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EFFECT OF OFLOXACIN ON TIZANIDINE PHARMACOKINETICS IN RATS

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ABSTRACT

Objective of this work was to study the effect of ofloxacin on bioavailability and other pharmacokinetic parameters of tizanidine in rats. A single dose parallel design was used with 36 animals randomly divided in reference group and test group. All the rats received 7 mg tizanidine orally and in test group 200 mg ofloxacin was co-administered with tizanidine. Nine blood samples were collected from each animal over a 24-hour period. Plasma tizanidine concentrations were determined by HPTLC using UV detection, and pharmacokinetic parameters were determined by non-compartmental method. The mean value of the peak plasma concentration (C_{max}) of tizanidine decreased significantly (8.47%, P value <0.001; 90% CI, 91.32% -91.72%) in animals who had given the drug with ofloxacin (C_{max} , 31.54 ± 0.16 µg/mL) than those who had given the drug with water (C_{max}, 34.46 ± 0.07 µg/mL). The area under the plasma concentration time curve from t=0 to time of the last measureable concentration (AUC_{0-t}) was also increased significantly (17.17%, P value <0.001; 90% CI, 116.99% -117.34%). Similarly, the value of area under the concentration-time curve from t=0 to infinity ($AUC_{0-\infty}$) value was increased significantly (5.24% %, P value <0.001; 90% CI, 103.77% -104.83%); these changes were not within the 90% CI range of 80.000 - 125.000 % which is the acceptable range of bioequivalence. T_{max}, $T_{1/2}$, terminal elimination rate constant (λ_z), CL/F value, V_d/F value, $AUMC_{0-t}$ and $AUMC_{0-\infty}$ values, MRT_{0-t} and $MRT_{0-\infty}$ values and % relative bioavailability (Fr) value for test group were also determined and compared with reference group. Form results the values of C_{max} and $AUC_{0-\infty}$ were not within the bioequivalence acceptable range and from statistical analysis the reference and test samples were found to be bio-in-equivalent, suggesting the improved tizanidine oral bioavailability and therapeutic efficacy due to co-administration of ofloxacin.

Keywords: Ofloxacin, Tizanidine, Pharmacokinetics, Drug-drug interaction.

INTRODUCTION

Drugs can interact to alter the ADME of a drug, or interact in a synergistic or antagonistic fashion altering their pharmacodynamics. Generally, the outcome of an interaction can be harmful, beneficial or clinically insignificant. It is a relatively current practice for prescribers to use known interactions to enhance efficacy in the treatment of several conditions such as epilepsy, hypertension or cancer [1]. An example illustrating beneficial effects rather than ADRs, involves the co-administration of carbidopa, together with levodopa to prevent its peripheral degradation to dopamine [2].

A drug interaction is a measurable modification in magnitude or duration of the pharmacological response of one drug, due to the presence of another drug that is pre- or co-administered [3]. Usually, this modification of the action of one drug by another is a result of one or more of four principal mechanisms: a) pharmaceutical, b) pharmacodynamic, c) pharmacokinetic, and d) metabolic [4].

Tizanidine may be susceptible to *in vivo* interactions with inhibitors or inducers of CYP1A2 enzymes [5]. This study aimed to determine the effect of ofloxacin co-administration on pharmacokinetics of tizanidine in rats.

MATERIALS AND METHODS

Materials: Tizanidine HCl was obtained as a gift sample from Blue Cross Pvt. Ltd. Nashik, India. Ofloxacin was obtained as a gift sample from Catapharma Chemicals Pvt Ltd. Nashik, India. AR grade toluene, methanol and acetone were obtained from Merck Chemicals, India and ammonia liquor was obtained from Qualigens Fine Chemicals, India.

Subjects: Wistar rats of either sex weighing between 200-250 g purchased from Bharat Serums & Vaccines Ltd., Thane were used. Institutional Animal Ethics Committee (IAEC) approved the protocol (protocol no. MGV/PC/XXV/02/2010-2011); animals were maintained under standard conditions in an animal house (M.G.V.'s Pharmacy College, Panchavati, Nashik-03.) approved by Committee for the Purpose of Control, and Supervision on Experiments on animals (CPCSEA).

Study design: A total of 36 healthy wistar rats of either sex were selected for studies. For noncompartmental pharmacokinetic analysis single-dose parallel study design was selected. Study involves two groups reference group and test group. Both groups receive 7 mg tizanidine orally and in test group ofloxacin (200 mg) was co-administered with tizanidine. Animals were fasted from 12 hr before the commencement of study and through the complete study but allowed to drink water.

Blood sampling: Animals were anesthetized and blood samples were drawn from retro-orbital plexus of rats using fine bored glass capillary. Blood samples were drawn (1.5 mL) ½ hr before the oral drug administration and ½, 1, 2, 3, 4, 8, 12, 24 hr after the drug administration. After each withdrawal, animals were replenished with same amount of dextrose normal saline. Blood samples were collected into micro-centrifuge tubes containing EDTA. Blood samples were centrifuged at 5000 rpm for 15 min and plasma was separated within 30 min after blood sampling and stored at -20^{0}C till the time of analysis.

Extraction procedure: Liquid phase extraction procedure was used for extraction of tizanidine from plasma samples for HPTLC analysis. To a fix quantity of plasma (0.5 mL), 100 µL acetonitrile was added; the mixture was shaken and allowed to stand for 5 min. Free drug was extracted with methanol by vortexing followed by centrifugation at 1000 rpm for 10 min. Organic layer was separated and evaporated carefully on water bath. After cooling residues were reconstituted with 1 mL methanol and these were used as sample solutions for further analysis [6].

Determination of plasma drug concentration: Plasma tizanidine concentrations were quantified by HPTLC system. The samples were spotted in the form of bands of width 6 mm with a Camag microlitre syringe on pre-coated silica gel aluminium plate 60F_{254} (20 cm×10 cm with 250 µm thickness) using a Camag Linomat IV. A constant application rate was 0.1 µL/s with bandwidth of 5 mm. Optimized mobile phase (12 mL) consisted of toluene: acetone: ammonia (6:6:0.4 v/v/v) was used. Linear ascending development was carried out in twin trough glass chamber saturated with the mobile phase for 10 min at room temperature.

The length of chromatogram run was 7 cm. Subsequent to the development; TLC plates were dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner 3 with Deuterium lamp and Wincats 1.4.2.81 software in the absorbance mode at 254 nm. The linearity of tizanidine (r^2=0.991±0.001) in plasma is found in range of 300–1100 ng/spot. Standard curve for plasma tizanidine concentrations range from 30-110 µg/mL with a mean correlation coefficient of 0.991±0.001 shows acceptable linearity, precision and accuracy. Intraday and interday precision is less than 15 % RSD while average % recovery is found to be 99.72 with average % RSD 0.4865. The specificity of the HPTLC method for tizanidine was determined by spiking the tizanidine plasma sample with internal standard. No interferences with the measurement of tizanidine by plasma constituents or internal standard were observed [6].

Pharmacokinetic analysis: The pharmacokinetics of tizanidine was characterized by plasma C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $T_{1/2}(z)$, λ_z, V_d/F, CL/F, $AUMC_{0-t}$, $AUMC_{0-\infty}$, MRT_{0-t}, $MRT_{0-\infty}$ and Fr values for reference as well as both the test groups. The pharmacokinetic calculations were performed with the "R software using bear version 2.5.3" developed by Hsin-ya Lee & Yung-jin Lee (Kaohsiung Medical University, Taiwan). Noncompartmental analysis (NCA) approach is used to compute AUCs and the terminal elimination rate constants λ_z for drug plasma concentration. The linear trapezoidal method is applied to calculate AUC (time 0 to the last measurable Cp). The extrapolated AUC (from time of the last measurable Cp to time infinity) is equal to

the last measurable Cp divided by λ_z. λ_z is calculated using the Adjusted R Square method that excludes data point of T_{max} and C_{max} [7].

Statistical analysis: Values were expressed as mean ± SD. Average bioequivalance data was analyzed from NCA using ANOVA for parallel study. The pharmacokinetic variables between the groups like AUCs and Cmax were compared by the ANOVA followed by Welch Two Sample t-test (% T/R); TOST and Anderson-Hauck Test to analyze statistical significance. The differences were considered statistically significant when $P < 0.05$. In both studies 90% CI were calculated for the mean differences of selected variables of the test and reference products. Bioequivalence acceptance criterion was set within the range of 80.000 - 125.000 % [7].

RESULTS

Effect of Ofloxacin on Tizanidine Pharmacokinetics in Rats:

The time-course of plasma tizanidine is shown in Figure 1. As per the figure, the bioavailability of tizanidine was improved to a small extent when it was co-administered with ofloxacin. Effect of ofloxacin on the pharmacokinetic parameters of tizanidine is given in Table 1. C_{max} of tizanidine was significantly decreased by 8.47% ($P<0.001$) when ofloxacin was co-administered orally with tizanidine as compared to reference. There was significant increase in AUC_{0-t}, and $AUC_{0-\infty}$ by 17.17% ($P<0.001$) and 5.24% ($P<0.001$) respectively. When tizanidine was co-administered orally with ofloxacin marked increase was observed in tizanidine T_{max} value from 1.5 hr to 3 hr (by 100%) and $T_{1/2}(z)$ value was decreased by 76.07% as compared to reference. At the same time terminal elimination rate constant (λ_z) of tizanidine was increased by 451.28% as compared to reference. In ofloxacin co-administered group CL/F value and V_d/F value of tizanidine were decreased as compared to reference by 5.12% and 78.63% respectively. $AUMC_{0-t}$ and $AUMC_{0-\infty}$ values were increased by 71.81% and 10.81% respectively as compared to reference. MRT_{0-t} and $MRT_{0-\infty}$ of tizanidine were increased by 46.92% and 5.19% as compared to reference respectively. Fr value of tizanidine was found to be 117.17%. Table 2 shows statistical analysis summary of pivotal parameters of bioequivalence Study.

DISCUSSION

Ofloxacin co-administration improves oral bioavailability of tizanidine to a greater extent. This was probably by inhibiting the CYP1A2-mediated presystemic metabolism of tizanidine in liver as reported by Granfors [4]. Ofloxacin decreases C_{max} of tizanidine significantly. Lewis et al. reported that various fluroquinolones like ciprofloxacin and ofloxacin are inhibitors of CYP1A2 enzymes [8, 9]. AUCs of tizanidine were increased by ofloxacin significantly but to a very small extent as compared to test group I, suggesting suppression of CYP1A2 to some extent but not strong inhibition of CYP1A2 enzymes by ofloxacin. Ofloxacin doubled the T_{max} of tizanidine and sustainined its action. This suggested the delayed onset of action related to inhibition of CYP enzymes by co-administered drug as reported by Ching et al. [10] and Giorgi et al. [11] The $T_{1/2}$ and CL/F of tizanidine were found to be decreased by ofloxacin co-administration. The reason behind this may be the inhibition of CYP1A2 mediated metabolism of drug in liver as suggested by Yamashi et al. [12] V_d/F value was also found to be decreased during study but its cause was unknown. Contrary to other pharmacokinetic determination the λ_z was found to be increased. This increase in elimination rate constant was may be due to increased rate of drug metabolism [13]. AUMC and MRT values were found to be increased indicating that tizanidine resides in its active form for longer time in body indicating its therapeutic action for longer duration. The % relative bioavailability of tizanidine was found to be 117.17% indicating very small or insignificant change in tizanidine oral bioavailability due to co-administration of ofloxacin. This is a 2-treatment parallel single-dosed study. Log-transformed bioavailability measures were analyzed. The pivotal parameters of parallel bioequivalence studies were established using linear model (ANOVA) - statistical analysis. Here the bioequivalence acceptance criterion was set within the range of 80.000 - 125.000 % as per FDA guidelines [7]. These parameters include log transformed C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values of tizanidine. The classical (shortest) 90% CIs for log transformed C_{max} ($\ln C_{max}$), log transformed AUC_{0-t} ($\ln AUC_{0-t}$) and log transformed $AUC_{0-\infty}$ ($\ln AUC_{0-\infty}$) were estimated. T/R % values for each of the above parameters were calculated. Then the statistical significance of differences in pharmacokinetic parameters was determined by analyzing the data of log transformed values by TOST and Anderson-Hauck Test. The null-hypothesis was rejected at an upper significance level of 0.05 if the two one-sided tests for testing the ratio was less than 80% and greater than 125%,

respectively, both were rejected at the significance level 0.05 (two one-sided test situation). It could be shown that this was equivalent to a CI for the true ratio; with confidence level 90% was entirely within the interval 80% to 125% [14]. In this case classical 90% CI for lnC_{max}, $lnAUC_{0-t}$ and $lnAUC_{0-\infty}$ for ofloxacin co-administrated group were 91.52% (91.32%-91.72%), 117.17% (116.99%-117.34%) and 104.30% (103.77%-104.83%) respectively, which were found to be within the interval 80% to 125%. In both TOST and Anderson Hauck Test all P values were less than 0.05, thus we could reject the Ho. From the statistical analysis the test (tizanidine + ofloxacin) and reference (tizanidine) were found to be bioequivalent, suggesting that there was no significant change in bioavailability of drug.

CONCLUSION

Concomitant oral administration of ofloxacin resulted in a significant increase in systemic tizanidine exposure after oral administration. The increased oral bioavailability of tizanidine may probably attribute to the inhibition of CYP1A2 mediated pre-systemic metabolism by oral ofloxacin. No serious adverse events were observed during the study period and tizanidine was well tolerated in all animals. But the plasma concentration of tizanidine if increasing drastically, it would lead to hazardous pharmacodynamic adverse effects, so we should aware the potential drug- drug interactions and the dose monitoring of both the co-administered drugs is necessary.

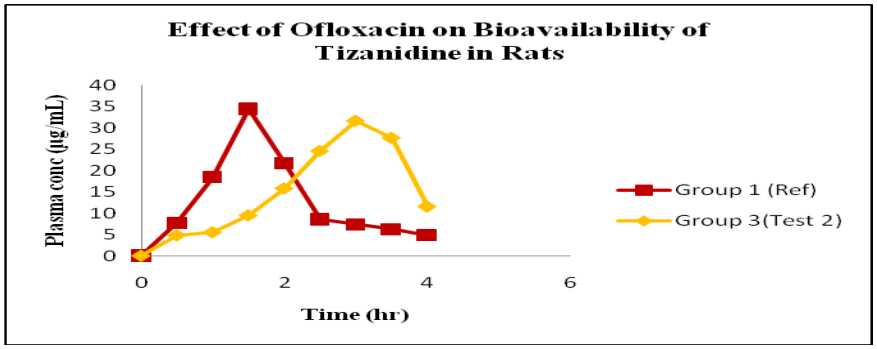

Figure 1: Effect of Ofloxacin on Bioavailability of Tizanidine in Rats

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Table 1: Effect of Ofloxacin co-administration on pharmacokinetic parameters of Tizanidine in rats

	Treatment groups	
Pharmacokinetic parameters	**Reference (Control)^n**	**Test 2(Ofloxacin co-administrated)^n**
C_{max} (µg/mL)	34.46 ± 0.07	31.54 ± 0.16
T_{max} (hr)	1.5	3
AUC_{0-t} (µg.hr/mL)	53.33 ± 0.07	62.49 ± 0.22
$AUC_{0-\infty}$ (µg.hr/mL)	65.56 ± 1.12	69.00 ± 0.42
$T_{1/2}$(z) (hr)	1.63 ± 0.18	0.39 ± 0.008
λ_z	0.39 ± 0.03	1.76 ± 0.03
V_d/F (mL/kg)	269.85 ± 17.79	57.67 ± 1.01
CL/F (mL/min)	106.86 ± 1.9	101.39 ± 0.54
$AUMC_{0-t}$ (µg.hr^2/mL)	95.87 ± 0.20	164.72 ± 0.49
$AUMC_{0-\infty}$ (µg.hr^2/mL)	175.66 ± 9.90	194.65 ± 1.46
MRT_{0-t} (hr)	1.79 ± 0.002	2.63 ± 0.003
$MRT_{0-\infty}$ (hr)	2.68 ± 0.10	2.82 ± 0.008
Fr (%)		117.17

Values are mean ± SD; n= 18; C_{max}: Maximum plasma concentration ; T_{max}: Time to reach the peak concentration; AUC_{0-t}: Area under the plasma concentration time curve (Time= 0 to time of the last measureable plasma concentration); $AUC_{0-\infty}$: Area under the plasma concentration time curve (time = 0 to infinity); $T_{1/2}$(z): Terminal elimination half life; λ_z: Terminal elimination rate constant; V_d/F: Volume of distribution; CL/F: Total plasma clearance; $AUMC_{0-t}$: Area under the first moment curve (Time= 0 to time of the last measureable plasma concentration); $AUMC_{0-\infty}$: Area under the first moment curve (time = 0 to infinity); MRT_{0-t}: Mean residence time (Time= 0 to time of the last measureable plasma concentration); $MRT_{0-\infty}$: Mean residence time (time = 0 to infinity); Fr: Relative bioavailability determined by comparing AUC_{0-t} of test group I and AUC_{0-t} of reference group.

Table 2: Statistical analysis and 90% Confidence Intervals (CI) for Different Pharmacokinetic Parameters from Log-Transformed Data for Assessment of Bioequivalence

Property	Classical 90% CIs	T/R (%)	TOST	Anderson-Hauck Test
lnC_{max}	91.52 (91.32-91.72)	97.49	Upper P<0.001 Lower P<0.001	P<0.001
$lnAUC_{0-t}$	117.17 (116.99-117.34)	103.98	Upper P<0.001 Lower P<0.001	P<0.001
$lnAUC_{0-\infty}$	104.30 (103.77-104.83)	101.00	Upper P<0.001 Lower P<0.001	P<0.001

REFERENCES

1. Corina I and Mino RC. Springer, 2005; 39(50):191.
2. Back DJ and Rogers SM. Aliment Pharmacol Ther, 1987; 1: 339-57.
3. Granfors M. Interactions of tizanidine with CYP1A2 enzyme inhibitors Studies in vitro and in humans. Academic Dissertation, University of Helsinki, Finland. 2005: 1-82.
4. Coleman MD. Human Drug Metabolism: An Introduction. New York; John Wiley & Sons: 2005.
5. Granfors MT, Backman JT, Laitila J and Neuvonen PJ. Br J Clin Pharmacol, 2004; 57: 349-53.
6. Sujatha K, Chitra K, Hettiarachchi DS, Krishna MV and Vasantha J. Indian J Pharm, 2003; 65: 519-20.
7. Nimje H, Wate SP, Dharkar DP and Razdan R. Indian J. Pharm, 2007; 69: 281-3.
8. Drug Bank, Tizanidine, Drug created on June 13, 2005 07:24 / Updated on November 10, 2010.
9. Lewis DF, Lake BG and Dickins M. *Drug Metabol Drug Interact*, 2004; 20(3): 111-42.
10. Ching H, Tsai SY, Hsiu SL, Wu PP and Chao PDL. J Chin Med, 2001; 12(1): 51-9.
11. Giorgi M, MacCheroni M, Del Carlo S, Yun HI and Saccomanni G. Iran J Vet Res, 2011; 12(1-34): 8-15.
12. Yamsani MR, et al. Acta Pharmaceutica Sciencia, 2010; 52: 461-8.
13. Helleberg L, Rubin A, Wolen RL, Rodda BE, Ridolfo AS and Gruber CM. Br J Clin Pharmacol, 1974; 1: 371-4.
14. Sultana R, Ullah A, Akbor MM, Azad MAK, Latif AHMM and Hasnat A. J Appl Res, 2008; 8(1): 34-42.