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Pharmacokinetic Parameters of Ofloxacin are Altered by Female Harmones

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ABSTRACT

The level of female Hormones is phase specific and the Pharmacokinetic parameters of drugs are altered by the cyclic changes in menstrual cycle. The luteal phase has high levels of progesterone levels and follicular phase has higher circulating α-1 acid glycoprotein which alters absorption and distribution respectively. The objective of the present research work is to study the influence of menstrual cycle (i.e., follicular phase, ovulatory phase and luteal phase) on pharmacokinetic parameters of most commonly prescribed antibiotic, Ofloxacin. Salivary levels of Ofloxacin in healthy volunteers were estimated during three phases of menstrual cycle for the evaluation of various pharmacokinetic parameters. 20 female healthy volunteers of 20 to 50 years age with regular menstrual cycle, were participated in the study, received 400 mg of Ofloxacin tablet at 9 am along with a glass of water 1 hr after breakfast on 3rd, 13th and 23rd day of menstrual cycle. Salivary samples (2ml) were obtained at 1, 2, 4, 6, 8, 12 & 24 hrs after dosing, were estimated for drug concentration by HPLC method. All the results were expressed as Mean ± S.D, data was analyzed using one way ANOVA, followed by Newman-Keuls multiple comparision test. A value of $P < 0.05$ was considered to be statistically significant. The mean salivary concentrations of Ofloxacin were decreased in ovulatory and luteal phases than follicular phase due to which bacteria may develop resistance in several infections and the diseases may not cured in these phases of menstrual cycle and may require dosage adjustments.

Key words: Pharmacokinetics, Ofloxacin, Female Harmones, Menstrual cycle.

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INTRODUCTION

Ofloxacin is a synthetic broad-spectrum antimicrobial agent and is the first generation flouroquinolone, its activity is comparable to or more potent than Ciprofloxacin for gram positive bacteria. Ofloxacin and other fluoroquinolones are widely used in veterinary and human medicine because of their broad spectrum activity against gram negative and gram positive bacteriae, aerobes, rickettsia, mycoplasma and against multi-drug resistant microorganisms[1]. It showed bactericidal activity against Mycobacterium tuberculosis in *invitro* studies[2]. It has good antibacterial activity at considerably low plasma/tissue concentrations. Monitoring Ofloxacin concentrations in body fluids is valuable to adjust the drug dosage to achieve minimum inhibitory concentration and protection from side effects.

The level of female hormones is phase specific and the pharmacokinetic parameters of drugs are altered by the cyclic changes in menstrual cycle i.e., luteal phase has high levels of progesterone which relaxes gastrointestinal smooth muscle, alters gastrointestinal transit time and drug absorption. Follicular phase has higher circulating levels of α-1 acid glycoprotein increases protein binding and decreases free drug concentration. Gender exerts influence on the clearance of some drugs by regulating cytochrome P450 isoenzymes. Estrogens inhibit the metabolism of many drugs by inhibiting liver microsomal enzymes, whereas progesterone has both inhibitory and inductive effect. Drugs metabolised by P450 CYP3A4 are extensively cleared in women, whereas drugs metabolised by other systems are usually cleared faster by men.

Ofloxacin has good activity against Chlamydia, Mycobacterium tuberculosis and Mycobacterium leprae and indicated for the treatment of mild to moderate infections caused by Streptococcus pneumonia, Haemophilus influenza, Staphylococcus aureus, Streptococcus pyogenes, Neisseria gonorrhea etc.

Antibiotics are second most commonly prescribed drugs indicating the incidence of microbial infection. Drug concentration modulation below or above minimum effective concentration and maximum tolerable concentration lead to resistance to antibiotics, development of resistant strains of microorganisms, therapeutic failure and reverse may cause antibiotic induced adverse effects.

Saliva is used for the monitoring of systemic levels of drugs, as it offers distinctive advantages over serum [3, 4]. It is a readily available specimen, which is collected by non-invasive procedures and is helpful when multiple serial samples are needed, provides a cost-effective approach for the screening of large populations The concentration of most drugs in saliva corresponds to the free or unbound plasma drug concentrations [5-8]. A good correlation is observed between serum and salivary levels of Ciprofloxacin and Ofloxacin [9].

In our study, salivary levels of Ofloxacin in healthy volunteers were estimated during three phases of menstrual cycle for the evaluation of pharmacokinetic parameters.

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MATERIALS AND METHODS

Protocol

20 female healthy volunteers with their weights ranging from 45 to 60 kgs, height between 140 to 161 cms and age 20 to 50 years participated in the study. Institutional Ethical Committee approved the study protocol. Female volunteers healthy as per physical examination with regular menstrual cycle, not using any other medication and non allergic to drugs were included in the study after obtaining written informed consent. Volunteers with a history of cardiac, pulmonary, hepatic, renal, haematologic or endocrinologic disorders or having irregular menstrual cycles, suffering from amenorrhea or women using oral contraceptive pills were excluded from the study.

Study design

20 female subjects who compiled inclusion criteria received 400 mg of Ofloxacin in the form of tablet at 9 am along with a glass of water 1 hr after breakfast on 3^{rd}, 13^{th} and 23^{rd} day of menstrual cycle. Salivary samples (2ml) were obtained at 1, 2, 4, 6, 8, 12 & 24 hrs after dosing along with a blank sample before administering the drug. Samples were collected after cleaning the tongue debris and mouth every time before sampling. The pH of the salivary samples were measured and stored at - 80°C until further analysis was done.

Assay

Ofloxacin in the biological samples was estimated by HPLC method [10]. To 1 ml of saliva in a 10 ml glass stoppered tube, 100ul of internal standard solution (10 ug/ml of Ciprofloxacin) was added. The tubes were vertexed for 30 seconds, then 0.5 ml of zinc sulphate solution (0.7M) and 0.1 ml of 1N sodium hydroxide solution was added. After mixing for 1minute and centrifuging at 2000 rpm for 10 minutes, supernatent was filtered by 0.45 um sample filter (Millipore filter). A volume of 25 ul of filtered supernatent was injected into HPLC column.

Chromatographic conditions

Mobile phase consisting of methanol, 0.03M potassium dihydrogen phosphate (30:70v/v) was prepared and mixed thoroughly, degassed and used for the HPLC analysis. 1.0ml per minute flow rate was maintained throughout the analysis. The eluent was monitored using a UV-VIS detector set at 294 nm and sensitivity was set at 0.001 a.u.f.s.

Preparation of standard graph

Standard solutions

Stock solutions of 50 µg/ml each of Ofloxacin and Ciprofloxacin were prepared in methanol. Working standard solutions were prepared daily by diluting the stock solution with 1

% hydrochloric acid solution. All solutions were stable for at least four weeks when stored at -15ºC. For the preparation of standard graph 0.1, 0.5, 1, 5, 10, 50 and 100 µg/ml were used.

To 1 ml human saliva in a glass screw-capped tube 100 µl of Ofloxacin working standard solution (0.1, 1, 5, 10, 25 & 50 µg of the drug) and 100 µl of internal standard solution (10 µg/ml of Ciprofloxacin) were added. The tubes were vertexed for 30 seconds and then they were treated as described in the assay procedure. The retention times were 5.0 min. and 6.2 min. for Ofloxacin and Ciprofloxacin respectively. The peak area ratios obtained at different concentrations of the drug were plotted against the concentrations of the drug. The slope of this plot was determined by least squares regression analysis and was used to calculate Ofloxacin concentrations in unknown saliva samples. Then various pharmacokinetic parameters of Ofloxacin were obtained using RAMKIN software.

Mean C_{max} and Tmax values were obtained directly from concentrations and time data. The various pharmacokinetic parameters like absorption rate constant (Ka), mean residence time (MRT), elimination half-life (T1/2), area under the concentration time curve (AUC), area under the first moment curve (AUMC), apparent volume of distribution for fraction of the dose absorbed (Vd/f), volume of distribution at steady state (Vss/f) and systemic clearance for fraction of the dose absorbed (Cls/f) for Ofloxacin were obtained in each volunteer from saliva concentration versus time data.

Statistical analysis

All the results were expressed as mean ± S.D, data was analyzed using one way ANOVA, followed by Newman-Keuls multiple comparision test. A value of
$P < 0.05$ was considered to be statistically significant.

Analysis of blank blood samples for hormones

The blank blood samples were collected from volunteers before administration of the drug (0h) and analyzed for concentrations of estrogen and progesterone hormones by the Chemiluminisence method.

RESULTS

None of the volunteers complained of any side effects following 400mg of Ofloxacin oral administration in any of the three (follicular, ovulatory and luteal) phases of the menstrual cycle. The mean salivary Ofloxacin levels versus time in three phases were shown in Fig.1, the salivary levels were higher following its administration in the follicular phase compared to ovulatory and luteal phases and its levels in the ovulatory phase were in between the levels of follicular and luteal phase. Mean concentrations of estrogen and progesterone in three phases of menstrual cycle were summarized in Table 1. Mean values of various pharmacokinetic parameters of Ofloxacin obtained in three phases (Table 2) were compared with the values obtained in other two phases and for the calculation of percentage increase or decrease, follicular phase was treated as reference (Table 3). The mean Cmax levels of salivary Ofloxacin

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were 2.57±1.37, 1.88 ± 0.86 and 1.74 ± 0.75 ug/ml for the follicular, ovulatory and luteal phases respectively. As compared with follicular phase, the mean Cmax value was decreased by 26.84% ($P < 0.05$) in the ovulatory phase and 32.3% ($P < 0.05$) in luteal phase (Table 3). The mean $t_{1/2}$ levels of salivary Ofloxacin were 11.29 ± 4.56, 12.90 ± 5.16 and 16.46 ±7.14 hr for the follicular, ovulatory and luteal phases respectively. The mean $t_{1/2}$ was increased by 14.26% in ovulatory phase and 45.8% ($P < 0.05$) in luteal phase compared to follicular phase.

Table 1: Mean levels of hormones in healthy volunteers in three phases of Menstrual cycle (n=20) in Ofloxacin study

Hormone	Follicular phase	Ovulatory phase	Luteal phase
Estrogen (pg/ml)	58.7 ± 38.5	180.56 ± 140.45	111.45 ± 98.34
Progesterone(ng/ml)	4.2 ± 2.0	7.7 ± 4.2	14.9 ± 8.2

ng – nanograms; pg – pictograms

Table 2: Pharmacokinetic parameters of Ofloxacin during three phases (n=20)

Pharmacokinetic parameter	Follicular phase Mean ± SD	Ovulatory phase Mean ± SD	Luteal phase Mean ± SD	Statistical Significance	p-value
C_{max} (µg/ml)	2.57 ± 1.37	1.88 ± 0.86	1.74 ± 0.75	LvsF < 0.05* OvsF < 0.05*	0.0306
T_{max} (hrs)	2.3 ± 1.08	1.9 ± 1.20	2.25 ± 0.97	NS	0.4546
AUC_{0-t} (µg/ml/hr)	16.20 ± 4.81	16.91 ± 4.23	16.37 ± 7.13	NS	0.9143
$AUC_{0-\infty}$ (µg/ml/hr)	21.6 ± 7.19	23.32 ± 5.57	26.19 ±12.24	NS	0.2575
$AUMC_{0-\infty}$ (µg/ml/hxh)	385.64 ± 224.86	461.33 ±195.28	706.83 ± 449.84	FvsL< 0.01** OvsL < 0.05*	0.005
$t_{1/2}$(hrs)	11.29 ± 4.56	12.90 ± 5.16	16.46 ± 7.14	FvsL < 0.05 *	0.0185
$V_{d/f}$ (ml/kg)	6093.94 ± 2414.73	6403.72 ±2940.19	8236.31 ± 4679.33	NS	0.1188
$V_{ss/f}$ (ml/kg)	6234.50 ± 2662.37	6968.13 ± 2893.11	8916.32 ± 4819.20	NS	0.0588
$CL_{s/f}$(ml/hr/kg)	396.32 ± 133.13	348.39 ± 76.28	363.07 ± 169.15	NS	0.5038
MRT(hr)	17.15 ±7.34	20.28 ± 7.14	25.94 ±10.73	FvsL< 0.01** OvsL< 0.05*	0.0070
K_a (h^{-1})	0.152 ± 0.06	0.142 ± 0.054	0.116 ± 0.055	NS	0.1181

Values are expressed as Mean ± SD,*P<0.05 is considered as statistically significant

Abbr: AUC - Area under the curve; AUC_{0-t}- Area under the curve from 0 hour to time point t; $V_{d/f}$ - Apparent volume of distribution for fraction of drug absorbed; MRT – Mean residence time; $AUC_{0-\infty}$ - Area under the curve from 0 hour to infinity time; C_{max} - Peak drug concentration; $t_{1/2}$ - Biological half life or elimination half life; BMI - Body mass Index; T_{max} – Time of peak concentration; CL –Clearance; $V_{ss/f}$ - Steady state volume of distribution for fraction of drug absorbed; AUMC - Area under the first moment curve; K_a -Absorption rate constant; $CL_{s/f}$-Systemic clearance for fraction of drug absorbed; CL_{total}-Total clearance; NS- not significant

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The mean $AUMC_{0-\infty}$ levels of salivary Ofloxacin were 385.64 ± 224.86, 461.33 ± 195.28 and 706.83 ± 449.84 µg/ml/hrxhr for the follicular, ovulatory and luteal phases respectively. The mean $AUMC_{0-\infty}$ value was increased by 19.62% in ovulatory phase and 83.28% in luteal phase compared to follicular phase. The difference between mean $AUMC0_{-\infty}$ values for follicular versus luteal phase ($P < 0.01$) and ovulatory versus luteal phase ($P < 0.05$) were statistically significant.

Table 3: Changes in pharmacokinetic parameters of Ofloxacin in different phases of menstrual cycle

Pharmacokinetic parameter	Follicular phase	Ovulatory phase	Luteal phase	Statistical Significance	p-value
C_{max} (µg/ml)	2.57 Reference	1.88 26.84 % ↓	1.74 32.3% ↓	LvsF < 0.05* OvsF < 0.05*	0.0306
T_{max} (hrs)	2.3 Reference	1.9 17.39% ↓	2.25 2.17 % ↓	NS	0.4546
AUC_{0-t} (µg/ml/hr)	16.20 Reference	16.91 4.38 %↑	16.37 1.05 % ↑	NS	0.9143
$AUC_{0-\infty}$ (µg/ml/hr)	21.6 Reference	23.32 7.96 % ↑	26.19 21.25 % ↑	NS	0.2575
$AUMC_{0-\infty}$ (µg/ml/hxh)	385.64 Reference	461.33 19.62% ↑	706.83 83.28% ↑	FvsL< 0.01** OvsL < 0.05*	0.005
$t_{1/2}$ (hrs)	11.29 Reference	12.90 14.26 % ↑	16.46 45.8 %↑	FvsL < 0.05 *	0.0185
$V_{d/f}$ (ml/kg)	6093.94 Reference	6403.72 5.08 % ↑	8236.31 35.15 % ↑	NS	0.1188
$V_{ss/f}$ (ml/kg)	6234.50 Reference	6968.13 11.76 %↑	8916.32 43.01 % ↑	NS	0.0588
$CL_{s/f}$ (ml/hr/kg)	396.32 Reference	348.39 12.09 % ↓	363.07 8.39 % ↓	NS	0.5038
MRT (hr)	17.15 Reference	20.28 18.25%↑	25.94 51.25% ↑	FvsL< 0.01** OvsL< 0.05*	0.0070
Ka (h^{-1})	0.152 Reference	0.142 6.57% ↓	0.116 23.68 % ↓	NS	0.1181

F = Follicular phase; O = Ovulatory phase; L = Luteal phase

Values are expressed as Mean ± SD,*P<0.05 is considered as statistically significant

Abbr: AUC - Area under the curve; AUC_{0-t}- Area under the curve from 0 hour to time point t; $V_{d/f}$ - Apparent volume of distribution for fraction of drug absorbed; MRT – Mean residence time; $AUC_{0-\infty}$ - Area under the curve from 0 hour to infinity time; C_{max} - Peak drug concentration; $t_{1/2}$ - Biological half life or elimination half life; BMI - Body mass Index; T_{max} - Time of peak concentration; CL –Clearance; $V_{ss/f}$ - Steady state volume of distribution for fraction of drug absorbed; AUMC - Area under the first moment curve; Ka -Absorption rate constant; $CL_{s/f}$-Systemic clearance for fraction of drug absorbed; CL_{total}-Total clearance; NS- not significant.

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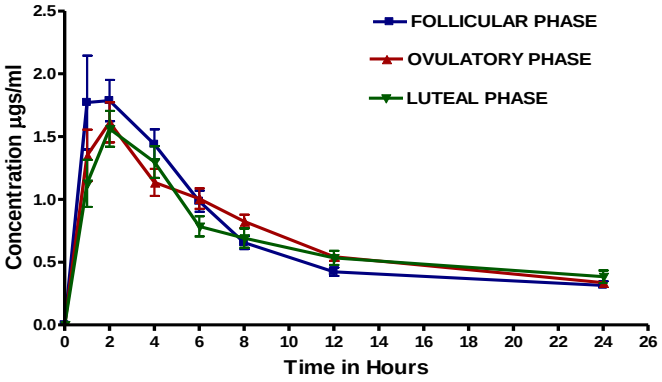

Fig 1. Mean salivary concentration versus time profile of Ofloxacin during three phases of menstrual cycle

The mean MRT values were 17.15 ± 7.34, 20.28 ± 7.14 and 25.94 ± 10.73 hr for the follicular, ovulatory and luteal phases respectively. As compared with follicular phase, the mean MRT value was increased by 18.25% in the ovulatory phase and 51.25% in luteal phase. The difference between mean MRT for follicular versus luteal phases ($P < 0.01$) and ovulatory versus luteal phases ($P < 0.05$) was statistically significant.

DISCUSSION

Variations in Hormonal levels and consequent changes in the menstrual cycle modifies the pharmacokinetics of drugs either through difference in gastric acidity, gastro intestinal motility and plasma protein levels which alter drug absorption, drug-binding and elimination.

Hormones alter gastrointestinal motility frequently during the luteal phase of the menstrual cycle and is well documented [11]. Relatively high levels of progesterone during luteal phase promote smooth muscle relaxation [12]. Absorption of drugs from the small intestine increases during this phase because of prolonged gastrointestinal transit time [13]. In this study, though non significant, $AUC_{0-\infty}$ of Ofloxacin i.e, bioavailability was increased in luteal phase compared to follicular and ovulatory phases due to increased gastro intestinal transit time induced by high levels of progesterone.

The Cmax of Paracetamol was significantly lowered in the ovulatory phase than in the follicular phase, AUC_{0-t} and $AUC_{0-\infty}$ values were significantly lowered in the ovulatory phase than those in the luteal phase. These changes were due to increased first-pass metabolism and decreased bioavailability during ovulatory phase [14]. In our study, mean Cmax of Ofloxacin was significantly decreased in ovulatory phase than in follicular phase and $AUC_{0-\infty}$ was lowered in ovulatory phase than in luteal phase.

Higher circulating levels of α-1 acid glycoprotein and decrease in free drug concentration was reported during the follicular phase of the menstrual cycle [15]. Volume of distribution of Ofloxacin in follicular phase was decreased than other two phases might be due to high protein binding in the present study. In the previous investigation Amikacin study, 15%

and 35% higher total clearance and volume of distribution was observed in mid luteal phase compared to mid-follicular phase [16]. In our study, 35% higher volume of distribution of Ofloxacin was observed in mid-luteal phase than in mid-follicular phase as progesterone retains body water due to increased synthesis of aldosterone [17].

Estrogens inhibit the metabolism of many drugs by inhibiting liver microsomal enzymes[18]. In our study, Ofloxacin clearance was decreased about 12% in ovulatory phase compared to follicular phase could be due to decreased metabolism by enzyme inhibition with high levels of estrogen in this phase. The metabolism of Theophylline and consequently its clearance was increased in healthy women around the onset of menses [19]. Ofloxacin clearance was increased and elimination half-life was decreased in follicular phase as compared to other two phases in our study.

Caffeine elimination was slowed in the luteal phase than in the follicular phase due to higher levels of estrogen and progesterone [20]. Similarly Ofloxacin clearance was slowed about 8% in luteal phase compared to follicular phase. The tendency of lower clearance of aminopyrine was observed in the luteal phase of the menstrual cycle (where progesterone levels are highest) as progesterone inhibits microsomal function [21]. Clearance of Ofloxacin was lower in luteal phase compared to follicular phase in our study.

No significant difference in the half-life, clearance or volume of distribution was observed for Nitrazepam in different phases of the menstrual cycle [22]. In contrast to this study, Cmax, $t_{1/2}$, MRT and $AUMC_{0-\infty}$ of Ofloxacin were significantly different and other parameters such as Tmax,Vd/f, Vss/f, $AUC_{0-\infty}$,CLs/f and Ka were not significantly different across the menstrual cycle phases in the present study.

Menstrual cycle variations occur in the renal, cardiovascular, hematological and immune systems [23, 24] and potentially impact the pharmacokinetics of medications by systemic oscillations, such as protein binding or the volume of distribution, thereby results in significant differences at various times of the menstrual cycle. Hormonal changes within the cycle influence drug absorption, distribution, metabolism and/or excretion[25].

Vd, $t_{1/2}$ and clearance of Ranitidine were increased, where as Cmax and AUC were decreased in luteal phase compared to follicular phase of menstrual cycle[26]. However, systemic investigations of the impact of physiological variations on medications in the menstrual cycle are limited. In our study,Vd and $t_{1/2}$ of Ofloxacin were increased in luteal phase compared to follicular phase and Cmax was decreased in luteal phase compared to follicular phase.

CONCLUSION

From the above observations, it can be concluded that, the mean salivary concentrations of Ofloxacin were decreased in ovulatory and luteal phases than follicular phase

due to which bacteria may develop resistance in several infections and the diseases may not cured in these phases of menstrual cycle and may require dosage adjustments.

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