50 ± 2.66 years, (mean ± SD), weighing 58.33 ± 5.61 kg were randomly selected. The protocol of the study was explained to each volunteer and his written consent was obtained. No drugs including alcohol were allowed two weeks before and throughout the duration of study.
On the day of each test and prior to oral ingestion of 400mg metronidazole tablet, each volunteer fasted for at least four hours before administration of drug. The study was in three phases, during which the volunteers were administrated with 400mg of metronidazole tablets at three specified dosing times (0700, 1300 and 1900 h).

Sampling time and collection. The subjects ingested two (2) tablets (400 mg) dose of metronidazole with 150 mL of water in the morning (phase one] at 0700 h after over night fast. Saliva samples [2mL] were collected with the aid of chewing a semi-solid paraffin at 0.0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 12.0 h respectively. The saliva sample were then centrifuged for about 5 minutes at 3 g and the clear supernatant harvested and analysed using the modified method of Liu *et al.* (1986). After a wash out period of two weeks between phases, the above procedures were repeated with drug administration starting at 1300 and 1900 h for phase two (afternoon) and phase three (evening) dosing time respectively.

Pharmacokinetic parameters were determined by non-compartmental methods. First order elimination rate constant Ke was determined from the slope of the best log-linear fit of the terminal phase by least square linear regression analysis. Elimination half-life was calculated using the formula 0.693/Ke. The area under the curve (AUC) and AUMC (area under the first moment of the plasma concentration- time curve were calculated by the linear trapezoid rule with extrapolation to time infinity. The pharmacokinetic parameters were presented as mean ± SD. Treatment effects were statistically evaluated using ANOVA, assuming the Null hypothesis that there is variation in the pharmacokinetic parameters with the three dosing times; 0700 h,1300 h and 1900 h.

Mean pharmacokinetic parameters derived from saliva concentration of metronidazole in six healthy volunteers following oral administration of 400 mg metronidazole at 0700 h, 1300 h and 1900 h.

Results and Discussion

The metronidazole tablets used in this study complied with BP 1988, quality assurance tests. The calibration curve was found to be linear over a concentration range of 2.0 – 15.0 mcg/mL with a correlation coefficient (r) of 0.999. The mean ± SD of the calibration curve slope was found to be $3.197 \times 10^{-3} \pm 1.5 \times 10^{-6}$. The very small value of the SD, shows excellent day-to-day precision. The limit of quantification was set at 2.5

mcg/mL. The percentage recovery (%R) of metronidazole from saliva of 97.9% was obtained, with a % RSD of 4.06 over the concentration range of the study [Table 1]. The analytical procedure is satisfactory enough for the work.

The lag time was found to be 0.13 h, 0.18 h and 0.24 h at 0700, 1300 and 1900 h respectively [Table 2, Figure 1], and all these fall within previous reports (Lau *et al.* 1992). There was 41.73% and 88.98% increase in lag time with dosing at 1300 and 1900 h respectively when compared with dosing at 0700 h. These changes are not statistically significant [P>0.05]. The increase could be due to increased gastric motility in the morning compared to afternoon and evening as proposed by Goo *et al.*, 1987.

The absorption parameters Ka and $t_{1/2a}$ vary considerably between individual volunteers however they fall within previously reported values (Liu *et al.*, 1986). There was 48.35% and 9.61% decrease in mean Ka, with dosing at 1300 and 1900 h respectively over Ka value at 0700 h. These differences in the values obtained at the various dosing time are statistically significant (P<0.05). There was 94.12% and 57.14% increase in $t_{1/2a}$ at dosing time 1300 over dosing time 0700 and 1900h respectively. These changes are statistically significant (p <0.05), while the 23.33% increase at 1900h over 0700h is not statistically significant (p>0.05). This might be due to circadian changes in gastric emptying rate, since it has been reported that there is decrease in gastric emptying rate at night compared to morning or day. This is in support of the report of Markiewicz, 1979 that some drugs show circadian variation in drug absorption at different time of the day.

The values of bioavailability parameters, C_{max}, T_{max} and AUCo-∞ are presented on Table 2. Peak plasma concentration, C_{max} (crest) values of 8.72, 9.02 and 8.32 mcg/ mL were obtained at dosing time 0700, 1300 and 1900 h respectively were obtained and these values are in harmony with the literature report for plasma concentration of metronidazole following 250, 500 or 2000 mg oral dose of 6, 12 and 40 mcg/ mL, which usually produce the required mean effective concentration (MEC) of 8mcg/mL (Katzung 1998; http://www.Nlist.com). There was 3.44% increase and 4.59% decrease in C_{max} with dosing at 1300 and 1900 h respectively when compared with dosing at 0700 h. The differences are not statistically significant (p>0.05). The T_{max} was found to range from 2.03 h and 0700 h to 2.77 h at 1900 h (Table 2, Figure 1). These values fall within the documented value of 1-3 h (Katzung, 1998). There was 1.97% and 36.45% increase in T_{max} with dosing at 1300 and 1900 h respectively when compared with dosing at 0700 h. There was no significant difference (p>0.05) between the values. The AUCo-∞ was found to range from 73.69 to 82.76 mcg.h/mL and in harmony with the report by Lau, *et al.* (1992). A 9.88% increase and 2.16% decrease was obtained for dosing time 1300 and 1900 h respectively over the value obtained for 0700 h. These changes are however not statistically significant (p>0.05).

The elimination rate constant (Ke) are 0.135 h, 0.130 h and 0.121 h at 0700, 1300 and 1900 h respectively and these values are close to the previously reported values (Katzung 1998). A 3.7% decrease and 10.37% decrease in Ke was obtained dosing at 1300 and 1900 h respectively when compared with dosing at 0700 h. No significant difference (p>0.05) was observed. The elimination half life ($t_{1/2e}$) obtained was found to range from 5.19 h at 0700 h to 5.86 h at1900 h. However, these values falls below 7-8 h reported by Katzung (1998) and this can be attributed to individual variations. The study showed 2.31% increase and 12.91% increase at the dosing times 1300 and 1900 h respectively over the value obtained at 0700 h. The

differences are not statistically significant ($p>0.05$).

The total body clearance (Clt) ranged from 81.88 to 92.57 mL/ min and all these fall within the range reported by Lau *et al.* (1992). There was 10.4% decrease and 1.30% increase with dosing at 1300 and 1900 h when compared with dosing at 0700h. These changes are not statistically significant. The differences might be due to circadian variation in production and binding of drugs to plasma proteins.

Table 1. Analytical characteristics of method; accuracy and precision (within day and day-to-day).

	Within day			Day-to-day	
	Conc. spiked (mcg/mL)	Conc. obtained (mcg/mL)	% RSD	Slope of calibration curve	Correlation coefficient.
1	2.50	2.42 ± 0.13	5.4	3.205×10^{-3}	1.080
2	5.00	4.93 ± 0.23	4.7	3.170×10^{-3}	0.997
3	7.50	7.38 ± 0.19	2.6	3.205×10^{-3}	0.980
4	10.00	9.76 ± 0.46	4.7	3.200×10^{-3}	0.930
5	12.50	12.26 ± 0.36	2.9	3.205×10^{-3}	1.030
Mean ± SD (n=5)				$3.197 \times 10^{-3} \pm 1.5 \times 10^{-6}$	1.003 ± 0.0043

Table 2. Mean pharmacokinetic values derived from saliva concentration of metronidazole in six healthy volunteers following oral administration of 400mg metronidazole at 0700 h, 1300 h and 1900 h.

	Mean ± SD		
	0700 h	1300 h	1900 h
Lag time(h)	0.127 ± 0.091	0.18 ± 0.10	0.24 ± 0.10
Ka (h^{-1})	1.394 ± 0.20	0.72 ± 0.13	1.26 ± 0.56
$t_{1/2a}$ (h)	0.51 ± 0.069	0.99 ± 0.22	0.63 ± 0.21
C_{max} (mcg/mL)	8.72 ± 0.90	9.02 ± 0.74	8.32 ± 0.47
T_{max} (h)	2.03 ± 0.052	2.07 ± 0.08	2.77 ± 0.96
AUCo-∞(mcg.h/mL)	75.32 ± 14.71	82.76 ± 10.70	73.69 ± 14.36
Ke (h-1)	0.135 ± 0.015	0.130 ± 0.17	0.12 ± 0.02
$t_{½e}$(h)	5.19 ± 0.563	5.31 ± 0.89	5.86 ± 1.00
Clt (mL/min)	91.38 ± 17.89	81.88 ± 12.48	92.57 ± 15.19

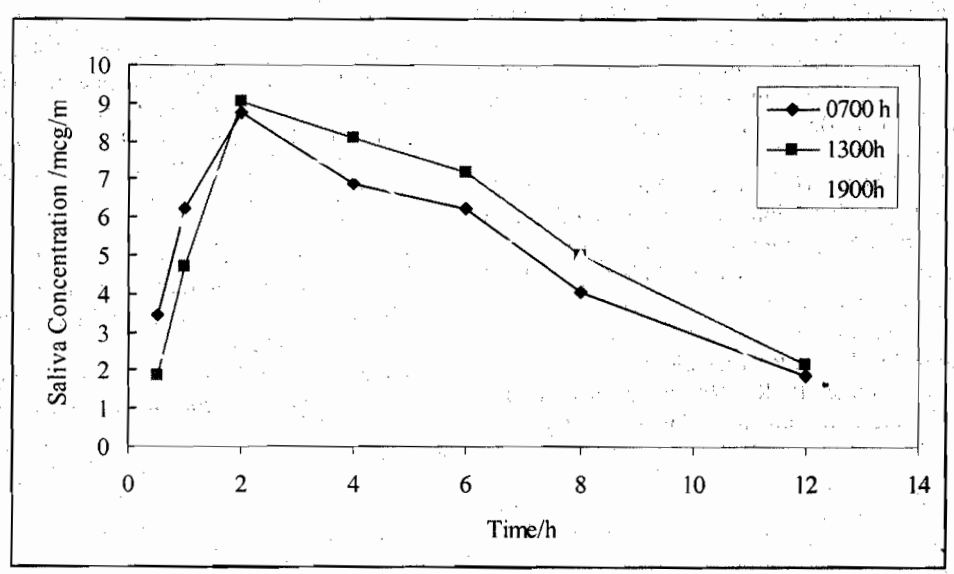

Figure 1. Saliva Concentration-Time curve of metronidazole in six healthy human volunteers following oral administration at different dosing times (0700 h, 1300 h and 1900 h)

Conclusion

The chronokinetics study of a single oral dose (400mg) of metronidazole in six healthy male volunteers reveals that there were statistical significant differences in the Ka and $t_{1/2a}$ with respect to time of dosing. The morning dosing showed a better absorption profile as compared with the afternoon and night dosing. This resulted in marginal but not statistically significant changes in C_{max} and AUC o-∞. These increased bioavailability by morning dosing may enhance therapeutic efficacy especially when single dose treatment or 'stat' dose is required. There were no significant differences in the rest of the pharmacokinetic parameters studied. Although the time of the dosing did not statistically affect the lag time, C_{max}, T_{max}, AUC o-∞, Ke, Clt and $t_{1/2a}$, there were marginal changes, which may be statistically significant if larger doses were employed in the study.

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