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ORIGINAL RESEARCH

Interaction of carbamazepine and chlorpromazine in rabbits

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ABSTRACT: The interaction of carbamazepine and chlorpromazine in rabbits has been studied. The drugs were administrated as single oral doses (200 mg of each drug). The sequence of administration of the drugs was varied. It has been established that by simultaneous administration these drugs decrease absorption of each other in plasma. This may be explained by competition of the drugs to transfer from the gastrointestinal tract into plasma, as well as by the formation of complexes, more or less stable and more or less bound to gastrointestinal tissues. Carbamazepine intensifies the biotransformation of chlorpromazine, which may be caused by the ability of carbamazepine to induce microsomal liver enzymes. Chlorpromazine suppresses the biotransformation of carbamazepine, however. This may be caused by intensive capture of chlorpromazine by liver tissues and by its intensive biotransformation, which in turn is conditioned by its surface-active nature and by the increase of its metabolism with carbamazepine. Therefore the biotransformation of chlorpromazine is increased and metabolism of carbamazepine is reduced. The sequence of administration of the drugs affects their pharmacokinetics significantly. Copyright © 1999 John Wiley & Sons, Ltd.

INTRODUCTION

The mechanism of drug interaction *in vivo* is considered via changes in absorption, biotransformation, excretion and influence on the receptors (Eadie and Tyrer, 1983). The presystem metabolism and degree of binding of drugs and their metabolites with plasma proteins are also important factors (Kivmann *et al.*, 1982; Markova and Nezhentsev, 1994). The surface-active nature of some drug molecules introduces their own contribution to these phenomena (Florence, 1980). Some tranquillizers, anesthetics and antihistamines (promethazine, chlorpromazine, etc.) belong to this group of surfactants. They preferentially adsorb on the surface of the gastrointestinal tract. When in some region of the organism the concentration of the drug becomes high, the formation of associates of different sizes is also possible. Chlorpromazine (CPM), a strong neuroleptic, is used in antiepileptic therapy when other drugs are unsuccessful (Mashkowsky, 1977). In some cases CPM stops the seizures or decreases their frequency, but sometimes the condition of the patient becomes worse.

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Abbreviations used: CBZ, carbamazepine; CPM, chlorpromazine; ECBZ, epoxycarbamazepine.

Our goal was to study the interaction of the widely used antiepileptic drug carbamazepine (CBZ) with CPM (possessing a surface active nature) in rabbits.

EXPERIMENTAL

Sample pretreatment. For the determination of CPM, 0.2 mL of phenothiazine solution (8 μg/mL; internal standard) and 0.3 mL of 0.3 M hydrochloric acid were added to 0.5 mL of blood plasma (Rukhadze *et al.*, 1998). For the simultaneous determination of CBZ and epoxycarbamazepine (ECBZ), 0.2 mL of halodiph solution in chloroform at concentration 14 μg/mL, and 200 μL of 4.0 M sodium hydroxide were added to 0.2 mL of blood plasma (Alexishvili *et al.*, 1997). The analytes were extracted with 3 mL of chloroform. The extraction mixture was agitated by hand for 1 min and centrifuged for 10 min at 3000g. The organic layer was filtered, transferred into a clean test-tube and evaporated to dryness at room temperature with a gentle flow of air. The residue was dissolved in 50 μL of mobile phase and 20–30 μL were injected for HPLC analysis.

Chromatographic procedure. The analyses were performed on a "Milichrom" (Nauchpribor, Oryol, Russia) microcolumn high-performance liquid chromatograph equipped with a UV absorption variable-wavelength (190–360 nm) detector and syringe-type pump. The column used was a Separon-C_{18} (Lachema, Brno, Czechoslovakia) column (62 × 2 mm, i.d.) with particle size 5 μm.

The mobile phase used for the determination of CPM was a mixture of ethanol:0.01 M potassium dihydrogen phosphate

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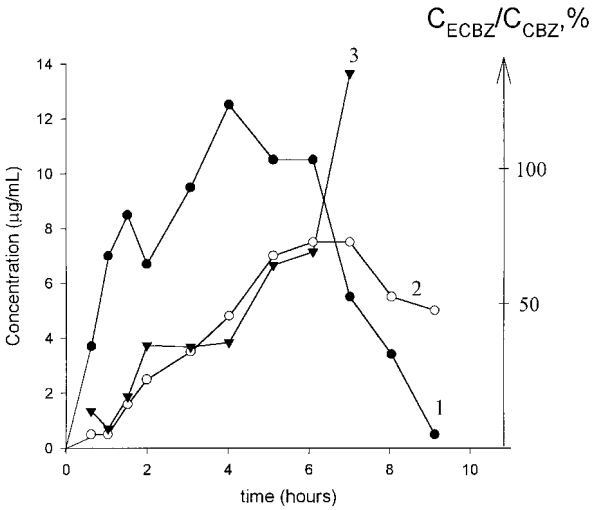

Figure 1. Plasma concentration–time curves in the rabbit after oral administration of 200 mg CBZ. (1) Concentration of CBZ; (2) concentration of ECBZ; (3) C_{ECBZ}; C_{CBZ} ratio in percentage from C_{CBZ}.

(52:48). As an ion-pairing agent, 0.25% triethylamine (v/v) was added to the mobile phase. The mobile phase pH was adjusted to 3 with phosphoric acid. A UV detector was operated at 260 nm.

The mobile phase composition for CBZ and ECBZ analysis was 0.05 M Na_2HPO_4: acetonitrile (40:60, v/v). The pH of the eluent was adjusted with phosphoric acid to 4.0. Detection of analytes was carried out at a wavelength of 210 nm.

The flow rate of eluent was 50 μL/min. Analyses were carried out at ambient temperature. Because of the light-sensitive character of CPM, sample preparation was carried out in dark test-tubes.

Experimental animal. Blood samples were collected from the ear of a rabbit (weight 2.7 kg) at several time points. CBZ (200 mg) and CPM (200 mg) were administered to the rabbit as single oral doses. Additional drugs were given sequentially with an interval of 15 min.

RESULTS AND DISCUSSION

Influence of chlorpromazine on the pharmacokinetics of carbamazepine

The pharmacokinetic curves of CBZ (curve 1) and its metabolite ECBZ (curve 2) after a single dose are given in Fig. 1. There are two peaks on the ascending branch of CBZ and elimination of the drug proceeds with almost constant rate. This is probably due to saturation of microsomal liver enzymes at high CBZ doses (74 mg/kg). Possibly, the elimination of CBZ obeys Michaelis–Menthen kinetics.

The kinetic curve of ECBZ is S-like and maximum concentration (C_{max}) is reached 2 h later than CBZ (Fig. 1, curve 2). The value of the C_{ECBZ}:C_{CBZ} ratio increases

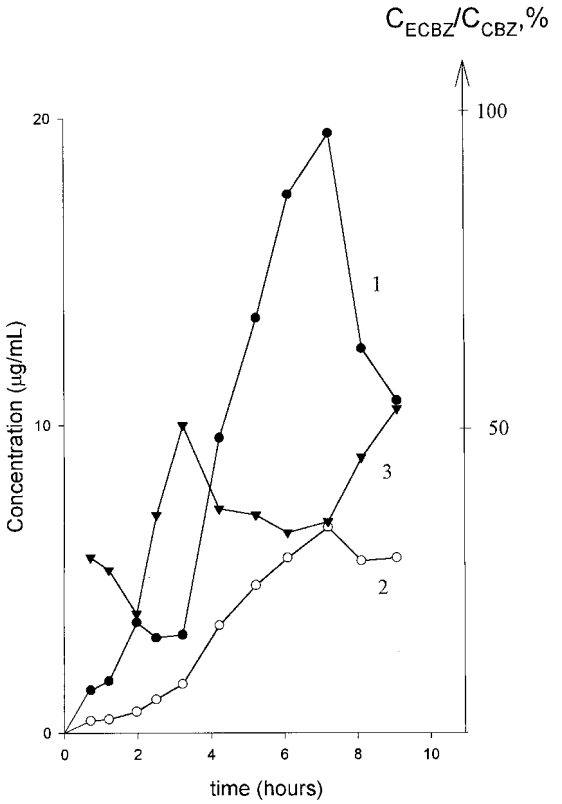

Figure 2. Plasma concentration –time curves in the rabbit after oral administration of 200 mg CBZ + 200 mg CPM. (1) Concentration of CBZ; (2) concentration of ECBZ. (3) C_{ECBZ}; C_{CBZ} ratio in percentage from C_{CBZ}.

from 15 to 40% until CBZ reaches C_{max}, but from t_{max} of ECBZ the metabolite concentration becomes higher than the CBZ concentration (Fig. 1, curve 3).

In order to study the interaction of CBZ and CPM the sequence of administration of the drugs was varied: (a) 200 mg CBZ were given and 15 min later 200 mg CPM (CBZ + CPM); and (b) 200 mg CPM were given and after 15 min 200 mg CBZ (CPM + CBZ). The pharmacokinetic curves for cases (a) and (b) are illustrated in Figs 2 and 3. In Figs 4–6 the kinetic curves of CBZ (Fig. 4), ECBZ (Fig. 5) and C_{ECBZ}:C_{CBZ} ratio vs time (Fig. 6) are given. Curves 1 correspond to the administration of CBZ only, curves 2 to CBZ + CPM and curves 3 to CPM + CBZ.

It is clear from Fig. 4 that CPM strongly affects the pharmacokinetics of CBZ: the concentration of CBZ is sharply decreased at the beginning of the process. In the case of CPM + CBZ the low concentration of CBZ is maintained until the end of the process (Fig. 4, curve 3), but in the case of CBZ + CPM, after the first peak of CBZ, a considerable increase of C_{CBZ} takes place (Fig. 4, curve 2); therefore the maximum concentration of CBZ is higher than in the case of administration of CBZ only.

CPM also significantly influences the kinetics of

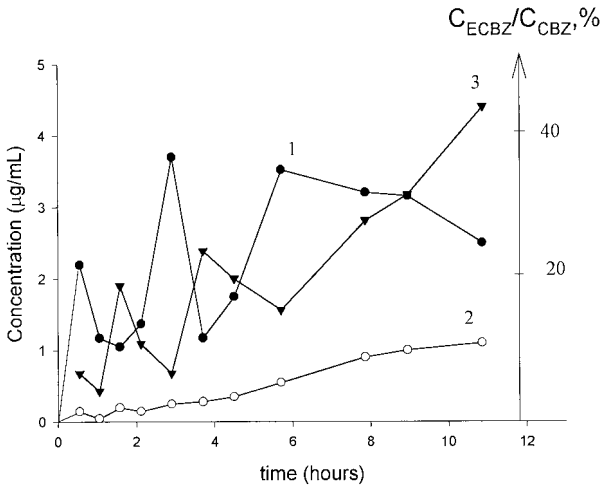

Figure 3. Plasma concentration–time curves in the rabbit after oral administration of 200 mg CPM + 200 mg CBZ. (1) Concentration of CBZ; (2) concentration of ECBZ; (3) C_{ECBZ}; C_{CBZ} ratio in percentage from C_{CBZ}.

ECBZ (Fig. 5): in the presence of CPM the formation of ECBZ is suppressed, especially in the case of CPM + CBZ (Fig. 5, curve 3).

A further significant increase in C_{CBZ} and W_{absCBZ} (rate of absorption of CBZ), considerable suppression of ECBZ formation (Fig. 5, curves 1 and 2) and decrease in the value of C_{ECBZ}:C_{CBZ} ratio (Fig. 6, curves 1 and 2) point to the reduction of biotransformation of CBZ.

Analogous results are obtained by the comparison of CPM + CBZ and CBZ only. A strong decrease of C_{ECBZ} (Fig. 5, curves 1 and 3) and a considerable reduction of the C_{ECBZ}:C_{CBZ} ratio (Fig. 6 curves 1 and 3) indicate that, in the case of CPM + CBZ, CPM suppresses the biotransformation of CBZ more strongly than in the case of CBZ + CPM. Taking this into account, the formation of multiple peaks of CBZ and a sharp decrease of C_{CBZ}

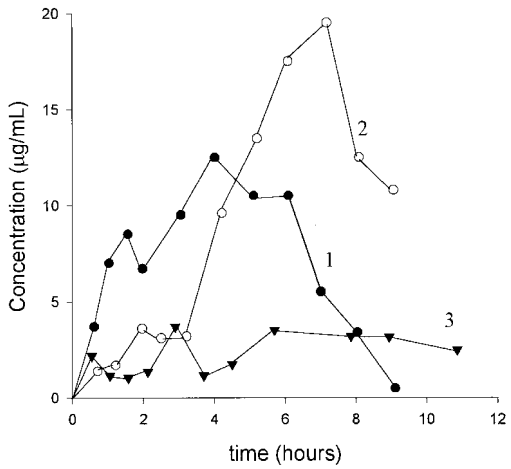

Figure 4. Plasma concentration–time curves of CBZ in the rabbit after oral administration of: (1) 200 mg CBZ; (2) 200 mg CBZ + 200 mg CPM; (3) 200 mg CPM + 200 mg CBZ.

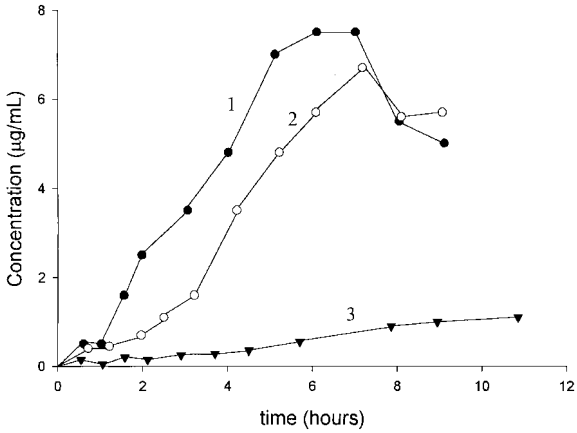

Figure 5. Plasma concentration–time curves of ECBZ in the rabbit after oral administration of: (1) 200 mg CBZ; (2) 200 mg CBZ + 200 mg CPM; (3) 200 mg CPM + 200 mg CBZ.

during whole processes points to the drastic reduction of absorption of CBZ in the presence of CPM.

Thus, independently of the sequence of drug administration, in the presence of CPM the degree of absorption and biotransformation of CBZ is significantly decreased.

The influence of carbamazepine on the pharmacokinetics of chlorpromazine

To elucidate the influence of CBZ on the pharmacokinetics of CPM, the results of administration of 200 mg CPM (74 mg/kg) and 200 mg CBZ + 200 mg CPM were compared (Fig. 7). In both cases the rather low concentration of CPM in blood plasma is significant. Because of the surface-active nature of CPM, it must be supposed that CPM intensively binds to the proteins of organs and tissues until it is transferred into plasma (Kholodov and Iakovlev, 1985). It is known that CPM is very intensively absorbed by liver and other eliminating

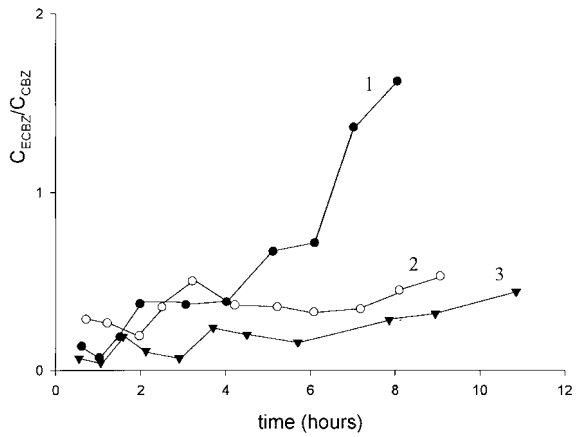

Figure 6. Curves of C_{ECBZ}; C_{CBZ} vs time in the rabbit after oral administration of: (1) 200 mg CBZ; (2) 200 mg CBZ + 200 mg CPM; (3) 200 mg CPM + 200 mg CBZ.

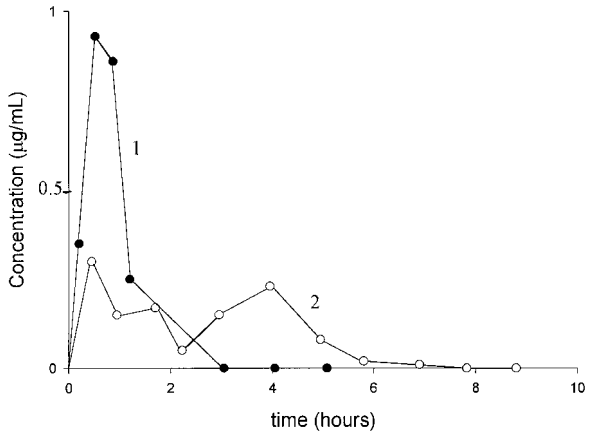

Figure 7. Plasma concentration–time curves of CPM in the rabbit after oral administration of: (1) 200 mg CPM; (2) 200 mg CBZ + 200 mg CPM.

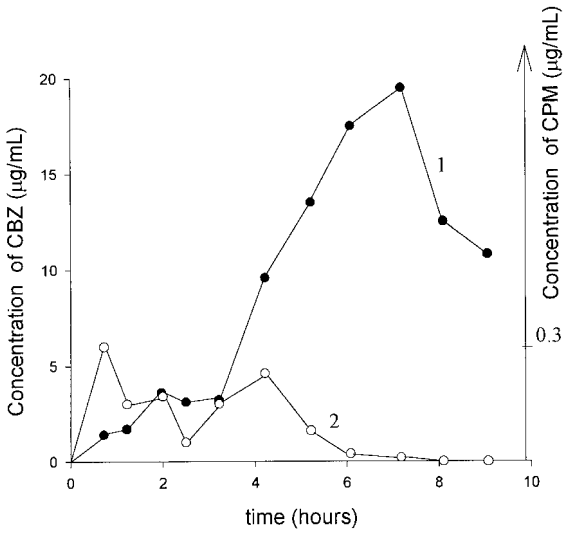

Figure 8. Plasma concentration–time curves in the rabbit after oral administration of: 200 mg CBZ + 200 mg CPM; (1) concentration of CBZ; and (2) concentration of CPM.

organs. The intensity of this capture is determined by a high degree of binding of CPM with these organs rather than by the level of free drug in plasma. Moreover, CPM undergoes strong presystem metabolism (Rukhadze *et al.*, 1998). The low level of CPM in plasma may be caused by the factors mentioned. As is shown from the comparison of curves in Fig. 7, the kinetics of CPM is sharply changed in the presence of CBZ: there are two peaks of CPM instead of one, and C_{max} of these peaks is 3–4-fold lower than with the administration of CPM alone. The prolongation of the drug takes place in the organism—the time of disappearance of CPM from blood plasma increases approximately 3-fold.

The existence of multiple peaks on the kinetic curve of CPM may be explained by the decrease in absorption of the drug in plasma (Adkin *et al.*, 1995; Alexishvili *et al.*, 1997). However, the influence of CBZ on the pharmacokinetics of CPM is not limited only by the delay of absorption. The increase in biotransformation of the drug also takes place. This is indicated by the significant increase in CPM metabolites and the change in the ratio between CPM and its metabolites in plasma in the presence of CBZ.

The results of interaction of CBZ and CPM are that both drugs decrease absorption of each other; CBZ intensifies the biotransformation of CPM, but CPM suppresses the metabolism of CBZ.

The enhancement of the biotransformation of CPM by CBZ is not unexpected. As is known, CBZ is the inductor of microsomal liver enzymes which are responsible for the biotransformation of other drugs. CBZ increases both its own metabolism and the biotransformation of other drugs (Levy and Pitlick, 1982; Eadie and Tyrer, 1983; Kholodov and Iakovlev, 1985; Markova and Nezhentsev, 1994). It should be noted that in our case CBZ reveals its inductive nature sharply and rapidly at the single dose, even when CPM is administrated first.

The suppression of biotransformation of CBZ with CPM may be explained by saturation of liver enzymes with CPM molecules. It was mentioned previously that low concentration levels of CPM in plasma are induced by their active capture by the liver and other organs, or by strong presystem (and general) metabolism. It was also emphasized that the high dose of CBZ (74 mg/kg) may be induce the saturation of liver enzymes. The CPM dose is the same. Therefore, it is quite possible that, until the basic mass of CBZ transfers into the circulation, the liver is already saturated with CPM and this is the reason for reduced biotransformation of CBZ. The decrease in CBZ and CPM absorption by sequential administration of drugs may be explained most simply by the competition of drugs for transfer from the gastrointestinal tract into plasma. We consider, however, that in addition the formation of a certain complex, more or less stable, and more or less bound to gastrointestinal tissues, is responsible for the decrease in absorption of these drugs. The simultaneous transfer of drugs into plasma after decomposition of this complex is possible. Our data on periodic and symbathic changes of CBZ and CPM concentrations until the second peak of CPM (Fig. 8) confirm this hypothesis to a considerable extent.

The amount of CBZ absorbed in plasma is considerably less in the case of CPM + CBZ than in the case of CBZ + CPM (Fig. 4, curves 2 and 3). We explain this thus: when CPM first enters the organism, because of its surface-active nature, it forms a sufficiently stable complex with gastrointestinal tissues; CBZ is arranged from above CPM. The association of molecules of CPM into micelles or the formation of surface half-micelles by which CBZ is solubilized is also possible. By the

decomposition of such complexes, CPM transfers into plasma first, and only then can CBZ transfer. However, when CBZ enters the gastrointestinal tract first, it cannot interact with tissues as strongly as CPM. It forms a less stable complex of CBZ with gastrointestinal tissues and CBZ absorbs into the plasma more intensively than in the previous case. Besides, the transfer of CBZ and CPM into plasma is symbathic.

The paradoxical result mentioned in the Introduction with antiepileptic drugs and CPM (Mashkowsky, 1977) may be caused by different sequences of administration of drugs: when CPM is administrated first, the absorption degree and therefore the concentration of CBZ in plasma decreases strongly, which results in increase in the frequency of seizures, but when CBZ is administered first, the concentration of CBZ becomes even higher than in the case of CBZ alone. This facilitates effective therapy.

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REFERENCES

Adkin, D. A., Davis, S. S., Sparrow, R. A., Huckle, P. D. and Wilding, I. R. 1995. *J. Pharma. Sci.* **84**:1405.

Alexishvili, M. M., Rukhadze, M. D. and Okujava, V. M. (1997). *Biomed. Chromatogr.* **11**:36.

Eadie, M. J. and Tyrer, J. H. 1983. Anticonvulsant Therapy (Russian translation) pp. 166, **177**, Medicine, Moscow.

Florence, A. T. 1980. In: Micellization, Solubilization and Microemulsions (Russian translation) p. 43. Mir, Moscow.

Kholodov, L. E. and Iakovlev, V. P. 1985. Clinical Pharmacokinetics. p. 137. Medicine, Moscow.

Kivmann, G. I., Rudzit, E. A. and Iakovlev, V. P. 1982. Pharmacokinetics of Chemical Therapeutic Drugs. (Pharmacokinetica Chimiotherapevticheskikh Preparatov) p. 57. Medicine, Moscow.

Levy, R. H. and Pitlick, W. H. 1982. In Antiepileptic Drugs. (Woodbury, D. M., Penry, J., K. and Pippenger, C. E., eds). p. 497. Raven Press, New York.

Markova, I. V. and Nezhentsev, M. V. 1994. Pharmacology, p. 14, Sotis, St Peterburg.

Mashkowsky, M. D. 1977. Medicinal Remedies. (Lekarstwennye sredstwa) vol. **1**, p. 44, Medicine, Moscow.

Rukhadze, M. D., Okujava, V. M., Alexishvili, M. M., Tsagareli, S. K and Makharadze, T. G 1998. *Chim-Pharm Zhurnal Russia* (accepted for publication).

