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A COMPARATIVE BIOEQUIVALENCE STUDY OF SOME BRANDS OF OFLOXACIN BY URINE AND SALIVARY ANALYSIS IN INDIA

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ABSTRACT

This study was aimed to assess the bioequivalence of randomly selected brands of ofloxacin tablets marketed in India. Bioavailability assessment was conducted by measuring the concentration of drugs in the urine as well as saliva and bioavailability data was presented as cumulative quantity of drugs recovered in urine in 24 hours. Simple and sensitive, accurate and economical spectrophotometric method was developed for the estimation of ofloxacin in urine and saliva samples using phosphate buffer (pH 6.8) at 288 nm. Microbiological assay technique was used to analyze urine samples. The linearity was obtained in the concentration range of 2-20 µg/ml for ofloxacin. The two different brands of ofloxacin with the strength of 200mg and 400mg each showed same minimum inhibitory concentration value against the test strain of Staphylococcus aureus of 0.468 µg /ml and 1.388 µg /ml respectively. The salivary ofloxacin concentration ratio was highly dependent on sampling time. The salivary half-lives showed significant correlation with each other while the area under curve of ofloxacin concentration in saliva failed to show significant correlation. The two brands of ofloxacin 200mg(X_1-X_2), 400mg(X_3-X_4) each in punjab (India) exhibited same bioavailability data in vivo and can be said to be bioequivalents.

Keywords: *Antibiotics, Bioequivalence, India, Microbiological assay, ofloxacin*

INTRODUCTION

Generic substitution is defined as dispensing of product that is generically equivalent to the prescribed product with the same active ingredients in the same dosage form, and identical in strength, concentration, and route of administration [1]. Nevertheless, opponents of generic substitution argue that the quality of generic drugs might be inferior to that of brand name products [2]. It is therefore important to ensure that generics substitutes are bio-equivalent. This is particularly important for developing countries like

India where drug distribution and supply is known to be erratic. Factors that often necessitate the need for adequate bioequivalence studies include treatment failures, high cost of patented products,

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increase in resistance strains and sub-standardization. Bioequivalence studies will ensure that the characteristics of finished product conform to the appropriate standard specifications in terms of product identity, purity, potency, bioavailability, pharmacokinetics and therapeutic activity **[3].** Ofloxacin which is the focus of this study is a second generation fluoroquinolone with a 6-fluoro substituent and a 7-piperazinyl substituent on the quinolone ring structure [4]. It is a well known fact now that all the clinically useful quinolones bear a fluorine group at the C-6 position and such quinolones which are described as fluoroquinolones are produced by laboratory synthesis. They have excellent pharmacokinetic profile and attain appreciable concentrationswell above their MICs in biological tissues [5]. The assay procedure mentioned in USP, BP, IP pharmacopoeias uses non aqueous titration for estimation of ofloxacin. Literature surveys reveals Spectrophotometric method [6], atomic absorption spectrometry, spectroflurometry[7] , HPLC[8] and microbiological method[9] for its determination. This paper presents simple, accurate and reproducible spectrophotometric methods for simultaneous determination of ofloxacin in tablet dosage form as well as urine and saliva.

Therefore, two pharmaceutically equivalent drug products are considered to be bioequivalent when the rates and extents of bioavailability of the active ingredient in the two products are not significantly different under suitable test condition [10, 11]. There are in vitro and in vivo tests that can be utilized to estimate the bioequivalence of drug product, but only the in vivo tests are employed in this study. This study was aimed to assess the bioequivalence of randomly selected brands of ofloxacin tablets marketed in Punjab India.

MATERIAL AND METHOD

Material

Pure ofloxacin was obtained as a gift sample from Cipla Ltd. . The tablets (referred as X_1-X_4) of the said combination were purchased

from a local pharmacy The label claim for both X_1-X_2 and X_3-X_4 contained ofloxacin 200 mg and 400 mg respectively All the chemicals were used of analytical grade

All Absorbance measurements were made on Shimadzu model UV 1601 double beam UV-Visiblespectrophotometer with matched quartz cuvettes.

Instrument

Table 1: Latin square crossover design treatment for ofloxacin (200mgX_1-X_2) and ofloxacin 400mg(X_3-X_4) brands through urine analysis

Group	Subject	Week 1	Week 2	Week 3	Week 4
1	1	X1	X2	X3	X4
	2	X1	X2	X3	X4
2	3	X2	X3	X4	X1
	4	X2	X3	X4	X1
3	5	X3	X4	X2	X1
	6	X3	X4	X2	X1
4	7	X4	X3	X1	X2
	8	X4	X43	X1	X2

ESTIMATION OF DRUG IN URINE

Two commercial brands of ofloxacin 200 mg (X_1-X_2) and 400mg(X_3-X_4) and were obtained randomly from different pharmacies and patent medicines stores Punjab India. Eight healthy adult male volunteers between the ages of 21-25, weighing between 55 to 70 kg body-weight were selected for the study. Informed consent was obtained from all subjects and the project was approved by the Ethical Committee of our Institution.

Informed consent was obtained from each subject. Prior to initiation of the study the participants were subjected to thorough physical examination and their medical history taken. Basic tests like liver function test (LFT), urine analysis, full blood count (FBC) and blood sugar levels were conducted for each subject, to certify that they were medically fit for the study. The subjects were not permitted to take any drug two weeks prior to trials and during the trials. The experimental design employed to determine comparative bioavailability (bioequivalence) of the drug brands was the Latin square crossover design [12].Eight subjects were randomly assigned to four different groups (Two per group) ensuring that uniformity existed between groups with respect of age, body weight and sex. Each group received a particular treatment or brand the treatment were separated by a 7-day washout period and the design was balanced over weeks. The volunteers were fasted overnight prior to and 4 hours immediately after administration of a 200 mg and 400 mg ofloxacin tablets. No beverage such as coffee, milk or diet drink was permitted during the fasting period. Alcoholic drink was also restricted. Each of the tablet brands was administered with 150 ml of water. An additional 100 ml of water was given each hour for the first three hours after dosing. The subjects were ambulatory for the first twenty-four hours of treatment, and for the remaining 12 hours were permitted to proceed with their normal daily routine in so far as possible, but they were not permitted to engage in any strenuous or athletic activities during the period of the study. Total urine voids were collected at intervals of 0-1,1-2, 2-3, 3- 6, 6-9, 9-12, 12-16, 16-20, 20-24 hours post administration. The total volume of each interval was recorded and a 10 ml sample each was frozen until assay. Spectrophotometric method was employed to determine the

cumulative quantities excreted in urine and the mean excreted amount of drug was computed. The microbiological assay technique was used for the analysis of urine samples. Molten Mueller-Hinton agar (MHA at 56^{O}C) was seeded with a standardized inoculum 0.5 MacFarland standard of a clinical strain of Staphyloccus aureus [13] was allowed to solidify. Thereafter, 5 mm holes were bored on each MHA using a sterile cork borer. Various concentrations of a standard solution of ofloxacin 200mg and ofloxacin 400mg and the various urine samples obtained from each subject at different intervals were randomly introduced into different holes (40 µl per hole). After allowing for 30 minutes pre diffusion at room temperature, the plates were then incubated at 37^{O}C for 24 hours. The inhibition zone diameters (IZDs) [14] were measured. The IZDs of the standard was used to construct a dose-response plot from which the concentration of ofloxacin in each urine sample was calculated by fitting their respective IZDs into a regression equation derived from the standard dose-response plot. Statistical analysis was carried out using SPSS for Windows (version 14; SPSS, Chicago, IL). Data were summarized as mean ± SD. Group comparison was conducted using ANOVA. Sub-group analysis was carried out using the Post hoc test, LSD. A two-tailed significance level of 0.05 was used.

Table 2: Minimum Inhibitory Concentration (MIC) and Cumulative quantity of drugs recovered in urine in 24 hours

Brands	Mean cumulative quantity excreted In 24hrs (mg)	Mean Recovery (%)	Mean maximum excretion rate(mg/hr)	MIC(µg/ml)
X1	22.80 ± 0.74	11.48	3.14 ± 0.73	0.468
X2	21.52 ± 0.55	11.47	2.76 ± 0.20	0.468
X3	27.83 ± 0.50	20.40	2.03 ± 0.36	1.388
X4	26.64 ± 0.75	19.41	1.82 ± 0.18	1.388

ESTIMATION OF DRUG IN SALIVA

Following overnight fast one tablet of two different brands containing 200mg and 400mg of ofloxacin was given orally along with 150 ml of water. The mouth was rinsed promptly with another 100 ml of water which was also swallowed. Food was with held for a further period of 2 h to ensure complete absorption of the drug. Saliva samples were collected simultaneously at 0, 15, 30, 45, 60, 90, 120, 150, 180 and 240 min. The salivary sample was collected by placing citric acid (about 10 mg) over the tongue and held in the mouth for one to two minutes after which the contents were spit into a centrifuge tube. (Preliminary experiments showed that citric acid did not interfere with the estimation). Samples were centrifuged to remove mucous and particulate matter from saliva. The salivary supernatants samples were stored at -20°C until analysed. All analyses were done on

the following day. The ofloxacin concentration in saliva was estimated by a modified method of Miceli et al. (1979) [15]. For the determination of ofloxacin concentration in saliva: 1 ml of 20% trichloracetic acid was added drop by drop to 1 ml saliva with constant shaking. It was then centrifuged at 4000 rpm for 5 min followed by 2000 rpm for 3 min. The supernatant (1.75 ml) was transferred to a screw-capped 10 ml test-tube followed by dilution with phosphate buffer (pH 6.8). The remaining procedure salivary estimation was the same as that of Miceli et al. (1979). With the above modification concentration up to 1µg/ml could be detected in saliva. The salivary concentrations of ofloxacin were plotted against time on a semi logarithmic graph paper and the elimination half life ($t_{1/2}$%) was calculated. The area under the curve (AUC) from 0 to 6 h was calculated by trapezoidal rule [15]. The statistical analysis was done using Student's t-test.

Table: 3 Mean and individual values of ofloxacin half lives and area under curves calculated from saliva concentrations

Subjects	Half-life(min) Saliva				Area under curve (0-6 h)(µg ml^{-1}'h) Saliva			
	$X_{1(SA_1)}$	**$X_{2(SA_2)}$**	**$X_{3(SA_3)}$**	**$X_{4(SA_4)}$**	**$X_{1(SA_1)}$**	**$X_{2(SA_2)}$**	**$X_{3(SA_3)}$**	**$X_{4(SA_4)}$**
1	240	243	237	251	31.6	29.4	34.3	33.2
2	234	232	233	254	33.4	32.1	36.3	31.2
3	245	223	229	221	29.6	31.2	30.2	31.3
4	210	245	245	223	23.4	34.3	33.2	32.3
5	232	248	267	256	32.1	33.8	31.2	29.3
6	237	234	251	229	33.2	35.4	29.8	35.8

7	243	256	253	228	31.2	30.6	33.4	38.3
8	246	267	267	243	33.2	32.2	25.4	28.3
Mean	235.8	243.5	247.75	238.125	30.96	32.75	31.72	32.46
+ s.e. mean	±5.0	±6.1	±6.2	±7.9	±3.2	±4.3	±4.2	±4.3
Correlation coefficient (r)	±0.64				±0.52			
P Value	<0.05				>0.05			

(SA_1), OFLOXACIN 200mg, (cipla) (SA_2), OFLOXACIN 200mg (alkem)

(SA_3) OFLOXACIN 400mg(cipla (SA_4) OFLOXACIN 400mg (alkem)

RESULTS

There were many brands of ofloxacin tablets in circulation in the country. They were of varying shapes, sizes and colours. Ofloxacin prize (200mg) range varies from ₹144.50 to 150 per pack of 10 tablets while Ofloxacin prize 400mg varies from ₹ 150.60 to 160. Cumulative quantity excreted in urine was directly related to the total amount of drug absorbed and indicates the extent of absorption. The percentage recovery indicated that brand X_3 was the brand with the highest recovery within 24 hrs of 20.40%, while the least was X_2 with 11.47%. The two brands of ofloxacin 200mg and the two brands of ofloxacin 400mg showed the same minimum inhibitory concentration (MIC) value against the test strain of Staphylococcus aureus of 0.468µg/ml and 1.388 µg /ml respectively. The details of these results are shown in Table 2.

Analysis of ofloxacin concentration in 160 paired samples of saliva collected at different time intervals showed significant correlation ($r = 0.64$, $P<0.05$). Salivary

ofloxacin levels; also the salivary concentrations were higher ofloxacin 400mg than in ofloxacin 200mg. In our study, the SA^1/SA^3 & SA^2/SA^4 ratio showed wide inter individual and intra individual variation. The intraindividual difference in ratio SA^1/SA^3 & SA^2/SA^4 was highly dependent on sampling time. The SA^1/SA^3 & SA^2/SA^4 ratio was higher in 30, 45 and 60 min samples. The SA^1/SA^3 & SA^2/SA^4 ratio of 30 min samples was found to be significantly higher ($P<0.05$) than that of 180 and 240 min samples. During the elimination phase the SA^1/SA^3 & SA^2/SA^4 ratio within individual subjects remained constant. Such a phenomenon was also observed in bioavailability studies of theophylline [16, 17]. We could not find any significant difference between the mean urine and salivary ofloxacin half lives (Table 3). Furthermore, the presence of a significant correlation between the two ($r = 0.64$, $P < 0.05$) suggests the reliability of using salivary ofloxacin concentrations for the calculation of its elimination half-life. On the other hand, although the mean AUC values of ofloxacin calculated from and salivary concentrations were not significantly different (Table 3) they showed poor correlation with each other ($r = 0.52$, $P>0.05$) which may be due to intraindividual variation in SA^1/SA^3 & SA^2/SA^4 ratio. This finding is in contrast to those of (Glynn & Bastain 1973) [18] who observed a significant correlation between the AUC values of salivary ofloxacin (different brands 200mg, 400mg) concentration.

DISCUSSION

Our study describes bioequivalence study of some brands by a simple spectrophotometric method for the estimation of ofloxacin salivary and urine levels which may be of practical value in the determination of its elimination kinetics and also in the identification and estimation

of ofloxacin concentrations in patients suffering from overdosage. It is probably not possible to derive other reliable pharmacokinetic parameters based upon salivary and urine data obtained from single dose studies**.** Oral ofloxacin are widely used in India with several new brands introduced into the Indian market in recent times. Variety of drugs in circulation often put clinicians and pharmacists into difficult situation of choice, and the possibility of interchangeability among brands. The question remains unsolved whether these brands can be substituted with each other especially considering that Indian drug market are filled with substandard products. This formed the major intent of our study which tried to establish the bioequivalence of ofloxacin marketed in India. Percentage cumulative quantity of drug recovered in urine after 36 hours for the two different brands of ofloxacin (200mg), ofloxacin 400mg were not significantly different. However, percentage cumulative quantity of drugs obtained in our result is less than what has been reported in literature. It has been reported that the recovery of a median range cumulative quantity of ofloxacin 84.3% (46.5% - 92.5%) in 144 hours [19]. The low quantity recovered in this work may be due to the shorter time of observation. The two different brands of ofloxacin (200mg) and the two brand of ofloxacin (400mg) each compared in our study had the same minimum inhibitory concentration (MIC). This is an indication that the brands were all very potent. Our results showed a higher MIC compared to another study which reported MIC range of 0.25µg/ml to 32 µg/ml for ofloxacin all against Staphylococcus aureus [20]. However currently published bioavailability studies conducted in india show some marked improvement in terms of bioequivalence of the same brands of

drugs available in the drug market. For example, a recently published study showed that five of the seven brands of metronidazole were physically and chemically equivalent and could be interchanged irrespective of the brands, while two could not [21]. This might be as a result of the intense campaign that has been carried out by National Agency for Food &Drug Administration in the country to sanitize the drug market. However, there is need for constant monitoring of new brands of drugs introduced into the drug market to ascertain bioequivalence and conformity with set standards.

CONCLUSION

The various brands of ofloxacin (X_1 X_4) in India exhibited same bioavailability data in vivo and can be said to be bioequivalents. This indicates that a low price product does not necessarily imply poor quality, and the brands can be prescribed interchangeably. This is an important pharmacoeconomics and essential drug principles. Also the mean cumulative quantity excreted in urine for the various brands and the maximum excretion rates do not show any statistical significant difference. The various brands of both ofloxacin(200mg,400mg) each exhibited maximum excretion rate at 6 hours post administration. Therefore, the various ofloxacin brands can be said to be bioequivalents.

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