The effect of norfloxacin on pharmacokinetics of carbamazepine at steady state in rabbits

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Abstract

Introduction: Carbamazepine (CBZ) is one of the most commonly prescribed antiepileptic drugs. Carbamazepine is rapidly absorbed with a bioavailability of 75.85% with plasma protein binding of 75%. It is extensively metabolized in the liver, primarily by CYP3A4, to carbamazepine-10, 11-epoxide. CBZ initial half-life elimination values ranges from 25-65 hours, decreasing to 12-24 hours on repeated doses, because of autoinduciton effect. Metabolism of drugs by cytochrome system can lead to several drug-drug interactions, which result in decrease pharmacological action, drug toxicity and adverse drug reactions. Norfloxacin (NFX) is an antibiotic, which exhibited a moderate CYP3A4 inhibitory effect. The aim of this study was to investigate the effect of NFX on pharmacokinetics of CBZ at steady state in rabbits.

Materials and Method: An in vivo drug-drug interaction, a randomized, crossover design study was conducted in six healthy male rabbits between NFX and CBZ. The study was carried out on two periods, first period (CBZ) was administered alone as daily single oral dose (40 mg/kg) for 10 days. In the second period, CBZ was administered as a single oral dose (40 mg/kg) for three consecutive days. On the fourth day a single dose of NFX (11.4 mg/kg) was given orally along with CBZ for the following seven days to each rabbit, after a washout period (10 days). Serial blood samples were collected over a period of 24 hours after the last dose of CBZ. Chemiluminescent enzyme immunoassay (CLEIA) was used to measure CBZ concentration in serum. Pharmacokinetic parameters as C_{max}, t_{max} AUC_{0-24}, $AUC_{0-\infty}$, $t_{1/2}$ and the constant rate of elimination K_e were determined for the two periods.

Results: Six rabbits were enrolled in the study which exhibited good tolerability to CBZ and NFX formulations. No statistical differences were found based on ANOVA between the two periods. The mean values of PK parameters for first and second periods were as follows: C_{max}= 9.970 versus 8.400 µg/ml, $AUC_{0-\infty}$= 154.1 versus 166.8 µg.h/ml, AUC_{0-24}= 130.3 versus 113.6 µg.h/ml, ke= 0.0587 versus 0.0419 h^{-1}, t_{max}= 4.330 versus 4.580 h and t½= 12.78 versus 21.34 h for the first and second periods, respectively.

Conclusion: No significant differences in PK of CBZ was found when CBZ was administered alone or in combination with NFX (P> 0.05).

Keywords: Carbamazepine, Norfloxacin, Drug interaction, Pharmacokinetic, CYP3A4, Cytochrome P450.

Introduction

Carbamazepine (CBZ) is one of the most commonly prescribed antiepileptic drugs. It is used on a long-term basis for control of generalized tonic clonic seizures and psychosis.[1,2] Carbamazepine is rapidly absorbed with a bioavailability of 75.85% and plasma protein binding of 75%. It is extensively metabolized in the liver, primarily by CYP 3A4, to carbamazepine-10, 11-epoxide which is pharmacologically active.[2,3]

CYP 450 is a family of isozymes responsible for biotransformation of numerous endogenous compounds such as steroids, bile acid, prostaglandins, fatty acids and leukotrienes and metabolises many of drugs, pollutant and environmental chemicals.[4-6]

Cytochrome P450 system is an evolutionary system to deal with the breakdown of endogenous and exogenous chemicals in the body. It is a family of isosymes responsible for metabolism of several drugs.[7,8]

CBZ is metabolized primarily by CYP 3A4. Also, other isoenzymes as CYP2C8, CYP2B6, CYP2E1, CYP1A2, and CYP2A6 are contributed. Less than 2% of an administered dose is excreted as unchanged carbamazepine in urine.[2]. Metabolism of drugs by cytochrome system can lead to several drug-drug interactions, which result in decreasing the pharmacological action, drug toxicity and adverse drug reactions.[7]

Norfloxacin (NFX) is a widely used representative member of flouroquinolone and is the first choice of drug for the treatment of bacterial infections of the urinary, biliary and respiratory tracts.[9] Norfloxacin is a synthetic, broad-spectrum fluoroquinolone antimicrobial agent. In vitro NFX has activity against a broad range of gram-positive and gram-negative aerobes but no activity against anaerobes.[10]

The effect of Norfloxacin (NFX) on human hepatic microsomal fraction that was prepared form a female, who exhibited a high CYP3A4 activity showed inhibition of activity by 64%.[11] NFX also inhibited the metabolism of cyclosporine A in human liver microsomes by 56%, when 1µm of cyclosporine A was incubated in presence of 100 µm NFX.[12] NFX had significantly also depressed the N-demethylation of erythromycin in human microsomes (mediated by CYP3A4) and in rat microsomes mediated by

CYP3A4.[11] Also NFX had affecting pharmacokinetics of some drugs in vivo like caffeine, theophylline and cyclosporine. The observed interactions were explained by inhibitory effect on CYP450 enzymes.[13-15]

The aim of this study was to investigate the effect of NFX on pharmacokinetics of CBZ at steady state in rabbits.

Materials and Method

Animals: Six healthy male adult rabbits (weighed 3.2-3.5 kg, aged: 8-10 months) should be fasted for 12 hours with free access to water (add libitum) before administration of the drug.[16,17] The rabbits were obtained from Asdda for animal production and welfare centre, where follow up care and clinical examination were performed.

Study design: An in vivo drug-drug interaction study was conducted in healthy male rabbits between Norfloxacin and Carbamazepine. The study was carried out on two periods, period one the CBZ will be administered alone as single daily oral dose and in period two the CBZ will be administered along with NFX with ten days washout period in-between the two periods. Pharmacokinetic parameters as Cmax, tmax AUC 0-t, AUC 0-∞, $t_{1/2}$ and the constant rate of elimination Ke was determined.

Blood sampling: Installation of IV-cannula to the ear marginal vein for each rabbit by a veterinary doctor.

In the first period CBZ suspension is given as daily single oral dose (40 mg/kg, equivalent to 7 ml tegretol suspension 2%) by a special oral gavage for 10 days. On the tenth day CBZ dose was given followed by collection of 1 ml of blood samples in vacutainer tubes according to the following time schedule 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0 and 24.0 hours. Centrifugation of blood samples at 3000 rpm for 5 minutes to separate serum and kept at 2-8 ^{0}C until being analyzed. After a Washout period for ten days the second period was started by given CBZ alone as a daily single oral dose (40 mg/kg) for three consecutive days and on the fourth day a single dose of NFX (11.4 mg/kg) was given orally along with CBZ for the following seven days to each rabbit. A suspension was prepared by mixing 400 mg NFX in 10 ml distilled water, 7 ml of this suspension were mixed with 49 ml tegretol 2% and from the final suspension 8 ml were given orally to each rabbit. On the tenth day CBZ and NFX are given at the same dose followed by collection of 1ml blood samples in vacutainer tubes according to the time schedule 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0 and 24.0 hours and the collected samples were treated as in the first period until being analyzed.

Analysis of serum samples: Analysis of rabbit serum samples to determine the concentration of CBZ was performed at laboratory of Medical Relief Society-Gaza using CBZ detection kit and IMMULITE 1000 System apparatus and (Siemens healthcare Diagnostics). The Kit is used for rapid quantitative detection of CBZ concentrations in serum for a large number of samples. IMMULITE 1000 CBZ is a solid phase, competitive, chemiluminescent enzyme immunoassay.

Pharmacokinetic analysis: The plasma pharmacokinetic parameters were estimated, which included the observed maximum plasma concentration C_{max}, the time to reach C_{max}, (T_{max}) and the area under the plasma concentration–time curve from 0 h to last measurable concentration (AUC $_{0-t}$) and 0 h to infinity (AUC $_{0-\infty}$). C_{max} and $_{Tmax}$ were directly determined from the serum concentration versus time curves. The area under the curve from 0 h to t (AUC $_{0-t}$) was calculated by the linear trapezoidal rule. The area under the curve from 0 h to infinity (AUC $_{0-\infty}$) was estimated by summing the area from AUC$_{0-t}$ and AUC $_{0-\infty}$, where AUC $_{0-\infty}$ =AUC0-t+Ct / ke, with 'Ct' defined as the last measured serum concentration at time t, and ke is the elimination rate constant. The elimination rate constant ke was estimated by the least squares regression of plasma concentration– time data points lying in the terminal region by using semilogarithmic dependence that corresponds to first-order kinetics. The half-life $t_{1/2}$ was calculated as 0.693/ke.

Pharmacokinetic analysis was performed by means of model independent method (Non-Compartmental Approach) WinNonlin Professional Software (Version 6.3, Pharsight Corporation, Cary, NC).

Statistical analysis: Analysis of Variance (ANOVA) was used to compare the calculated pharmacokinetic parameters of carbamazepine for the two periods using general linear model procedures, in which sources of variation were subject and period. The statistical analysis was performed using SPSS, version 16. (P-value) of 0.05 was considered statistically significant.

Results

The mean plasma concentrations of carbamazepine when administered alone or in combination with Norfloxacin are shown in Fig. 1. The concentration time profile obviously indicated that the two periods are comparable. The mean pharmacokinetic parameters of carbamazepine administered alone or in combination with Norfloxacin as well as the statistical significance following their comparison are given in Table 1.

Table 1: Paired-samples t-test for the equality between the means of the pharmacokinetic parameters of CBZ in both periods. first period: CBZ was administerd orally as daily single dose (40 mg/kg) for ten days and in the second period CBZ was administered as in the first period and on the fourth day NFX (11.4 mg/kg) was given concurrently

Parameter	Group	N	Mean	Standard deviation	Difference	T	P-value
C_{max}	CBZ+alone	6	8.40	2.42	5	1.43	0.210
	CBZ+ NOR	6	9.97	2.50			
t_{max}	CBZ-alone	6	4.33	0.516	5	-0.620	0.562
	CBZ+ NOR	6	4.58	1.20			
$t_{1/2}$	CBZ-alone	6	12.77	4.100	5	-1.246	0.268
	CBZ+ NOR	6	21.33	14.67			
Ke	CBZ-alone	6	0.0587	0.0171	5	1.429	0.212
	CBZ+ NOR	6	0.0419	0.0184			
AUC_{0-24}	CBZ-alone	6	0.0132	42.51	5	1.265	0.262
	CBZ+ NOR	6	0.0136	29.26			
$AUC_{0-\infty}$	CBZ-alone	6	0.0154	54.31	5	-0.771	0.476
	CBZ+ NOR	6	0.0166	67.51			

Significant statistical difference ($P \leq 0.05$)
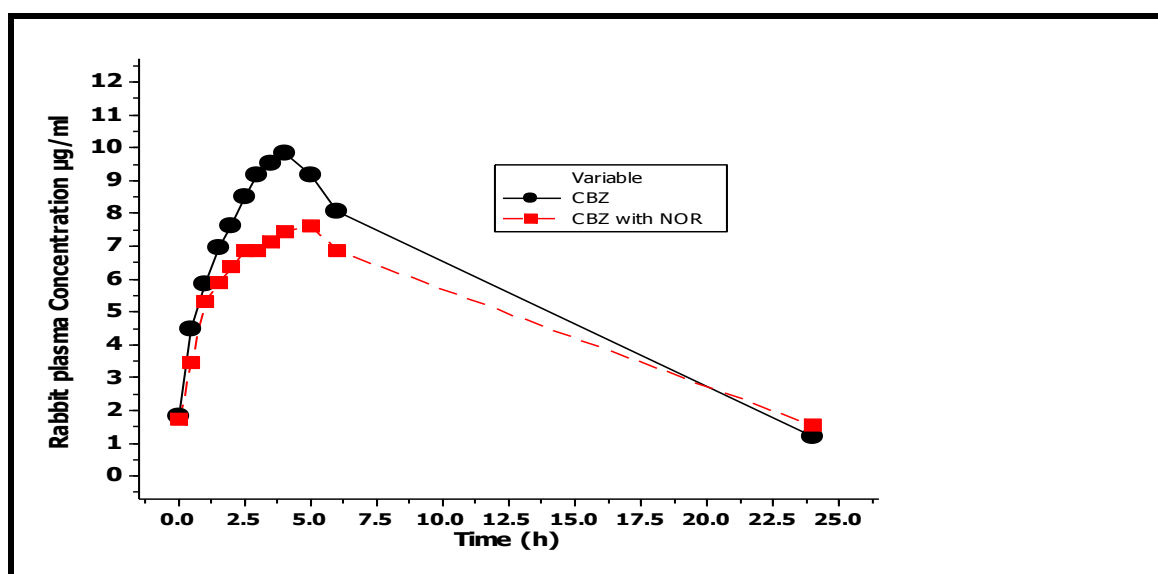
Fig. 1: Mean serum concentration-time profile of CBZ after administration of 140 mg CBZ suspension alone and in the presence of Norfluxacin (140 mg) for rabbits (n=6)

Discussion

CBZ as anti-epileptic drug which is used lifelong. It is a substrate of CYP3A4. CBZ has narrow therapeutic index. Inhibition of CYP3A4 can result in the accumulation of parent drug that can put the patient at increased risk for side effects or possible toxicity. Rabbits were ideal animals for studying the pharmacokinetic parameters and drug-drug interaction between CBZ and NFX. They were easy to handle for giving multiple oral doses of drugs and collecting blood samples. In rabbits it has been seen that the isoenzyme CYP3A6 correspond to the CYP3A4 activity in human hepatocytes.[18] Hence, drugs like CBZ that are metabolized by CYP3A4 in human will be biotransformed by CYP3A6 in rabbits. Any drug interaction occurring due to an effect on this particular cytochrome i.e. CYP3A6 in rabbits will correlate to an interaction at CYP3A4 levels in humans.

Statistical analysis of PK of CBZ showed no significance difference between the two periods by using paired sample t-test (**Table 1**). From statistical treatment, insignificant difference in **Cmax** was observed. The mean **Cmax** was decreased from 9.970 μg/ml in the second period to 8.400 μg/ml in the first period (P value = 0.210).

A slight decrease in the elimination phase was observed (ke = 0.0587 and 0.0419 h^{-1} for the first and second period, respectively). This effect was

statistically insignificant (P value = 0.212). Similar results were found by studying the effect of NFX on PK of caffeine[15] and theophylline at steady state.[19]

The elimination half-life $t_{1/2}$ was increased ($t_{1/2}$ =12.77 h and 21.33h for the first and second period, respectively). This is due to inhibitory effect of NFX on CBZ metabolism which was little and remained statistically insignificant (p=0.268).

These results are similar to those recorded in drug interaction studies, applied to investigate the effect of NFX on PK of warfarin[20] and theophylline.[19] In contrast, a fluoroquinolone antibiotic ciprofloxacine had significantly increased Cmax, AUC and $t_{1/2}$ of CBZ, while CL and Vd of CBZ were decreased significantly when CBZ was administered concurrently with ciprofloxacin (single dose 500 mg) in adult volunteers.[21]

CBZ is one of the drug after multiple doses, can stimulate the synthesis of enzymes that catalyze its own metabolism by a process known as auto-induction.[2,3] An autoinduction effect can be observed after 2 weeks of treatment.[22] This can explain the insignificant effect of NFX on CBZ. CBZ may increase its metabolism during the ten days of treatment which may abolish the effect of NFX on the PK of CBZ. Autoinduction of CBZ metabolism appeared to be complete within 1 week of starting CBZ therapy or dose change, and its degree was linearly related to CBZ daily dose.[23]

In this study CBZ was given for ten days during which metabolism of CBZ can be induced. This can abolish the expected inhibitory effect of NFX on metabolizing enzymes. In addition, flouroquinolone antibiotics as Norfloxacin and Ofloxacin have been shown to be a subject to active efflux.[24,25] An inhibitory effect of NFX is dose dependent. NFX may be effluxed back to intestine. A crossover study design is recommended to minimize the subject variability between the two group of treatments.[26]

Conclusions

No significant differences in Pharmacokinetic parameters of CBZ was found when CBZ was administered alone or in combination with NFX (P> 0.05).

Conflict of Interest

There is no conflict of interest.

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