Research Paper

Ofloxacin pharmacokinetics in saliva

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ABSTRACT

Objective: To study the pharmacokinetics of ofloxacin using salivary drug concentration when administered alone or in combination with rifampicin (R), isoniazid (H) and pyrazinamide (Z) and also to assess the saliva to plasma concentration ratio.

Material and Methods: Twelve healthy male volunteers were investigated on four different occasions with an interval of at least one week between occasions. They were administered ofloxacin, either alone or in combination with R, H and Z. A partially balanced incomplete block design was adopted and the subjects were randomly allocated to each group. Salivary and plasma concentrations of ofloxacin were measured at 1, 2, 3, 6 and 8 h after drug(s) administration using validated methods.

Results: There were no significant differences between various pharmacokinetic parameters when ofloxacin was administered alone or in combination with R, H and Z. The mean saliva to plasma ratio of ofloxacin concentration was around 0.6. The bioavailability indices of ofloxacin in the saliva and plasma were similar in all the groups.

Conclusion: Several pharmacokinetic parameters could be calculated using salivary concentrations of ofloxacin. The determination of ofloxacin levels in saliva may be useful in therapeutic drug monitoring and pharmacokinetic studies.

KEY WORDS: Anti-TB drugs, ofloxacin, pharmacokinetics, saliva.

Introduction

Ofloxacin is a fluorinated carboxy quinolone exhibiting a marked bactericidal effect by inhibiting DNA gyrase.[1,2] *In Vitro* studies on bactericidal activity against *Mycobacterium tuberculosis* have suggested that ofloxacin is likely to be the most useful drug in the early stages of treatment and in preventing the emergence of resistance to other drugs.[3] Its favorable pharmacokinetic features include good oral absorption and lack of metabolism resulting in less drug interactions.[4]

The determination of drug concentrations in the saliva has gained widespread acceptance in a variety of settings.[5] The estimation of drugs in the saliva has been employed for therapeutic drug monitoring and for calculation of pharmacokinetic variables.[6] The rational use of such determinations can provide knowledge of patient-specific pharmacokinetic parameters leading to improved therapy.[7]

Saliva can serve as an alternative body fluid for pharmacokinetic investigations. It can be collected with minimal patient discomfort and can be easily obtained on multiple occasions. It is particularly suitable for investigations in geriatrics and pediatrics. Pharmacokinetic studies have shown that ofloxacin penetrates into saliva and its concentration correlates well with serum levels.[8] However, the data available in the published studies are inconsistent and results reported for the ratio of saliva to serum concentrations show wide variations.

The present study was undertaken to evaluate the pharmacokinetics of ofloxacin in saliva when administered alone or in combination with other antituberculosis (TB) drugs, *Viz.* rifampicin (R), isoniazid (H) and pyrazinamide (Z) and to study the ratio of the saliva to plasma concentration by measuring ofloxacin concentrations in simultaneous saliva and plasma samples in healthy volunteers.

Material and Methods

Study participants

Twelve healthy male volunteers with a mean age of 23 years (range 19-27 years) and a mean body weight of 56 kg (range 45-69 kg) participated in the study. The volunteers were assessed to be healthy on the basis of medical history, hepatic and renal function tests. None of the subjects was taking any medication one week before or during the study for any ail-

ment and were non-alcoholics. The study protocol was approved by the Institutional Ethics Committee and written informed consent was obtained from all the volunteers.

Assay for the stated contents of the drugs

Before starting the study, six tablets of ofloxacin (Dec-Pharma Ltd.), R (Asojsofcaps Pvt. Ltd.), H (Pfizer) and Z (Lupin Laboratories) were assayed on five different occasions to check for the quantity and the stated contents. The drugs employed in the study were drawn from the same batch.

Study design

The volunteers were investigated on four different occasions with an interval of at least one week between occasions. They were administered ofloxacin on one occasion and ofloxacin in combination with R, H and Z on other occasions, as shown in Table 1. A partially balanced incomplete block design was adopted. Thus, the volunteers received either ofloxacin alone or in combination with R, H, Z and RHZ. After an overnight fast, the drugs were administered on an empty stomach after emptying the bladder. The dosages of the drugs administered to the volunteers were approximately 10 mg/kg body weight for R, 35 mg/kg for Z and the dosages of ofloxacin and H were 10 mg/kg for subjects weighing $\leq$ 40 kg and 15 mg/kg for subjects weighing > 40 kg.

Sample collection

On each occasion, 2-3 ml of paired samples of saliva and heparinized blood were collected at 1, 2, 3, 6 and 8 h after oral administration of the drug(s). Plasma was separated and stored. The study participants were instructed to wash their oral cavity and a piece of unsweetened, unflavored chewing gum was used to stimulate salivary secretion. The saliva samples were frozen at -20°. They were thawed the following day, centrifuged and the supernatant was separated. The saliva and plasma samples were stored at -20°C. By storing biological samples under these conditions, no detectable drug decomposition takes place.

Table1

Ofloxacin administered alone and in combination to healthy volunteers

Volunteer no.	*Occasions*			
	1	2	3	4
1	0	OH	OZ	OR
2	OH	OZ	O	ORHZ
3	OR	0	OH	OZ
4	ORHZ	OZ	O	OH
5	OZ	OH	O	OR
6	ORHZ	O	OZ	
7	O	OR	OZ	
8	OR	OZ	O	
9	OZ	O		
10	OR	O		
11	O	ORHZ		
12	O			

O: Ofloxacin, R: Rifampicin, H: Isoniazid, Z: Pyrazinamide
The number of volunteers in each group: O=12, OR=6, OH-5 OZ=9,ORHZ=4

Assay of ofloxacin

Ofloxacin concentrations in saliva and plasma were determined by the plate diffusion method employing a strain of *E.coli* as described elsewhere.[9] In brief, the organism seeded in the autoclaved nutrient agar was poured into sterile petriplates. Wells of 2 mm diameter were cut. Ofloxacin standards ranging from 0.25-8.0 mg/ml in pooled saliva and plasma and the drug in sativa and plasma samples were set up in quadruplicate. The concentrations of the drug were obtained from the regression line of the zone of inhibition on log concentrations of the standards. The assays were undertaken after coding the samples.

Pharmacokinetic analysis

Peak ofloxacin concentration (C_{max}) in saliva and plasma as well as the time at which peak concentration was attained (t_{max}) were obtained from the measured data. The elimination rate constant (Kel) was calculated from the terminal log-linear decline of concentrations in the measured fluid. Terminal elimination half-life ($t_{1/2}$) was calculated as 0.693/Kel. The areas under the concentration-time curve (AUC) (0-8 h) for 0 were calculated by the trapezoidal rule. Bioavailability of 0 for each drug combination was expressed as an index (B.I.) and calculated as the ratio of drug combination to that of 0 alone (control).

Data analysis was performed using SPSS (version 10.5) package. Differences among the treatment groups were determined using one-way ANOVA. *P values <0.05* were considered significant.

Results

Based on the salivary concentrations of ofloxacin at different time points, several pharmacokinetic variables were calculated (Table 2). The mean C_{max} values of ofloxacin when administered alone and in combination with R, H, Z and RHZ were 4.88,5.38,4.36,4.63 and 5.18 mg/ml respectively. There were no significant differences in the C_{max} and the t_{max} of ofloxacin between the different groups.

The concentrations of ofloxacin in saliva were much lower than those in plasma. The C,, values of ofloxacin in plasma when administered alone and in combination with R, H, Z and RHZ were 7.14, 6.98, 7.19, 6.06 and 6.38 mg/ml respectively. The mean saliva to plasma ratio of ofloxacin concentrations was calculated at all time points and it was around 0.6. The correlation coefficient between plasma and saliva concentration of ofloxacin was 0.94. The mean saliva to plasma (S/P) ratio of ofloxacin concentration when given alone and in combination was compared (Table 2). There was no significant difference between the groups. The bioavailability indices of ofloxacin in saliva and plasma calculated on the basis of C, and AUC (in comparison with various combinations) are shown in Table 3. They are similar in all the groups.

Discussion

Ofloxacin has proved to be a particularly valuable addition

Table 2

Pharmacokinetic parameters of ofloxacin in saliva when administered alone and in combination

Parameter	*O (n=12)*	*OR (n=6)*	*OH (n=5)*	*OZ (n=9)*	*ORHZ (n=4)*
C_{max} µg/ml	4.80±1.51	5.38±1.26	4.36±1.65	4.63 ± 1.05	5.18 ±2.42
t_{max}h	1.33±0.65	1.50±0.55	2.00±0.71	1.78 ± 0.67	1.50± 1.00
$t_{1/2}$ h	4.23±0.76	4.84±1.49	4.26±0.81	3.07 ± 0.47	7.54±5.22
AUC µg/rnl.h (0-8 h)	15.59±5.41	18.94±2.80	13.49±4.83	15.77 ± 4.37	17.34±3.98
Saliva/plasrna ratio	0.6±0.1	0.6±0.1	0.5±0.1	0.6 ± 0.1	0.6±0.1

Values are mean±SD. O: Ofloxacin, R: Rifampicin, H: Isoniazid, Z: Pyrazinamide

Table 3

Bioavailability indices of ofloxacin in saliva and plasma

Group	*Bioavaiiability index*			
	C_{max}		AUC	
	Plasma	Saliva	Saliva	Plasma
O *Vs* OR	1.1	10	1.2	1.2
0 *Vs* OH	0.9	1.0	0.9	11
0 *Vs* OZ	1.0	0.9	1.0	1.0
0 *Vs* ORHZ	1.1	0.9	1.1	10

to the available anti-TB drugs with a MIC of 1.0 mg/ml against *Mycobacterium tuberculosis.* The effectiveness of ofloxacin against *Mycobacterium tuberculosis* was studied by Crowle *et al*[10] and their results reveal its clinical usefulness.

Using saliva instead of blood for pharmacokinetic investigations has obvious practical advantages. It is a painless, non-invasive procedure, hence suitable for the collection of multiple specimens. Variable results have been reported for the ratio of saliva to serum concentrations. Koizumi *et al*[11] reported a ratio of 1.0 between 2-8 h after administration of a 300 mg dose. Fujita *et al*[12] in their studies on patients with renal impairment had reported a ratio of 1:1 for saliva to serum concentrations. However, Warlich *et al,*[13] have reported a ratio of 0.6 and a close relation of levels in the saliva and serum (r= 0.99), which is consistent with our findings. The mean S/P ratio of ofloxacin was around 0.6 and this remained the same when ofloxacin was given in any combination. The S/P ratio is a possible measure of the amount of protein binding to the drug. There was a good correlation (0.94) with respect to drug concentrations.

The present study shows that several pharmacokinetic parameters can be calculated using salivary concentrations of ofloxacin as can be done with plasma. The estimation of ofloxacin levels in saliva seems to be useful in therapeutic drug monitoring and pharmacokinetic studies.

Based on the salivary levels, the present study compares the pharmacokinetic parameters of ofloxacin when given alone and in combination with other anti-TB drugs namely R, H and Z. The pharmacokinetic parameters such as C_{max}, t_{max}, AUC, and $t_{1/2}$ did not change when given in combination with other anti-TB drugs suggesting that the pharmacokinetics of ofloxacin do not get affected or altered when administered with the other anti-TBdrugs.

When ofloxacin was administered in combination with three other drugs, namely R, H and Z, there were variations in the pharmacokinetic parameters calculated based on the salivary estimates. This could be due to the small sample size in this group (n=4) and interindividual differences in the absorption and metabolism of the drug.

The microbiological assay (MBA) employed for the estimation of ofloxacin in the saliva and plasma is very simple, specific, sensitive and reproducible, and does not require sophisticated equipment. This method was evaluated in samples wherein drug levels were also measured by HPLC according to the method of Immanuel *et al*[14] A very good correlation (0.99) was observed suggesting either method may be useful for pharmacokinetic studies.

It can, therefore, be concluded that for bioavailability studies, invasive blood collection can be replaced by simple non-invasive saliva collection. Further investigations are required to determine whether the concentration of ofloxacin in saliva can act as a valid index after multiple dosing. In this regard, a larger population sampling is required before any general statements regarding the utility of salivary measurements of ofloxacin can be made.

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