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Full Length Research Paper

Drug-drug interaction between ciprofloxacin and diclofenac ophthalmic drops at ocular level

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In present times, there has been interest in multi-drug combinations for treatment of ocular infections, which are more commonly antibiotics and analgesics. Ciprofloxacin and diclofenac sodium were used concomitantly and pharmacokinetic drug-drug interactions between them were studied. To investigate disposition kinetics of ciprofloxacin eye drops (0.3%) alone and with co-administration of diclofenac sodium eye drops (0.1%) for pharmacokinetic drug-drug interaction(s). A randomized clinical trial was conducted in 84 human volunteers and the aqueous humor samples were collected from the eyes at various time intervals and concentration of ciprofloxacin in aqueous humor was determined using a validated reversed-phase high performance liquid-chromatography/ultraviolet detection method. The results were subjected to statistical analysis to determine whether a statistically significant difference was observed between the two groups. The mean age of the patients in this study ranged from 40 to 83 years, and gender distribution was 46.0% female and 54.0% males. The maximum concentration of ciprofloxacin in aqueous humor observed was 0.47 ± 0.057 µg/ml at 0.83 h. While the maximum concentration with concurrent administration of ciprofloxacin with diclofenac sodium was 0.44 ± 0.00577 µg/ml at 0.25 h. Time to reach maximum concentration was decreased to 0.3 h. $t_{1/2abs\ and}$ $t\alpha_{1/2}$ were not significantly affected by co-administration of the ciprofloxacin eye drops with the diclofenac sodium eye drops (P = 0.73), however, $t_{1/2}\beta$ was significantly affected (P = 0.036). Similarly, apparent volume of distribution, volume of central compartment, steady-state distribution volume, AUC_{0-t} and MRT were also significantly reduced due to concurrent administration of the diclofenac sodium eye drops with the ciprofloxacin eye drops (P = 0.021, 0.0.017, 0.05, 0.01 and 0.04). On the other hand, the ocular Cl_t was significantly elevated (P = 0.04), while absorption rate constant (P = 0.58) was non significantly decreased due to concurrent administration of the diclofenac sodium eye drops, while C_{max} was not significantly affected. Diclofenac sodium affects the pharmacokinetics of ciprofloxacin when co-administered with ciprofloxacin eye drops.

Key words: Ciprofloxacin ophthalmic drops, pharmacokinetics drug-drug interaction, aqueous humor.

INTRODUCTION

With the seemingly constant use of new therapeutic agents and new treatment indications for existing medications, polypharmacy is increasingly common. The use of multiple medications increases the risk for medication-related adverse events (Es) and drug interactions (DIs) (Allard et al., 2001). The risk of DIs can increase from approximately 6% in patient taking only two medications to 50% in those taking five medications and 100% in those taking 10 medications (Grymonpre et al., 1988). Although, non linear pharmacokinetic may sound irrelevant to busy clinicians, but it can have significant impact on their patients. Doubling the dose of a given medication

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does not translate into just doubling of the blood level or effect of the medication. For many medications, dose increase can produce exponential rises in blood level. So, even a small change in dose could mean a significant rise in the drugs blood level and causes potential drug interaction and side effects (Kampmann et al., 1972).

Recently, there has been interest in many drug combinations, including antibiotics and non-steroidal anti-inflammatory drugs (NSAIDS), for treatment of various ocular infections. Ciprofloxacin is one of the most commonly used broad spectrum antibiotic frequently used for the treatment of various ocular infections (Campoli-Richards et al., 1988; Cover and Mueller, 1990; Hooper, 2000; Yalvac et al., 2003). It exists mainly as a zwitterion in biological fluids that allows it to cross physiological barriers by passive diffusion. In additions to passive diffusions, P-glycoprotein (P-gp) is also involved in the trans-epithelial secretion of ciprofloxacin (Fern et al., 1990; Behrens-Baumann and Martell, 1987). It is distributed throughout the extra-vascular spaces and has been detected in most of the body tissues and fluids. It is potentially a very useful antibiotic in ophthalmology because it is one of the few antibiotics that enter the human eye after oral administration (Dalhoff and Eickenberg, 1985). Reported concentrations of ciprofloxacin varied from 0.1 to 0.65 in aqueous and from 0.17 to 0.51 µg/ml in vitreous humor after the oral administration of various doses to humans (Fern et al., 1990; Davis et al., 1987; Mounier et al., 1988; Keren et al., 1991; Kowalski et al., 1993; Lesk et al., 1993; Serdarevic, 1993; Morlet et al., 2000).

However, beneficial effects of the ciprofloxacin are now bracketed with interaction, so it is necessary to take care in the prescription of this drug with combination to other classes, including NSAIDs, to preserve its potential therapeutics. Oral quinolones have been reported to possess potent convulsant activity, which is enhanced when they are administered concurrently with antiinflammatory drugs (Simpson and Brodie, 1985). In normal clinical practice, limited data is available regarding DIs at ocular level because it is difficult to obtain a full concentration-time profile of a drug in the eye. Keeping in mind these facts, a study was designed to investigate the disposition kinetics of ciprofloxacin eye drops alone and with co-administration of diclofenac sodium eye drops for pharmacokinetic drug-drug interaction(s).

MATERIALS AND METHODS

Study design

A randomized open-labeled clinical trial was designed to evaluate the potential pharmacokinetic drug-drug interaction(s) between the two co-administered drugs. The study was conducted as per the principles of the *Declaration of Helsinki* and its amendments. The protocol of the study was approved by the Ethical Committee of the Mardan Medical Complex, Khyber PakhtunKhwa, Pakistan.

Subjects undergoing extra capsular cataract extraction (ECCE) surgery in the eye department of the Mardan Medical Complex, Khyber PakhtunKhwa, Pakistan were recruited for the study. The objectives of the study and effects of the drugs were explained to the volunteers before obtaining the informed consent from them. Samples obtained were then analyzed at the Department of Pharmacy, University of Peshawar, Pakistan.

Subjects

Subjects were patients with cataract who were scheduled for ECCE surgery. A detailed medical history and clinical examination were performed for all the volunteers at the beginning of the study under the supervision of a qualified physician. In addition, 12-lead electrocardiography (ECG), complete blood count (CBC), blood pressure (BP), blood sugar level, liver function tests (LFTs), lipid profile and renal function tests (RFTs) were also evaluated for all of them at the hospital's pathology department.

Subjects having any other ocular or systemic pathology, such as diabetes mellitus, gastrointestinal, renal, hepatic or cardiovascular disease, etc or any recent history of surgical intervention were not eligible for the study. Subjects with abnormal clinical laboratory tests values were also excluded from the study. Similarly, subjects with a history of allergic responses to any class of drugs were also excluded from the study. Moreover, they were not taking any medicines or special diets at least 2 weeks before the trial.

Protocol for drug administration

The volunteers were divided into two groups; each comprised of 42 patients (three patients for each sampling time). Drugs were instilled onto their cornea at 0, 5, 10, 15, 20, 30, 40, 50, 60, 90, 120, 180, 240, 360 and 480 min (0, 0.083, 0.167, 0.250, 0.333, 0.5, 0.667, 0.833, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 8.0 h) prior to ECCE surgery. Volunteers in group I received single dose, equivalent to 222 µg of ciprofloxacin, topical instillation of ciprofloxacin eye drops (Ciloxan®; 0.3%; Batch #. 42738F; Mfg. date: 09/08; Alcon Laboratories Inc. Fort Worth, Texas, USA). While, group II received concurrent topical instillation of ciprofloxacin eye drops with single dose, equivalent to 75 µg diclofenac, topical instillation of diclofenac sodium eye drops (Dofanac®; 0.1%; Batch #. F13054; Mfg. date: 08/08; Ethical Pharma. Pvt. Ltd. Lahore, Pakistan), using same protocol as adopted for group I.

Sample collection and handling

Aqueous humor (50 to 200 µl) was carefully aspirated through a paracentesis, using a 27-gauge needle on a tuberculin syringe under an operating microscope with special care to avoid blood contamination and was stored at -80°C until analyses without any further treatment.

Analyses of samples

Samples were analyzed by a validated reversed-phase high performance liquid-chromatography/ultraviolet (RP-HPLC/UV) detection method, the detail of which is given subsequently.

Chemicals and reagents

The ciprofloxacin and paracetamol standards were kind gifts of Ferozsons Laboratories Pvt. Ltd. Nowshera, Pakistan. HPLC-grade solvents such as acetonitrile and methanol and all other analytical-

grade chemicals and reagents were purchased from Sigma-Aldrich (Oslo, Norway). Ultra-pure water was prepared by a Millipore ultra-pure water system (Milford, USA). All these reagents and chemicals were used without further purification.

Preparation of standard solutions

To prepare ciprofloxacin and paracetamol (internal standard) stock solutions, weighed amounts of each were dissolved in acetonitrile. Internal standard solution (to give a final concentration of 1.0 µg/ml), to be added to all standard solutions and sample matrices, was then prepared by dilution of the corresponding stock solution with acetonitrile. Similarly, standard solutions of ciprofloxacin in the range of 0.005 to 2.0 µg/ml (eleven concentration levels), each containing 1.0 µg/ml of internal standard solution, were also prepared by dilution of the ciprofloxacin stock solution. Similarly, standard solution containing 1.0 µg/ml each of ciprofloxacin and internal standard (1:1 mixture) was also prepared.

Sample preparation

Aqueous humor

At the time of analysis, the aqueous humor samples were thawed at room temperature and 50 µl of each of the sample was vertex-mixed with 50 µl paracetamol solution (1.0 µg/ml) used as the internal standard. These samples were then treated with 150 µl acetonitrile by further vertex-mixing for 5 min and then centrifuged at 5,000 rpm for 10 min at room temperature. The clear supernatant was collected and evaporated to dryness at 60°C under the stream of nitrogen gas. The residue was then reconstituted in mobile phase (100 µl) and 20 µl samples were injected into the HPLC system.

Ophthalmic solutions (Eye drops)

Ophthalmic solution of ciprofloxacin (Ciloxan®, containing 0.3% ciprofloxacin hydrochoride) was also prepared as described for aqueous humor and analyzed to calculate the percent purity and amount of drug in the dose instilled onto the cornea.

Instruments

Chromatography was carried out on Perkin Elmer (Norwalk, USA) HPLC system equipped with a serious 200 pump, vacuum degasser, column oven and ultraviolet-visible detector and Rheodyne 7725i manual injector. The chromatographic data was analyzed using Totalchrom Chromatographic Workstation (version 6.3.1.) interfaced with HPLC system through Network Chromatographic Interface (NCI) 900.

Chromatographic conditions

The analytes were separated using Hypersil C18 Column (250 × 4.6 mm, 4.5 µm) protected by a Bondapak™ RP18 (30 × 4.6 mm, 10 µm; Merck kGaA, Germany) pre-column guard cartridge. Analyses were performed at ambient temperature using acetonitrile of 0.25% H_3PO_4 (aqueous) (60:40 *v/v*) as the isocratic mobile phase pumped at a flow rate of 1.0 ml/min. The injection volume was kept 20 µl and the eluents were monitored at 275 nm. As mentioned, paracetamol (at the level of 1.0 µg/ml) was used as the internal standard.

Method validation

The chromatographic method was validated according to international guidelines with emphasis on linearity within the expected concentration range, sensitivity, recovery and precision (Epshtein, 2004). Bovine aqueous humor was used in the method development and validation studies.

Concentration of the samples *C* was calculated by the following formula:

$$C = \left(A^{Analyte}_{Sample} / A^{IS}_{Sample}\right) x \left(A^{IS}_{CS} / A^{Analyte}_{CS}\right) x C_{CS} x F_D$$

where $A^{Analyte}_{Sample}$ and $A^{Analyte}_{CS}$ are peak areas of the analyte in serum samples and 1:1 mixture, respectively; A^{IS}_{Sample} and A^{IS}_{CS} are peak areas of the internal standard in serum samples and 1:1 mixture, respectively; C_{CS} is the concentration of analyte in the 1:1 mixture and F_D is the dilution factor.

Pharmacokinetic and statistical analyses

The mean ± standard deviation (SD) of the ciprofloxacin concentration in the aqueous humor alone and in presence of diclofenac sodium for every data point was calculated and plotted as a function of time. Using both the compartmental and non-compartmental approaches, various pharmacokinetic parameters were then calculated with the help of *Microsoft Excel*® and *PK-solution*® softwares. The differences in these pharmacokinetic parameters between the two groups of volunteers were then evaluated statistically.

RESULTS

Volunteers

The human volunteers (n = 84) who visited Mardan Medical Complex for ECCE surgery were randomly selected. All the subjects were otherwise healthy as assessed by physical examination, medical history and laboratory tests. The mean ± SD of the patients' age was 62.4 ± 16.7 years, ranging from 40 to 83 years. Out of the total of 84 subjects, females were 46.0% and males 54.0%.

Analytical method validation

The HPLC method developed for the quantification of ciprofloxacin using paracetamol (1.0 µg/ml) as the internal standard was linear in the range of 0.005 to 2 µg/ml. The correlation coefficients of all standard curves were more than 0.999 for both the aqueous humor and the ophthalmic solutions (Figure 1). Lower limit of quantification (LLOQ) was 5 ng/ml and limit of detection

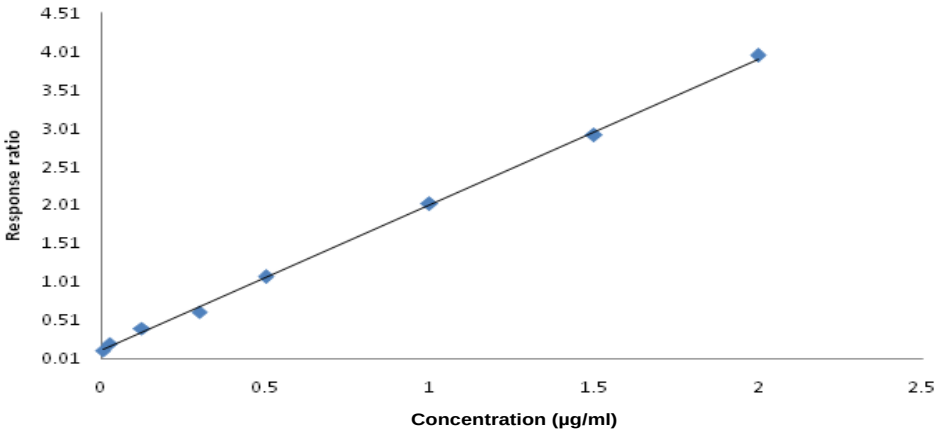

Figure 1. Calibration curve of ciprofloxacin eye drops in spiked aqueous humor samples

Table 1. Recovery, precision, sensitivity, calibration range and linearity of the developed method.

Validation parameter	Ciprofloxacin
	Mean (SD); RSD (%)[e]
Recovery	
0.05 µg/ml[a]	[c]97 (1.58); 1.63
0.01 µg/ml[a]	[c]98.4 (0.98); 0.98
Precision	
Repeatability	
Analysis repeatability	
0.05 µg/ml [b]	[d]0.049 (0.005); 0.94
Intermediate precision	
Intra-day reproducibility	
0.025 µg/ml	[d]0.024 (0.002); 1.17
0.05 µg/ml	[d]0.049 (0.005); 1.01
Inter-days reproducibility	
0.025 µg/ml	[d]0.0245 (0.005); 2.04
0.05 µg/ml	[d]0.0491 (0.008); 1.55
Sensitivity	
Lower limit of quantification (LLOQ)	5 ng/ml
Limit of detection (LOD)	1.5 ng/ml
Calibration range	0.005-2 µg/ml
Linearity	$y = 1.897x + 0.116$, $r^2 = 0.999$

[a]n = 5, [b]n = 10, [c] recovery (%), [d]quantity recovered in µg/ml and [e]residual standard deviation (%).

(LOD) was 1.5 ng/ml for ciprofloxacin. The mean percent recovery (n = 5) was found to be above 97% at the two nominal concentration levels. Results of the analysis repeatability and intermediate precision (intra-day and inter-days reproducibility) show complete harmony among the repeated analyses and intra-day and inter-days studies, as depicted in Table 1.

Ciprofloxacin concentration in ophthalmic drops and aqueous humor

When analyzed by the proposed validated analytical method, the concentration (mean ± SD) of ciprofloxacin in ophthalmic drops was found to be 0.296 ± 0.004% (that is, percentage purity was found to be 98%).

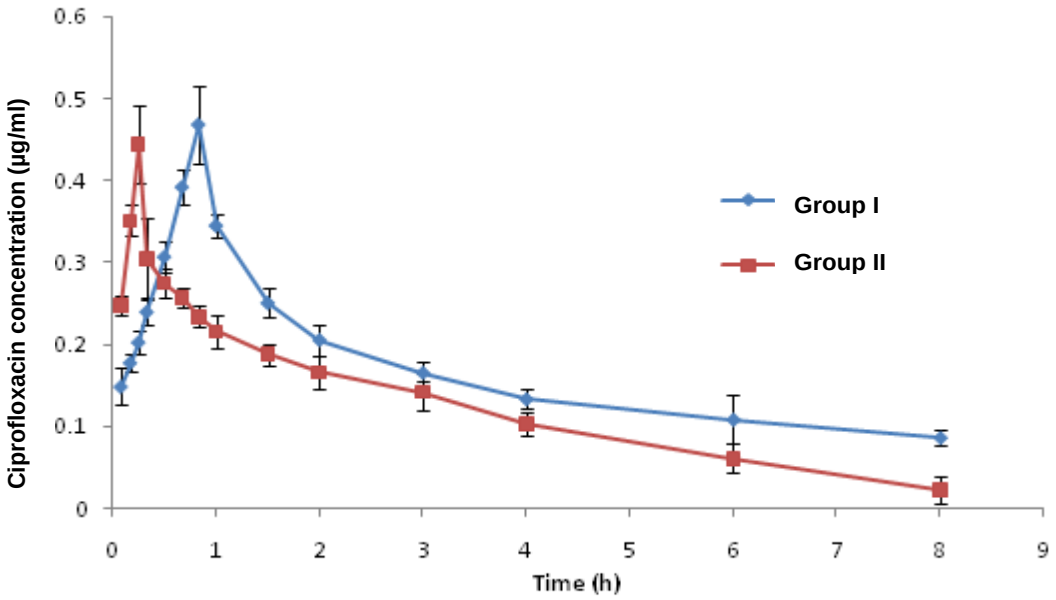

Figure 2. The concentration of ciprofloxacin in aqueous humor following instillation of ciprofloxacin alone, with co-instillation with diclofenac sodium eye drops. (Group I, ciprofloxacin only; Group II, ciprofloxacin + diclofenac sodium).

The concentration of ciprofloxacin in aqueous humor at various time intervals following the instillation of ciprofloxacin eye drops alone (Group I) and after concurrent instillation with diclofenac sodium eye drops (Group II) are given in Figure 2, showing that its concentration in aqueous humor after 0.08 h of drug administration was 0.149 ± 0.023 and 0.248 ± 0.012 µg/ml in Groups I and II volunteers, respectively. It slowly increased with passage of time, and the maximum concentration (C_{max}) of ciprofloxacin in the aqueous humor was observed at 0.83 and 0.25 h in the two groups, respectively, where its concentration was 0.47 ± 0.0577 and 0.44 ± 0.0577 µg/ml in Groups I and II, respectively. Samples collected after 8 h of topical application showed very low, that is, 0.087 ± 0.009 and 0.022 ± 0.017 µg/ml ciprofloxacin concentrations in groups I and II, respectively. In other studies, the C_{max} for ciprofloxacin was found to be 0.38 µg/ml after administration of the same 222 µg ophthalmic dose in human eye (Hehl et al., 1999). Similarly, in dogs, the C_{max} was found to be 0.69 µg/ml following topical administration of the 0.3% ciprofloxacin eye drops (Yu-Speight et al., 2005).

Pharmacokinetic and statistical analyses

The aqueous humor ciprofloxacin concentration versus time data for ciprofloxacin eye drops alone and in combination with diclofenac sodium eye drops were plotted on semi-logarithmic scale, which showed a bi-exponential decline, that is, two-compartment model (Figure 2). However, data was analyzed by both non-compartmental and two-compartmental approaches.

Non-compartmental analyses

The pharmacokinetic parameters, such as AUC (P = 0.01) and AUMC (P = 0.01) are significant and were decreased in the ciprofloxacin concentration when given with diclofenac sodium eye drops, while mean resident time (P = 0.0676) is non-significant, but decreased the ciprofloxacin concentration. The total body clearance (P = 0.0276) and elimination rate constant (P = 0.0421), significantly increased and decreased ciprofloxacin concentration and non-compartmental approach are given in Table 2 showing significant difference among the two groups of volunteers.

Compartmental analyses

A modified two-compartment model (Figure 3) that can be applied for the analysis of the various pharmacokinetic parameters following the instillation of the drug onto the cornea may be: the rate of distribution of drug from aqueous humor and tissue compartment can be expressed as shown in Equations 1 and 2, respectively.

$$\frac{dC_{aq}}{dt} = K_{21}C_t - k_{12}C_{aq} - kC_{aq} \quad (1)$$

Table 2. Non-compartmental analysis.

Parameter	Ciprofloxacin alone	Ciprofloxacin plus diclofenac sodium	Significance *P* value
Area under the curve AUC (0-t) (µg.h/ml)	1.3321 ± 0.0578	0.975 ± 0.127	0.01
Area under movement curve (AUMC, µg.h^2.ml)	4.075 ± 0.224	2.526 ± 0.542	0.01
Elimination rate constant (*kel*, h)	0.327 ± 0.007	0.3909 ± 0.0336	0.04
Clearance (CL, ml/kg/h)	166.86 ± 7.06	230.43 ± 30.0413	0.02
Mean residence time (MRT, h)	3.059 ± 0.068	2.5723 ± 0.2313	0.06
Time to peak T_{max} (h)	0.83 ± 0.00	0.25 ± 0.00	0.00
Peak concentration C_{max} (µg/ml)	0.47 ± 0.5	0.45 ± 0.05	0.52

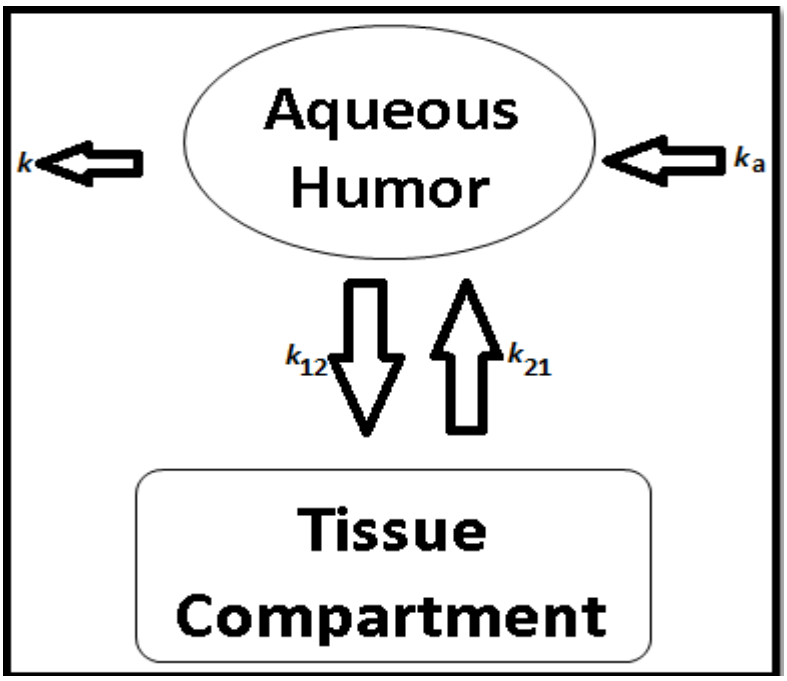

Figure 3. Modified two-compartment model. Where *k*a is the absorption of the drug through cornea: 1 is the aqueous humor and 2 is the tissue compartment; *k*12 is the rate constant at which drug move from aqueous humor compartment to the tissue compartment; *k*21 is the rate constant at which drug is transported from tissue to aqueous humor compartment; and *k* is the elimination of drug from the aqueous humor compartment.

$$\frac{dC_t}{dt} = k_{12}C_{aq} - k_{21}C_t \quad (2)$$

where k_{12} is the movement of drug from aqueous humor to tissue compartment and K_{21} is the movement of drug from tissue compartments to aqueous humor, C_{aq} and C_t are the concentrations of drug in aqueous humor and tissue compartments, respectively, and k is the elimination rate constant of the drug from aqueous humor chamber.

The C_{aq} and C_t can be calculated by means of the following equations.

$$C_{aq} = \frac{D_{aq}}{V_{aq}} \quad (3)$$

$$C_t = \frac{D_t}{V_t} \quad (4)$$

where D_{aq} is the drug in aqueous humor, V_{aq} is the

volume of aqueous humor, D_t is the drug in tissue compartment and V_t is the volume of tissue compartment.

Substitution of Equations 3 and 4 in 1 and 2, respectively will give us Equations 5 and 6.

$$\frac{dC_{aq}}{dt} = K_{21}\frac{D_t}{V_t} - k_{12}\frac{D_{aq}}{V_{aq}} - k\frac{D_{aq}}{V_{aq}} \quad (5)$$

$$\frac{dC_t}{dt} = k_{12}\frac{D_{aq}}{V_{aq}} - K_{21}\frac{D_t}{V_t} \quad (6)$$

The C_{aq} and C_t can also be calculated by Equations 7 and 8, respectively.

$$C_{aq} = \frac{FDo}{V_{aq}}\left(\frac{k_{21}-a}{b-a}e^{-at} + \frac{k_{12-b}}{a-b}e^{-bt}\right) \quad (7)$$

$$C_t = \frac{FDo}{V_t}\left(\frac{k_{12}}{b-a}e^{-at} + \frac{k_{21}}{a-b}e^{-bt}\right) \quad (8)$$

Total drug in aqueous humor (D_{aq}) and tissue (D_t) compartments can be calculated using Equations 9 and 10, respectively.

$$D_{aq} = FDo\left(\frac{k_{21}-a}{b-a}e^{-at} + \frac{k_{12}-b}{a-b}e^{-bt}\right) \quad (9)$$

$$D_t = FDo\left(\frac{k_{12}}{b-a}e^{-at} + \frac{k_{21}}{a-b}e^{-bt}\right) \quad (10)$$

where F is the fraction of drug absorbed from cornea to aqueous humor and Do is the dose of the drug. The a and b are hybrid constant for distribution and elimination phase, respectively.

$$a + b = k_{12} + k_{21} + k \quad (11)$$

$$ab = k_{21} \quad (12)$$

$$C_{aq} = Ae^{-at} + Be^{-bt} \quad (13)$$

where A and B are constant for the distribution and elimination phases and can be calculated from the intercept on the Y-axis, using method of residual. a and b are first order rate constant for distribution and elimination phase, respectively and calculated from the slope of the distribution and elimination curves.

$$A = \frac{FD_o\ (a-k_{21})}{V_{aq}\ (a-b)} \quad (14)$$

$$B = \frac{FD_o\ (k_{21}-b)}{V_{aq}\ (a-b)} \quad (15)$$

With the passage of time, absorption reaches to zero ($Ae^{-at} = 0$), then the concentration of the drug in the aqueous humor can be calculated using Equation 16.

$$C_{aq} = Be^{-bt} \quad (16)$$

Taking log:

$$\log C_{aq} = \frac{-bt}{2.303} + \log B \quad (17)$$

The half life ($t_{1/2}$) can be calculated using the following equation:

$$t_{1/2} = 0.693/b \quad (18)$$

Various rates constant can also be calculated using the values of A, B, a, and b.

$$k = \frac{ab\ (A+B)}{Ab+Ba} \quad (19)$$

$$k_{12} = \frac{Ab(b-a)^2}{(A+B)\ (Ab+Ba)} \quad (20)$$

$$k_{21} = \frac{Ab+Ba}{A+B} \quad (21)$$

The aqueous humor ciprofloxacin drug concentration-time curve showed that ciprofloxacin follows two-compartment model in the eye (Figure 2), the disposition kinetic parameters of ciprofloxacin eye drops, including $t_{1/2}$ (absorption, $t_{1/2abs}$; distribution, $t_{1/2\alpha}$; and elimination, $t_{1/2\beta}$), volume of distribution (apparent volume of distribution, volume of central compartment and steady-state distribution volume), peak concentration (C_{max}), absorption rate constant (k_{ab}), time to reach maximum concentration (T_{max}), AUC, Cl_t and lag time were also calculated by two- compartmental approach using least

Table 3. Two-compartmental analysis.

Kinetic parameter	Ciprofloxacin alone (Mean ± SD)	Ciprofloxacin plus diclofenac sodium (Mean ± SD)	Significance P value
Area under the curve AUC (0-t) [µg.h/ml]	1.34 ± 0.05	0.98 ± 0.100	0.015
Ocular clearance (CL) [ml/h]	105.95 ± 15.5	218.20 ± 36.7	0.040
Volume of distribution comp.1 [ml]	1477.42 ± 328	413.90 ±195	0.017
Volume of distributed steady state (Vss) (ml)	850.69 ± 84	622.84 ± 37.2	0.050
Volume of distribution (ml)	923.33 ± 111	389.60 ± 174	0.021
D Half life (h)	1.26 ± 1.02	0.99 ± 0.466	0.72
E Half life (h)	6.18 ± 1.50	1.31 ± 0.694	0.036
Rate constant k10 (1/h)	0.26 ± 0.139	0.01 ± 0.00850	0.35
Rate constant k12 (1/h)	0.29 ± 0.211	0.80 ± 0.519	0.49
Rate constant k21 (1/h)	1.38 ± 0.503	0.77 ± 0.553	0.95
Mean Residence Time (MRT) (h)	8.20 ± 1.82	2.91 ± 0.529	0.040
Absorption rate constant (ka) (1/h)	1.18 ± 0.936	0.80 ± 0.532	0.58
Absorption half-life (h)	1.57 ± 1.96	1.11 ± 0.542	0.73
Time to peak Tmax (h)	0.83 ± 0.05	0.25 ± 0.05	0.000
Peak concentration Cmax (µg/ml)	0.47 ± 0.0577	0.44 ± 0.0577	0.52

square regression analysis (Table 3). The two compartmental studies showed that $t_{1/2abs}$ was not significantly affected by co-administration of the ciprofloxacin eye drops with the diclofenac sodium eye drops (P = 0.73), however, the $t_{1/2\alpha}$ (P = 0.72) were not significantly affected, while $t_{1/2\beta}$ were significantly affected (P = 0.03). Similarly, apparent volume of distribution, volume of central compartment, steady-state distribution volume, mean residence time and AUC were also significantly reduced due to concurrent administration of the diclofenac sodium eye drops with the ciprofloxacin eye drops (P = 0.01, 0.05, 0.02, 0.04 and 0.01).

On the other hand, the total ocular clearance (Cl_t) was significantly increased (P = 0.04) and absorption rate constant and (P = 0.58) was non-significant, due to concurrent administration of the diclofenac sodium eye drops, while C_{max} and lag t_{max} were not significantly affected.

DISCUSSION

After the absorption phase, the initial part of the curve (Group II) showed a rapid decline in aqueous humor drug concentration when compared with the second part of the curve. The initial steep decline in aqueous humor drug concentration may be due to distribution of drug from the central to peripheral compartment (*α*-phase, distribution phase). Once apparent distribution equilibrium had been established, the rate of decline in aqueous humor drug concentration reduced, and it is determined mainly by irreversible elimination of drug from central compartment, termed as *β*-phase or elimination phase. The present studies showed the slow second phase as compared to the first phase, which indicated the slow elimination of drug from aqueous humor. According to this model, drug may distribute and equilibrate after reaching the aqueous humor to various tissues in anterior and posterior chambers and then eliminated through trabecular meshwork and uveoscleral routes. The rate of distribution of drug from aqueous humor to tissue compartment is related with changes in drug concentration in aqueous humor compartment and tissue compartment.

Drug transporters may play an important role in the penetration of drug through ocular barriers (Mannermaa et al., 2006). The P-gp, an ATP-binding cassette encoded by MDR1 gene, has been identified in cornea, retina, iris and conjunctiva (Kwatra et al., 2010; Dey et al., 2003, 2004; Yang et al., 2007) that is known to be the major barrier in the drug transportation. It may restrict the ocular absorption of the drugs, which may be the cause of the lower ocular bioavailability of the drugs. P-gp is an important determinant in the pharmacokinetics of the drugs in various body tissues; however, no sufficient data is available regarding its role in the pharmacokinetics of drugs in eye; although, many drug substrates of P-gp have been identified. The abundance of the MDR1, MRP2 and BCRP is low but MRP3 and particularly MRP1 in human cornea may be the potential barriers for the transport of the drug across the cornea. Transporters can alter the pharmacokinetics of the drugs by the following mechanisms: (1) drug is a substrate of transporters that may facilitate or impair the drug delivery, (2) drug may modulate the transporters of another drug by inhibition or expression and (3) polymorphism of the transporter of the drug (Dey et al., 2004). Studies have shown that the fluoroquinolones are good substrates of MRP (Karla et al., 2007), and that various MRP efflux transporters are

inhibited by NSAIDs at therapeutically relevant concentrations (El-Sheikh et al., 2007), thus may play important role in drug-drug interactions when these groups of drugs are concurrently administered.

Conclusion

It was clear from the data that T_{max} shift from 0.83 to 0.25 h occurred, but when ciprofloxacin eye drops were applied onto the cornea in combination with diclofenac sodium eye drops, a statistically significant difference were observed in AUC, VD, E-half life and MRT, and indicating a decreased absorption rate of the ciprofloxacin in the presence of the diclofenac sodium, while total ocular clearance will increase with combination significantly. However, no significant difference was observed in the C_{max} and T_{max} when ciprofloxacin eye drops were administered to the eye in combination with diclofenac sodium eye drops.

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