



CURCUMIN'S IMPACT ON CARBAMAZEPINE PHARMACOKINETICS AT VARIOUS DOSES IN RABBITS

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The purpose of this study is to explore the influence of preadministration of Curcumin (CUR), at two distinct dosages (60 and 90 mg/kg body weight per day, 8 days, p.o.), on the pharmacokinetic (PK) profile of Carbamazepine (CBZ) in rabbits. Eighteen rabbits were divided randomly into 3 groups (6 rabbits per group). Control group were given (20 mg/kg of CBZ, as single dose, p.o.). The first and second test groups were given CUR (60 and 90 mg/kg body weight per day, 8 days, p.o.). On the eighth day, CBZ (20 mg/kg) was administered one hour after giving CUR's last dose. Blood was drawn from a marginal ear vein at various intervals (0.5, 1, 1.5, 2, 2.5, 3.5, 4, 6, 12, and 24 hr.). CBZ levels in serum were determined using a Chemiluminescent Enzyme Immunoassay (CLEIA). C_{max}, t_{max}, AUC₀₋₂₄, AUC_{0-∞}, K_e and CL/F were calculated. Statistically insignificant differences were found in the different PK parameters of CBZ. The results demonstrated that prior treatment of CUR at both concentrations, had no influence on PK parameters of CBZ in rabbits.

INTRODUCTION

Carbamazepine (CBZ) is used to treat partial seizures, generalized tonic-clonic seizures, trigeminal neuralgia, and bipolar disorders all over the world¹. Because it has a significant low therapeutic index and a wide inter-individual variation at tolerable doses, therapeutic drug monitoring is necessary^{2&3}. Regardless of its clinical approval, CBZ has several PK properties, making it convenient for interaction with co-administered substances, including drugs, herbal products, and food⁴. CYP3A4 is the main enzyme that contributes to CBZ metabolism, leading to the formation of CBZ 10,11-epoxide, which is an active metabolite that participates in the efficacy and toxicity of CBZ^{5&6}. Drug interactions are classified into two categories: pharmacodynamic and PK interactions⁷⁻⁹.

In general, the variation in drug PK is related to the absence of a consistent relationship between the used dose of the medicine and its concentration at the action¹⁰. Medicinal remedies are believed to be natural and secure. However, some of their

constituents can modify various metabolizing enzymes and transport systems, which play a significant role in the absorption and disposition of co-administered drugs. Consequently, herb-drug co-administration may initiate herb-drug interactions by altering the function of drug-metabolizing enzymes and/or drug transporters^{11&12}.

Curcumin (Diferuloylmethane, CUR), a polyphenol constituent in the spice turmeric, is a specific secondary metabolite of *C. Curcuma longa*, *C. zedoaria*, *C. aromatica*, *C. wenyujin*, and *C. kwangsiensis*¹³. Curcumin is the main chemical compound of Turmeric and proven for its anti-inflammatory, antioxidant, antimutagenic, antidiabetic, antibacterial, hepatoprotective, expectorant and anticancerous pharmacological activities¹⁴. Several in vitro studies have indicated that CUR inhibits the activity of the CYP3A4 enzyme. Based on these findings, CUR co-administration should increase the oral bioavailability of CYP3A4 substrates¹⁵. The impact of preadministration of CUR at various doses on the PK profile of CBZ, a CYP 3A4 substrate, is investigated in the present study.

MATERIALS AND METHODS

Animals

Eighteen male rabbits weighted (3.1-3.4 kg) divided into three groups (six per group) were selected and used as an animal model for the present PK interaction study. The animals were placed in normal lab circumstances for 12 hours day/night at room temperature, fed improvisationally with nutritional pills and water, while fasting was followed the night before the blood draw.

Design of the study and blood sampling

Healthy male rabbits (n= 6) were used in PK interaction research experiments involving CUR and CBZ in three groups of animals in a parallel designed manner. In the control group, rabbits were given a volume equivalent to CBZ (20 mg/kg as single dose, p.o.) from an oral suspension of 2% (Tegretol, Novartis). Serial venous drawing blood samples (1.0-1.5 ml) obtained from rabbits ear marginal vein using special cannula (21G) at various time intervals: 0.5, 1, 1.5, 2, 2.5, 3.5, 4, 6, 12, and 24 hr. post-dosing¹⁶. Meanwhile, rabbits of the first and second test groups were given a volume of CBZ (20 mg/kg as single dose, p.o.) at the same conditions as in the control group along with prepared in aqueous saline suspension a volume equivalent to (60 and 90 mg/kg body weight per day, 8 days, p.o) from CUR capsules (CUR Turmeric 550 mg Jamieson) for eight consecutive days. On the 8th day, CBZ was administered one hour after administering the last dose of CUR suspension, and blood samples were collected from all rabbits of the first and second test groups at the intervals mentioned as in the control group. The serum was collected by centrifuging the blood samples and was stored at (-80°C) until analysis for CBZ.

Analytical method for CBZ

The analysis was carried out using an ARCHITECT analyzer 1000 Abbott Laboratories, Abbott Park, IL, USA, utilizing CBZ detection kits that rely on Chemiluminescent Enzyme Immunoassay (CLEIA).

Pharmacokinetic Analysis

The PK profiles of CBZ in control and herb treated groups were plotted between serum changed the concentration of CBZ versus sampling time. The PK parameters of control and treated test groups were obtained through an independent method (Non-Compartmental Approach) WinNonlin Professional Software (Version 6.3, Pharsight Corporation, Cary, NC) and (GraphPad Prism versión 4.00, San Diego, CA, USA). The following PK parameters were calculated, including C_{max} , t_{max} , AUC_{0-24} , $AUC_{0-\infty}$, K_e and CL/F for control and test groups. The serum concentrations were used to construct PK profiles by plotting drug concentration-time curves. To determine PK parameters for control and treated with CUR groups of CBZ, all obtained data was subsequently fed into WinNonlin Professional Software (Version 6.3, Pharsight Corporation, Cary, NC) and (GraphPad Prism versión 4.00, San Diego, CA, USA). The non-compartmental PK parameters including maximum serum concentration (C_{max}) and time to reach maximum concentration (t_{max}), area under the concentration curve from 0 to 24 hrs. (AUC_{0-24}), area under the concentration curve from 0 to infinity ($AUC_{0-\infty}$), terminal elimination rate constant (K_e) and total body clearance (CL/F) were calculated. For each time point in each group, the data is presented as a mean with standard deviation (SD). The differences in CBZ PK parameters between the control and test groups were examined using general linear model techniques and an independent (unpaired) t-test. SPSS (Version 22.0) was used to undertake data analysis that met the significance requirements ($P < 0.05$).

RESULTS AND DISCUSSION

Serum CBZ concentration-time profiles obtained after p.o. administration of 20 mg/kg CBZ to rabbits in control and co-administered with CUR (60 and 90 mg/kg) in the first and second test groups is shown in Figure 1. The corresponding PK parameters, including C_{max} , t_{max} , AUC_{0-24} , $AUC_{0-\infty}$, K_e , and CL/F for control and CUR treated test groups are summarized in Table 1.

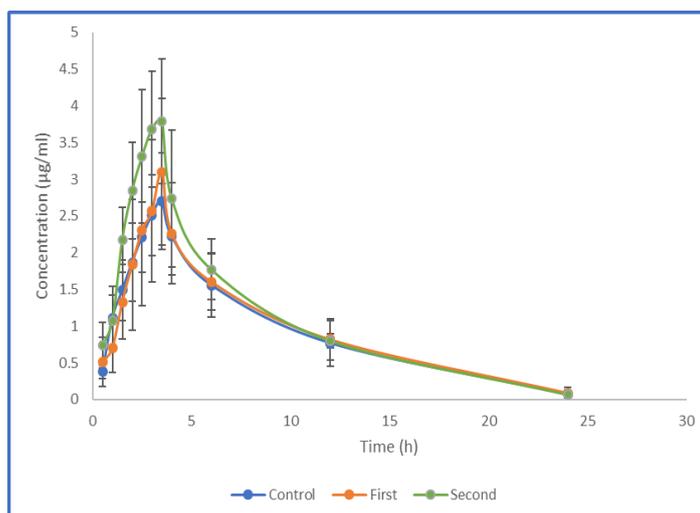


Fig. 1: The plot of the CBZ serum concentration-time profile. Control group: CBZ (20 mg/kg) was p.o administered alone. First and second test groups: CBZ (20 mg/kg) was co-administered along with CUR (60 and 90 mg/kg), respectively, in rabbits (n = 6).

Table 1: Calculated PK parameters of the control, first and second test groups (6 for each).

PK Parameters	Groups	Mean \pm SD	<i>P</i> -value
^a C _{max} (µg/mL)	Control group	2.70 \pm 0.84	0.361 [¥]
	First test group	3.07 \pm 1.00	
	Second test group	3.78 \pm 0.85	0.083 [§]
^b t _{max} (hr)	Control group	3.10 \pm 0.90	0.374 [¥]
	First test group	3.40 \pm 0.22	0.529 [§]
	Second test group	3.50 \pm 0.04	
^c AUC ₀₋₂₄ (µg*hr/mL)	Control group	22.80 \pm 6.49	0.680 [¥]
	First test group	23.25 \pm 7.26	0.562 [§]
	Second test group	27.10 \pm 5.36	
^d AUC _{0-∞} (µg hr/mL)	Control group	23.46 \pm 7.10	0.686 [¥]
	First test group	24.13 \pm 7.94	0.421 [§]
	Second test group	27.54 \pm 5.39	
^e Ke (hr ⁻¹)	Control group	0.136 \pm 0.04	0.840 [¥]
	First test group	0.123 \pm 0.03	0.136 [§]
	Second test group	0.148 \pm 0.07	
^f Cl/F (mL h ⁻¹)	Control group	2.76 \pm 0.91	0.838 [¥]
	First test group	2.88 \pm 0.98	0.185 [§]
	Second test group	2.24 \pm 0.43	

(¥): *P*-value of the differences between the control and first test group; (§): *P*-value of the differences between the control and the second test group.

- $P \leq 0.05$ Statistical significance, SD: Standard deviation, ^amaximum blood concentration, ^btime of peak concentration, ^carea under the concentration-time profile curve from 0 to 24 hours, ^darea under the concentration-time profile curve from 0 to infinity, ^eelimination rate constant and ^ftotal body clearance.

In the control group, the C_{max} of CBZ was found to be $2.70 \pm 0.84 \mu\text{g/mL}$, and the t_{max} was $3.10 \pm 0.90 \text{ hr}$. Meanwhile, in the first test group, the C_{max} and t_{max} were $3.07 \pm 1.0 \mu\text{g/mL}$ and $3.40 \pm 0.22 \text{ hr}$., respectively ($P > 0.05$). The systemic exposures defined by $AUC_{0-\infty}$ were also similar between the control and first test groups ($23.46 \pm 7.10 \mu\text{g}^*\text{hr/mL}$ vs. $24.13 \pm 7.94 \mu\text{g}^*\text{hr/mL}$; $P = 0.686$). Furthermore, the remaining PK parameters showed no significant variations, including AUC_{0-24} , K_e , and CL/F between the two groups (Table 1). Moreover, the comparison of serum concentration-time profiles of CBZ alone (control group) and treated with CUR 90 mg/kg (second test group) is shown in Figure 1. Their corresponding PK parameters are given in Table 1 as well. In this case, despite the observed increase in C_{max} and t_{max} of the second test group compared with the control group, the slight increase in C_{max} and t_{max} of CBZ were statistically non-significant ($P > 0.05$). The extent of CBZ absorption (measured by $AUC_{0-\infty}$) between the control and second test groups was insignificant ($P > 0.05$). Similarly, the PK parameters k_e , CL/F , and AUC_{0-24} between both groups were statistically insignificant ($P > 0.05$).

Traditional herbal medicine plays an essential role in the treatment of many diseases, such as epilepsy. However, herbal medicine should undergo evidence-based scrutiny due to the deficiency of clear and strong evidence for most herbs' efficacy and toxicity¹⁷. Besides, the herbs can perform their actions through various mechanisms that may alter the PK profile of the co-administered drug¹⁸. Therefore, it is highly critical to be aware of the possible interactions of the frequently used herbal medicines¹⁹. Treatment failure and exacerbation of side effects of drugs with a narrow therapeutic index are related to co-administration of an agent inhibiting or inducing the activity of the cytochrome P450 (P450) enzyme system^{20&21}. CYP3A4 is primarily involved in CBZ metabolism, where the end product of its metabolism is the formation of CBZ 10,11-epoxide, an active metabolite that contributes to the toxicity and efficacy of CBZ^{5&6}.

Based on the PK herb-CBZ interactions, the findings summarized in a review article published by Fong and his collaborators

showed that a reduction in the plasma level of CBZ results in a decrease in CBZ bioavailability due to the over-activity of the CYP3A4 enzyme when some herbal medicines interact with CBZ. On the contrary, the inhibitory effect on CYP3A4 activity led to an elevation of CBZ plasma level with increased CBZ bioavailability. Furthermore, the PK interaction was statistically insignificant on CBZ bioavailability when other herbs were co-administered with CBZ in vitro/in vivo and human models were shown to be studied⁴.

Previous herb-drug interaction experiments on CBZ, theophylline, and cyclosporine (narrow therapeutic indexed drugs) were published utilizing five to six rabbits that were pretreated with herbal products for 7-8 days²²⁻²⁶.

In the present study, statistically insignificant differences in the PK profile of CBZ were observed in the following parameters: C_{max} , t_{max} , AUC_{0-24} , $AUC_{0-\infty}$, K_e , and CL/F compared to the control group when pretreated with CUR at different doses (in the first and second test groups) on the PK profile of CBZ ($P > 0.05$). Similar results were obtained by Alkharfy and his research group when they studied the PK interaction between *Nigella Sativa* and CBZ by using rabbits as an animal model ($n = 5$ and for eight days). They found that the PK of CBZ does not change with and without *Nigella sativa*²². Also, in another study published by Abushammala *et al.*, demonstrated that the different PK parameters of CBZ were not altered when it was given alone or concurrently with *Panax Ginseng* by using rabbits as an animal model ($n = 6$ and for eight days)²³.

Regarding CUR, the combination of CUR with prescribed drugs should increase the oral bioavailability of CYP3A4 substrates due to the CYP3A4 inhibition effect¹⁵. The PK data revealed that CUR-treated animals had significantly altered the PK profile of Norfloxacin in rabbits²⁷. Meanwhile, the slight increase in the PK parameters C_{max} and t_{max} in the CUR treated group when co administered with theophylline was statistically insignificant. Also, the PK profile of Glucilazide was not altered by single-or multiple-doses of CUR pretreated rabbits²⁸.

Finally, the study published by Liu and collaborators (when using wistar rats as an animal model for 7 days) found that the

interaction between Warfarin (a narrow therapeutic index drug) and CUR at a small and medium dose (25 and 50 mg/kg) had no substantial inhibition effect on the PK parameters of Warfarin. In contrast, the effect was statistically significant at a larger dose of CUR (100 mg/kg)²⁹.

According to our experimental results, the CUR at tested concentrations (60 and 90 mg/kg) for eight days had not altered the PK profile of CBZ or produced a remarkable effect on its profile at a dose of 20 mg/kg. Otherwise, more research projects should be conducted with the aim of giving more explanation of the effect of CUR on the PK of CBZ by using higher CUR doses and/or a longer period of CUR co-administration.

Conclusion

Pretreatment herb-drug interaction involving CUR and CBZ is the subject of the current study. In the animal model, the interaction between CUR and CBZ was found to be pharmacokinetically insignificant. The PK profile of CBZ was unaffected by co-administration of CBZ with CUR at two different doses. Based on these findings, using CUR in combination with CBZ in an animal model appears to be safe. We need to confirm our findings by using a larger number of animals to reduce inter-individual variability and providing greater CUR doses to check if the CBZ/CUR interaction is significant before they can be used in patients. Simultaneously, delivering CUR and CBZ simultaneously should be done with caution until prospective clinical studies indicate that there is no significant interaction in humans.

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نشرة العلوم الصيدلانية جامعة أسيوط



تأثير الكركومين معاملات الحرائك الدوائية للكاربامازيبين في جرعات مختلفة في الأرناب

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الهدف من هذه الدراسة هو تحديد كيفية تأثير الكركمين (CUR) على معاملات الحرائك الدوائية (PK) للكاربامازيبين (CBZ) في الأرناب. تم تشكيل ثلاث مجموعات من الأرناب (ن = ١٨). وأعطيت ستة أرناب في المجموعة الضابطة ٢٠ مغ / كغ من CBZ عن طريق الفم ومن ثم تم سحب الدم من وريد الأذن الهامشي للأرناب على فترات زمنية مختلفة. وفي الوقت نفسه ، تم إعطاء الأرناب المتبقية ٦٠ و ٩٠ مجم / كجم (مجموعتي الاختبار الأولى والثانية) على التوالي ، عن طريق الفم لمدة ثمانية أيام. في اليوم الثامن ، تمت إعادة تناول (٢٠) CBZ مجم / كجم) بعد ساعة واحدة من إضافة آخر جرعة من المنتجات العشبية المحتوية على الكركمين. تم الحصول على (١.٠-١.٥ مل) من عينات الدم الوريدي من الأوردة الهامشية لأذان الأرناب في فترات مختلفة محددة مسبقًا. تم تحديد مستويات الأدوية في المصل باستخدام المقاييس المناعية الإنزيمية المضيفة (CLEIA) ، وتم حساب معاملات الحرائك الدوائية للكاربامازيبين باستخدام التحليل غير الجزئي للمجموعات الثلاث. لوحظت اختلافات ووجد أنها غير ذات دلالة إحصائية في معاملات PK المختلفة لـ CBZ ، بما في ذلك: C_{max} ، t_{max} ، AUC_{0-24} ، $AUC_{0-\infty}$ ، Ke و CL / F بعد الإعطاء المشترك مع وبدون CUR. توضح النتائج أن الاستخدام المترام لـ CUR في كلا التركيزين المستخدمين في الدراسة مع CBZ ليس له أي تأثير على معاملات الحرائك الدوائية الخاص بـ CBZ في الأرناب ، مما يعني أنه لا توجد احتياطات عند إعطاء CBZ باستخدام CUR.