

DISPOSITION KINETICS OF AMOXYCILLIN IN NORMAL AND FASTED HUMAN VOLUNTEERS BY URINE EXCRETION DATA

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ABSTRACT

Purpose : The disposition and pharmacokinetics of amoxicillin trihydrate administered orally was investigated in 7 control and food-deprived human volunteers by urinary excretion data. **Method :** The urine samples were collected up to 12 hours following drug administration and analyzed for the drug by disc diffusion method using *Sarcina lutea*. The data was analyzed for computation of pharmacokinetic parameters by standard methods. **Results :** Student t-test revealed a non significant decrease in urinary excretion of drug in control and overnight fasted volunteers at 0.5 and 1.0 hours after oral drug administration pointing to no effect of food on amoxicillin absorption during this interval. At 12 hours, urinary recovery of the drug in control volunteers was 7.3% more than that in the fasted volunteers which was significant. The volume of urine in control and overnight fast volunteers up to 12 hours averaged 1210 ± 30.26 ml and 948 ± 16.80 ml, respectively. Elimination rate constant of 0.60 ± 0.03 hr⁻¹ in control volunteers was significantly ($P < 0.05$) higher than 0.51 ± 0.02 hr⁻¹ in the overnight fasted volunteers. A shorter ($P < 0.05$) t_{1/2} of 1.18 ± 0.05 hours in control volunteers than 1.38 ± 0.05 hours in overnight fasted subjects was concluded. Food and water deprivation seems not to have influenced absorption of the drug but the water deprivation affected a decreased urinary recovery and volume of urine leading to decreased elimination rate constant and increased half life of the drug. **Conclusion :** The urine data may be used as an alternative to the blood data for the estimation of pharmacokinetics of drugs.

Key Words: Disposition Kinetics, Amoxicillin, Urine Excretion Data.

INTRODUCTION

Plasma is a body-fluid of choice for evaluation of pharmacokinetics of drugs. Most pharmacokinetic studies are based on the measurement of drug concentrations in plasma (1). However, some drawbacks of this technique have been reported. The collection of blood plasma is not entirely without risk and subjects, patients or healthy volunteers feel inconvenience

and discomfort. The subjects may become fearful about repeated sampling resulting in slowing of the rate of drug absorption from the gastrointestinal tract (2) and possibly, the alterations in pharmacokinetics of drugs (3). Pharmacokinetic assessment with the help of urinary excretion data, a non-invasive technique has been recommended as an alternative to blood sampling. Additionally, this is an important procedure for comparing different dosage forms,

routes of administration and drug preparations of various manufacturers. Amoxicillin was selected to compare the pharmacokinetic parameters in control and overnight fasted human volunteers by urinary excretion data. This data can also be helpful in dose adjustment in the month of Ramadan during which Muslims continue a day-long fast.

MATERIALS AND METHODS

Volunteers

Seven healthy male human volunteers ranging in age from 23 to 35 years and in weight 57-75 kg, participated in this study. Volunteers were advised not to take any medication one week prior to and during the study. The volunteers were informed about the objectives of the study and written consent was obtained from each volunteer.

Study design

The study was performed in two sessions. In the first instance, the experiments were performed in control volunteers who were not restricted to being without food or water. The studies were conducted on the same volunteers after starvation for a period of 12 hours in the second session which continued till the 3rd hour of sampling (about 15 hours fast). A washout period of 10 days was given between the two sessions.

Drug Administration

In both instances, each volunteer was given a single Amoxil capsule (Smith Kline Beecham Pvt. Ltd., Karachi-Pakistan) orally containing 250 mg of amoxicillin trihydrate. Before drug administration (at zero time) the volunteers emptied their bladders.

Sample collection

The measured amount of urine samples were collected at 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, and 12.0 hours after drug administration. The urine samples were immediately used for drug analysis.

Analysis of amoxicillin

Amoxicillin concentrations in urine samples were measured by employing microbiological assay (disc diffusion method) using *Sarcina lutea* (ATCC 9341) as described by Arret et al (4).

Data Analysis

The excretion of amoxicillin in urine expressed in milligrams was determined by multiplying the drug concentration in samples with the volume of respective urine. The percentage of dose excreted in urine samples was determined for each experimental period as follows:

$$\text{Percentage dose excreted} = \frac{\text{Total dose excreted (mg)}}{\text{Total dose (mg)}} \times 100$$

The cumulative excretion in milligrams as well as cumulative percentage of the dose excreted in urine at different time intervals after drug administration was determined. Elimination rate constant (K) and half-life from urine excretion data were calculated by standard method (5).

Statistical analysis

Mean values of amoxicillin concentration along with their standard error of mean (\pm SEM) were calculated to evaluate the significance of the difference between control and overnight fasting states. The data was subjected to student's paired t-test which was performed on SPSS, a Windows based computer package.

RESULTS

Table 1 represents the average \pm SEM values for the amount of amoxicillin excreted, percentage of dose excreted, cumulative amount excreted and cumulative percentage of the drug excreted in urine after administration of amoxicillin to control and overnight fasted volunteers. A non-significant ($P < 0.05$) decrease in urinary excretion of the drug in control and overnight fasted volunteers at 0.5 and 1.0 hour after oral drug administration has been observed. At the 12th hour, the urinary recovery of amoxicillin in control and overnight fasted volunteers showed a highly significant ($P < 0.01$) difference between the two conditions.

On the basis of urinary excretion, the pharmacokinetic data analysed from urine samples of control and overnight fasted volunteers has been compared and presented in Table 2. The control value for the elimination rate constant (K) of $0.60 \pm 0.03 \text{ hr}^{-1}$ is significantly ($P < 0.05$) higher than that of $0.51 \pm 0.02 \text{ h}^{-1}$

Table 1: Mean \pm SEM values of amount excreted, percentage amount excreted, cumulative amount excreted and percentage of cumulative amount excreted in urine of control (C) and overnight fasted (F) volunteers after administration of Amoxil-250 mg capsule.

Time (hrs)		Amount excreted (mg)	Percentage dose excreted	Cumulative amount excreted (mg)	Cumulative percentage excreted
0.5	C	7.70 \pm 1.78 ^{ns}	2.51 \pm 0.29 ^{ns}	6.27 \pm 0.78 ^{ns}	2.51 \pm 0.31 ^{ns}
	F	5.33 \pm 0.42	2.13 \pm 0.17	5.33 \pm 0.45	2.13 \pm 0.18
1.0	C	7.30 \pm 1.94 ^{ns}	2.34 \pm 0.39 ^{ns}	12.14 \pm 1.75 ^{ns}	4.86 \pm 0.31 ^{ns}
	F	4.88 \pm 0.52	1.95 \pm 0.21	10.21 \pm 0.57	4.08 \pm 0.23
1.5	C	17.51 \pm 2.51 ^{**}	7.01 \pm 1.00 ^{**}	29.65 \pm 3.31 ^{**}	11.86 \pm 1.32 ^{**}
	F	4.65 \pm 0.30	1.86 \pm 0.12	14.86 \pm 0.54	5.94 \pm 0.22
2.0	C	19.32 \pm 9.28 ^{**}	7.67 \pm 0.69 ^{**}	48.83 \pm 3.21 ^{**}	19.53 \pm 1.28 ^{**}
	F	9.28 \pm 0.55	3.71 \pm 0.22	24.14 \pm 0.90	9.66 \pm 0.36
3.0	C	22.58 \pm 2.68 ^{**}	9.03 \pm 1.07 ^{**}	71.40 \pm 5.69 ^{**}	28.56 \pm 2.28 ^{**}
	F	13.75 \pm 0.81	4.64 \pm 0.77	37.89 \pm 1.31	15.15 \pm 0.52
4.0	C	29.15 \pm 3.01 ^{ns}	11.66 \pm 1.20 ^{ns}	100.56 \pm 4.84 ^{**}	40.22 \pm 1.93 ^{**}
	F	22.56 \pm 3.41	10.57 \pm 0.48	64.30 \pm 2.04	25.75 \pm 0.82
6.0	C	28.66 \pm 2.98 ^{ns}	11.46 \pm 1.19 ^{ns}	129.22 \pm 5.99 ^{**}	51.69 \pm 2.40 ^{**}
	F	28.51 \pm 1.69	11.40 \pm 0.68	92.81 \pm 1.82	37.12 \pm 0.73
8.0	C	18.78 \pm 2.02 ^{**}	7.46 \pm 0.78 ^{**}	141.01 \pm 4.38 ^{**}	59.20 \pm 1.75 ^{**}
	F	26.65 \pm 0.94	10.66 \pm 0.38	119.46 \pm 2.26	47.48 \pm 0.91
10.0	C	18.38 \pm 2.10 ^{**}	7.35 \pm 0.84 ^{**}	166.38 \pm 3.20 ^{**}	66.55 \pm 1.28 ^{**}
	F	24.27 \pm 0.56	9.71 \pm 0.22	143.73 \pm 2.54	57.49 \pm 1.02
12.0	C	16.97 \pm 1.32 ^{**}	6.79 \pm 0.53 ^{**}	183.35 \pm 2.58 ^{**}	73.34 \pm 1.03 ^{**}
	F	21.35 \pm 0.92	8.54 \pm 0.37	165.08 \pm 2.69	66.03 \pm 1.08

ns - non-significant difference statistically (p > 0.05) ** - highly significant difference statistically (p < 0.01).

Table 2 : Mean \pm SEM (n=7) values for the pharmacokinetic parameters obtained from the urinary excretion data of the control and overnight fasted volunteers after the administration of Amoxil-250 capsule.

Pharmacokinetic Parameters	Volunteers		Statistical Difference
	Control	Overnight fasted	
K (hr ⁻¹)	0.60 \pm 0.03	0.51 \pm 0.02	S
t _{1/2} (h)	1.18 \pm 0.05	1.38 \pm 0.05	S

S = significant difference (P < 0.05)

reported in the overnight fasted volunteers. The half-life (t_{1/2}) of 1.18 \pm 0.05 hours is significantly (P < 0.05) shorter in the control volunteers when compared with the value of 1.38 \pm 0.05 hours in the overnight fasted subjects.

The control values of the amount of amoxicillin excreted demonstrated significant differences at sampling times of 1.5, 2, 3, 8 and 12 hours when compared with the fasted volunteers.

DISCUSSION

This study was conducted to evaluate any alteration in pharmacokinetics of amoxicillin in fasted humans as compared to the normal state using urine excretion data. The study was

completed with 7 human subjects without any dropout. A non-significant (P > 0.05) decrease observed in urinary excretion of amoxicillin in control and overnight fasted volunteers at 0.5 and 1.0 hours after oral drug administration shows that food may have no effect on amoxicillin absorption. This has been supplemented by the findings of Eshelman and Spyker (6) in which an equal absorption of amoxicillin in fasting and non-fasting adults was observed. At the 12th hour, the urinary recovery of amoxicillin in control and overnight fasted volunteers show a highly significant (P < 0.01) difference between the two conditions. About 7.3% more of the drug was excreted in urine of the control volunteers which may be attributed to a higher total body

clearance of the drug in control volunteers than that in the overnight fasted volunteers. In control volunteers, the increased diuresis as compared to overnight fasted volunteers who did not take water and meals for an overnight period and was continued up to the 3rd hour of the sampling (about 15 hours fasting).

The high rate of urine flow in control volunteers prevented any possible back diffusion of the drug and caused higher elimination of the drug (7), and that is why the average volume of urine in normal and overnight fasting volunteers until 12 hours was 1210 ± 30.26 ml and 948 ± 16.80 ml, respectively, which was concluded to be non-significant.

The normal value for the elimination rate constant is significantly higher in the overnight fasted volunteers when compared with the normal human subjects (0.60 ± 0.03 vs 0.51 ± 0.02 hr⁻¹).

The half-life ($t_{1/2}$) in control and fasted volunteers (1.18 ± 0.05 vs 1.38 ± 0.05) is significantly ($P < 0.05$) different. The present $t_{1/2}$ values are comparable with 60 ± 9 minute after a bolus intravenous administration in normal humans (8). Very limited literature reports this parameter in normal human volunteers, while a couple of reports on the half-life after blood data has been documented. This current value is slightly longer than 0.88 hr in sick children after an iv infusion of amoxicillin (9). In another study in elderly patients, the half life was observed to be 1.6-3.0 hours (10). In a subsequent study in pre-term infants with gestational ages of less than 32 weeks, $t_{1/2}$ of amoxicillin was measured as 6.7 ± 1.7 hours after iv administration of drug (11). The value of $t_{1/2}$ of 2.15 ± 0.20 and 1.20 ± 0.16 hours in goats after an oral and intravenous routes respectively was observed (12). 1.05 ± 0.09 hrs in goat with the same dosage level after i/v administration (13) and 66 min in Columba livia pigeons (14). In sheep and goat the half lives of amoxicillin were noted to be 1.43 ± 0.16 and 1.13 ± 0.19 hr, respectively (15). In another study in the same animals, the half life was determined to be 46.3 min in sheep and 66.9 min in goats (16). In a healthy adult horse after iv administration at the rate of 10 mg/kg, the half life was observed to be 1.43 hours (17).

The normal values of amount of amoxicillin

excreted demonstrated significant differences at sampling times of 1.5, 2, 3, 8 and 12 hours when compared with the fasted volunteers. In terms of percent dose excreted, statistical dissimilarities were observed at the same intervals. The cumulative dose and percent cumulative dose excreted of amoxicillin consistently differed at all the sampling points except at 0.5 and 1.0 hours. Both the pharmacokinetic parameters were statistically different, which warrants further elucidation of the results.

In conclusion, food and water deprivation seems to have no influence on the absorption of amoxicillin but the water deprivation led to a decreased urinary recovery and volume of urine and thus results in decreased elimination rate constant and elevation of drug half-life. Furthermore, the urinary excretion data is likely to be used as an alternative to blood data if the drug is excreted mainly in the urine.

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