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ORIGINAL RESEARCH

Interaction of carbamazepine and promethazine in rabbits

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ABSTRACT: The interaction of carbamazepine and promethazine in rabbits has been investigated. The influence of this interaction on the processes of biotransformation in the liver was revealed. The drugs were administered as single oral doses (100 mg of each drug) as well as simultaneously with an interval of 15 min. The sequence of administration of the drugs was varied. The influence of promethazine on the pharmacokinetics of carbamazepine is expressed by: (a) strong suppression of carbamazepine's level in plasma and appearance of multiple peaks of carbamazepine; (b) suppression of biotransformation of carbamazepine into carbamazepine-10,11-epoxide at the initial stages and its increase in the intermediate stages. These data are explained by the active capture of carbamazepine by liver at its primary transferal through the liver and sufficient presystem elimination of carbamazepine in the presence of promethazine. The character of kinetic curves of promethazine varies substantially under the influence of carbamazepine. However, this change is not as strong as in case of carbamazepine. The concentration of promethazine in plasma varies slightly and multiple peaks are not observed. The rate of terminal elimination of promethazine varies and abrupt prolonged segments of elimination appear at the initial and terminal stages of the process in return. These data perhaps indicate the induction of biotransformation of promethazine in the presence of carbamazepine—an inductor of microsomal liver enzymes. The changes of kinetics of promethazine and carbamazepine by simultaneous administration as compared with their administration separately, as well as a comparative consideration of pharmacokinetics of promethazine and carbamazepine by simultaneous administration show the existence of competition in the elimination between these drugs and the periodic saturation of liver for their biotransformation.

KEYWORDS: Antiepileptic; biotransformation; carbamazephine 10,11-expoxide; competition; elimination; microsomal liver enzymes.

INTRODUCTION

Carbamazepine (CBZ) is used as an antiepileptic drug. Its metabolite carbamazepine-10,11-epoxide (ECBZ) also possesses an anticonvulsive effect. CBZ is frequently used in combination with other drugs. Interaction of drugs influences the absorption, biotransformation, excretion and other processes (Eadie and Tyrer, 1983).

Drugs with a surface-active nature may interact with other drugs unusually since they intensively bind to blood plasma and tissue proteins. Previously we have studied the interaction of CBZ with chlorpromazine—a strong neuroleptic with a surface-active nature, which possesses presystem metabolism, a high degree of binding with plasma and tissue proteins and unusual interaction with other drugs (Kholodov and Yakovlev, 1985; American Hospital Formulary Service 1991; Rukhadze *et al.*, 2001). The results obtained indicate an induction of biotransformation of chlorpromazine by CBZ and vice versa—the suppression of metabolism of CBZ under the influence of chlorpromazine (Rukhadze *et al.*, 1999).

Promethazine (PMZ), a drug with antihistamine and sedative action, like chlorpromazine belongs to the group of surfactants. PMZ is almost wholly metabolized in the liver. Less than 1% of the dose is excreted unchanged in the urine, but it is affected by minimum metabolism in the gastrointestinal tract (Taylor *et al.*, 1983). PMZ is characterized by low bioavailability (12–40%) and considerable presystem metabolism in humans and rabbits (Taylor *et al.*, 1983; Taylor and Houston, 1983; DiGregorio and Ruch, 1980; Koytchev *et al.*, 1994). As known, the presystem metabolism introduces a significant contribution in the interaction of drugs (Kivmann *et al.*, 1982; Markova and Nezhentsev, 1994). Therefore it is topical to study the interaction of CBZ, capable of inducing microsomal liver enzymes with surfactant PMZ, which is characterized by considerable presystem metabolism (Levy and Pitlick, 1982; Eadie and Tyrer, 1983; Markova and Nezhentsev, 1994). Our goal was to study this interaction in rabbits.

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Abbreviations used: CBZ, carbamazepine; ECBZ, carbamazepine-10,11-epoxide; PMZ, promethazine.

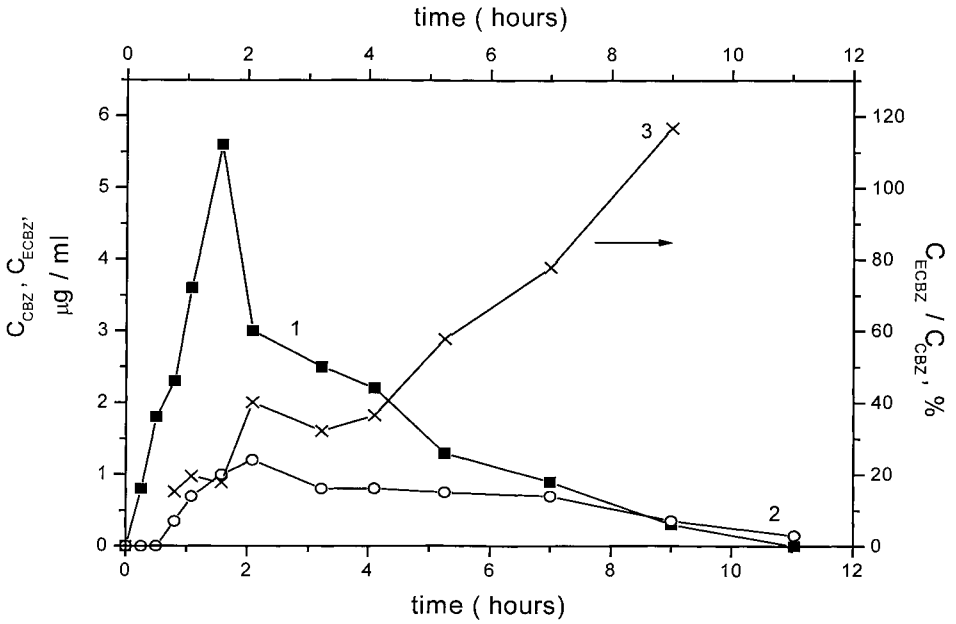

Figure 1. Plasms concentration–time curves in the rabbit after oral administration of 100 mg CBZ. (1) Concentration of CBZ; (2) concentration of ECBZ; (3) C_{ECBZ}/C_{CBZ} ratio in percentage from C_{CBZ}.

EXPERIMENTAL

Experiments in rabbits. Blood samples were collected from the ear of a rabbit (weight 3.0 kg) at several time points. Doses of 100 mg CBZ and 100 mg of PMZ were administered to the rabbit as single oral doses. Additional drugs were given sequentially with an interval of 15 min.

Sample pretreatment. For the determination of PMZ, 0.2 mL of phenothiazine solution (8 μg/mL; internal standard), 20 μL of 1 M sodium hydroxide and 2 mL cyclohexane were added to 0.2 mL of plasma (Rukhadze *et al.*, 2000). For the simultaneous determination of CBZ and ECBZ, 0.2 mL of halodiph solution in chloroform at concentration 14 μg/mL, 0.2 mL of 4.0 M sodium hydroxide and 2 mL chloroform were added to 0.2 mL of blood plasma (Alexishvili *et al.*, 1997). The extraction mixtures were agitated by hand for 1 min and centrifuged for 10 min at 3000***g***. 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 conditions. The analyses of the above mentioned drugs were carried out using a microcolumn high-performance liquid chromatograph Milichrom (Nauchpribor, Oryol, Russia) with a UV detector, which permitted fixation of the entire spectrum of absorption within the range 190–360 nm. Detection of PMZ was realized at a wavelength of 260 nm. CBZ and ECBZ were detected at 210 nm. The column used was a Separon-C_{18} (Lachema, Brno, Czech Republic) column (62 × 2 mm, i.d.) with particle size 5 μm. The mobile phase used for the determination of PMZ was a mixture of ethanol–0.1 M K_2HPO_4 at a ratio of 50:50 (v/v). As an ion-pairing agent, 0.25% triethylamine (v/v) was added to the mobile phase. The pH of the eluent was adjusted with phosphoric acid to 6.5. Separation of CBZ and ECBZ was carried out by mobile phase with composition 0.05 M KH_2PO_4 with acetonitrile at a ratio of 40:60 (v/v). The pH of the eluent was adjusted with phosphoric acid to 4.0. The mobile phase was delivered from syringe-type pump with flow-rate 50 μL/min.

RESULTS AND DISCUSSION

Influence of promethazine on the pharmacokinetics of carbamazepine

The pharmacokinetic curves of CBZ and its metabolite ECBZ are presented in Fig. 1. Three stages are distinguished on the kinetic curve of CBZ: fast, intermediate and terminal (Fig. 1, curve 1). The terminal elimination of CBZ obeys the first-order law. The kinetic curve of ECBZ has an S-shape (Fig. 1, curve 2). The maximum concentration of ECBZ is reached later than for CBZ. The elimination of ECBZ represents a first-order process. The concentration of ECBZ at the initial stages of its formation is equal to 15% of the concentration of CBZ (Fig. 1, curve 3). However the percentage of ECBZ increases later abruptly: it reaches 40% at the peak of ECBZ and exceeds the concentration of CBZ at the end of elimination.

In order to study the interaction of CBZ and PMZ the sequence of administration of the drugs was varied: (a) 100 mg CBZ were given and 15 min later 100 mg PMZ (CBZ + PMZ); and (b) 100 mg PMZ were given and

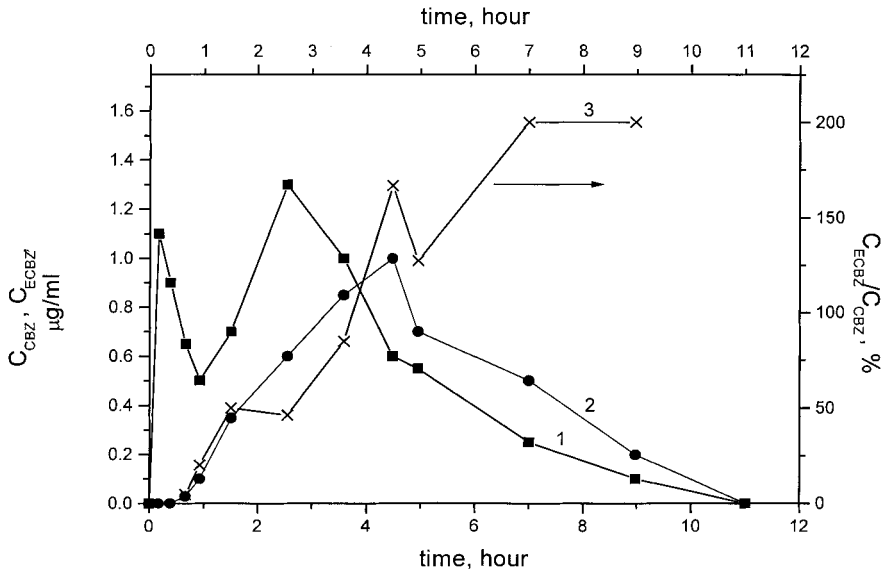

Figure 2. Plasma concentration–time curves in the rabbit after oral administration of 100 mg CBZ + 100 mg PMZ. (1) Concentration of CBZ; (2) concentration of ECBZ; (3) C_{ECBZ}/C_{CBZ} ratio as a percentage from C_{CBZ}.

after 15 min 100 mg CBZ (PMZ + CBZ). The pharmacokinetic curves of CBZ (curves 1) and ECBZ (curves 2) for cases (a) and (b) are illustrated in Figs 2 and 3.

In Fig. 4 the kinetic curves of CBZ are given. Curve 1 corresponds to the administration of CBZ only, curve 2 to CBZ + PMZ and curve 3 to PMZ + CBZ. As Fig. 4 shows, the concentration of CBZ in plasma is suppressed very strong in the presence of PMZ, independently of sequence of administration of drugs. At the same time the multiple peaks of CBZ are observed in both (a) and (b) cases.

The formation of ECBZ is suppressed at the initial stages, but it increases sharply in (a) and (b) cases in comparison with administration of CBZ only (Fig. 5). This results in the reduction of percentage of ECBZ at the initial stages and its increase at the intermediate stages (Fig. 6).

A strong suppression of CBZ in plasma at the initial

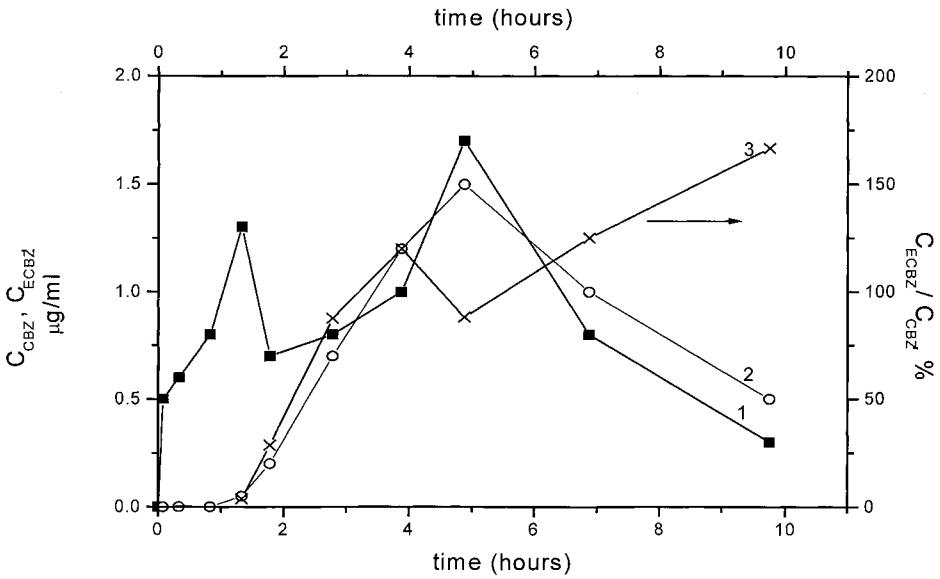

Figure 3. Plasma concentration–time curves in the rabbit after oral administration of 100 mg PMZ + 100 mg CBZ. (1) Concentration of CBZ; (2) concentration of ECBZ; (3) C_{ECBZ}/C_{CBZ} ratio as a percentage from C_{CBZ}.

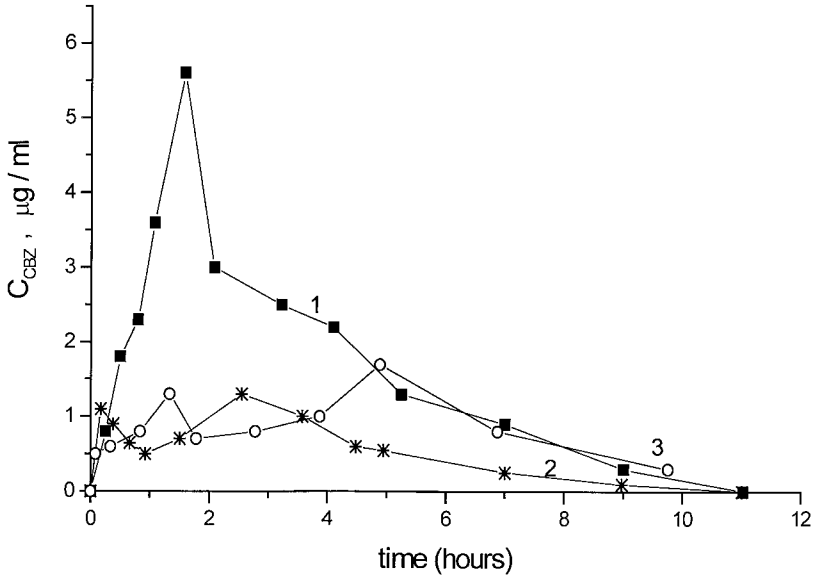

Figure 4. Plasma concentration–time curves of CBZ in the rabbit after oral administration of (1) 100 mg CBZ; (2) 100 mg CBZ + 100 mg PMZ; (3) 100 mg PMZ + 100 mg CBZ.

stages of process cannot be explained by decreasing absorption of CBZ in the presence of PMZ, since the rate of absorption (W_{abs}) of CBZ is approximately the same in all cases (see Fig. 4). It also cannot be explained by increasing biotransformation of CBZ in (a) and (b) cases, since, as emphasized above, the formation of ECBZ is suppressed on the contrary at the initial stages of process (see Fig. 5). The reduction of concentration of CBZ at the initial stages of process may be explained by rapid binding of CBZ with tissues and organs in the presence of PMZ. It is known that PMZ itself undergoes significant presystem elimination in the liver (Taylor *et al.*, 1983; Taylor and Houston, 1983; DiGregorio and Ruch, 1980). It is possible, that PMZ somehow prepares the liver for the binding of CBZ and the liver captures CBZ at its first transferal through the liver at the presence of PMZ.

Thus, the peculiarities of pharmacokinetics of CBZ and ECBZ in (a) and (b) cases may be explained as follows: the capture of CBZ by the liver (without biotransformation) takes place at the initial stages of

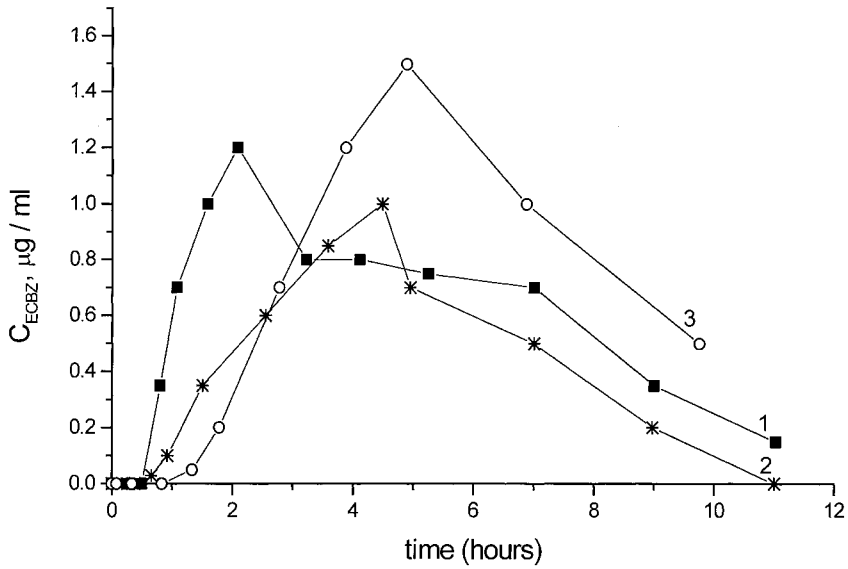

Figure 5. Plasma concentration–time curves of ECBZ in the rabbit after oral administration of (1) 100 mg CBZ; (2) 100 mg CBZ + 100 mg PMZ; (3) 100 mg PMZ + 100 mg CBZ.

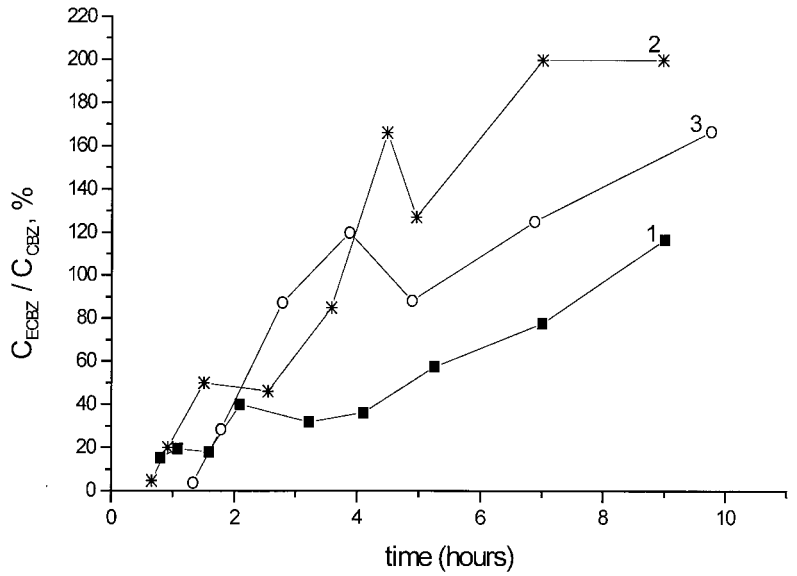

Figure 6. Curves of C_{ECBZ}/C_{CBZ} vs time in the rabbit after oral administration of (1) 100 mg CBZ; (2) 100 mg CBZ + 100 mg PMZ; (3) 100 mg PMZ + 100 mg CBZ.

the process, therefore concentration of both CBZ and ECBZ is very low at the beginning. The reinforced presystem (and also systemic) biotransformation of the captured CBZ proceeds further, hence concentration of CBZ in plasma remains low again but concentration and percentage of ECBZ increase greatly in comparison with administration of CBZ alone (Figs 5 and 6).

It is interesting to note that an opposite situation is observed in the study of interaction of chlorpromazine and CBZ: the negligible reduction of concentration and percentage of ECBZ at the initial stages and rather strong suppression of these quantities at the intermediate stages of the process (Rukhadze *et al.*, 1999).

The presence of the second peaks of CBZ in plasma in cases (a) and (b) may be explained by saturation of the liver for the biotransformation of CBZ, due to which a certain part of CBZ appears in plasma. In fact, decreasing power of biotransformation of CBZ (reducing the

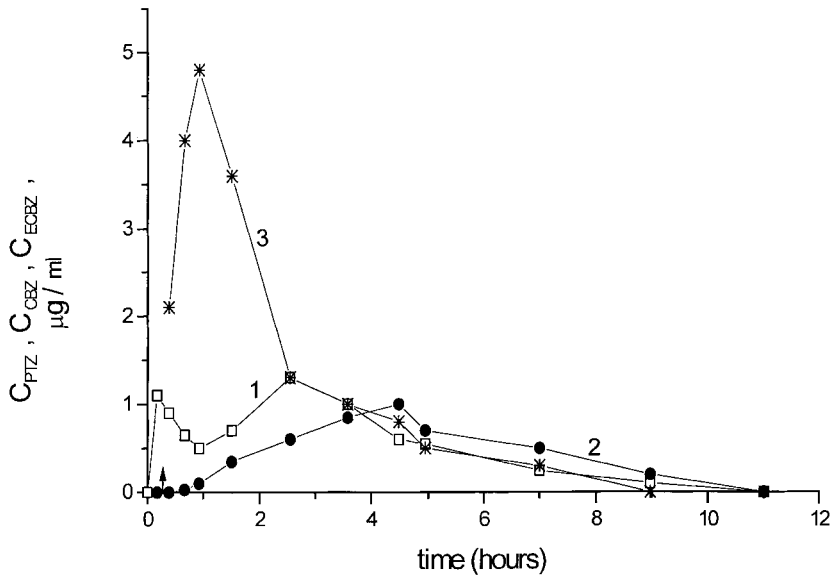

Figure 7. Plasma concentration–time curves in the rabbit after oral administration of 100 mg CBZ + 100 mg PMZ. (1) Concentration of CBZ; (2) concentration of ECBZ; (3) concentration of PMZ. The arrow indicates time of administration of PMZ.

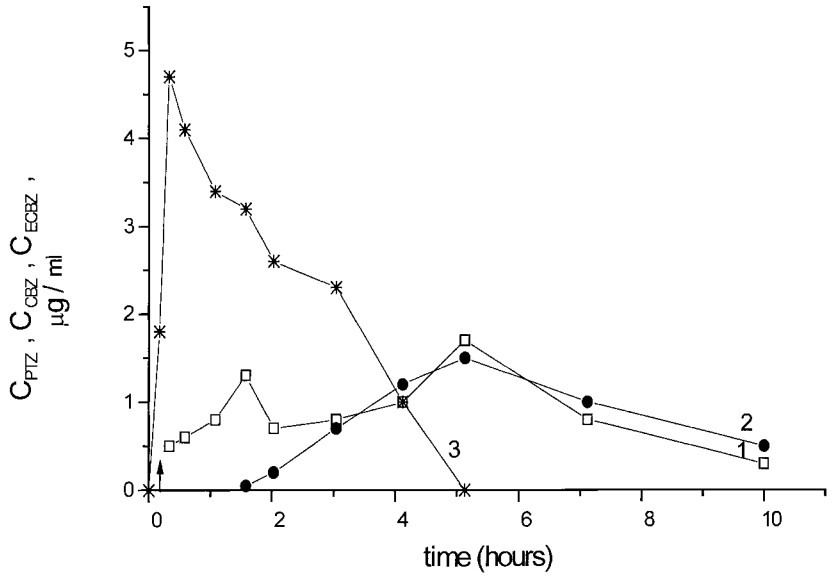

Figure 8. Plasma concentration–time curves in the rabbit after oral administration of 100 mg PMZ + 100 mg CBZ. (1) Concentration of CBZ; (2) concentration of ECBZ; (3) concentration of PMZ. The arrow indicates time of administration of CBZ.

percentage of ECBZ) takes place on the second ascending branches of CBZ, as shown in Figs 2, 3 and 6.

The comparison of pharmacokinetics of CBZ and PMZ by simultaneous administration [cases (a) and (b)] leads to conclusions about saturation of liver and existence of competition in biotransformation of these drugs. Actually, in the case of CBZ + PMZ the second ascending branch of CBZ (ie 'lassitude' of liver for biotransformation of CBZ) starts simultaneously with the beginning of the rapid elimination of PMZ (Fig. 7, curves 1 and 3). However, the second descending branch of CBZ (ie the general biotransformation of CBZ) starts simultaneously with the termination of the above-mentioned rapid elimination of PMZ. In the case of PMZ + CBZ, the sharp increase of concentration of CBZ on the second ascending branch proceeds simultaneously with abrupt elimination of PMZ (Fig. 8, curves 1 and 3), but the terminal elimination of CBZ and a rise of biotransformation of CBZ into ECBZ converges exactly in time with the end of elimination of PTZ. It is interesting that biotransformation of PMZ in both cases possesses the prioritized character: the basic biotransformation of CBZ starts when the elimination of PMZ is finished [case (b), Fig. 8] or the largest segment of its elimination terminates [case (a), Fig. 7].

As concerns the terminal elimination of CBZ, its first-order and approximately the same values of constants of elimination of CBZ and ECBZ with both simultaneous administration and in the case of administration of CBZ alone, indicate identical mechanism of elimination of CBZ and ECBZ in all cases (Table 1).

Influence of carbamazepine on the pharmacokinetics of promethazine

The kinetic curve of PMZ in the case of administration of PMZ only is given in Fig. 9 (curve 1). The concentration of PMZ reaches a maximum rather quickly. Three stages may be distinguished in the elimination of PMZ (as well as in the elimination of CBZ in the case of administration of CBZ only): fast, intermediate and terminal. The terminal elimination of PMZ obeys a zero order and $W_{el} = K_{el} = 0.505$ μg/ml h (Table 1).

The character of kinetic curves is considerably changed under the influence of CBZ (Fig. 9, curves 2 and 3). The maximal concentration of PMZ is somewhat reduced in comparison with the case of administration of PMZ only. The rate of the terminal elimination decreases in the case of CBZ + PMZ, $W_{el} = 0.153$ μg/ml h (Table 1). On the contrary, the rate of terminal elimination increases in the case of PMZ + CBZ, $W_{el} = 1.095$ μg/ml h (Table 1). In addition, the number of steps rises at

Table 1. The rate constants of terminal elimination of CBZ, ECBZ and PMZ after oral administration of CBZ, PMZ, CBZ + PMZ and PMZ + CBZ

Drugs	K_{CBZ}, h^{-1}	K_{ECBZ}, h^{-1}	K_{PMZ}, μg/ml h
CBZ	0.377	0.381	—
PMZ	—	—	0.505
CBZ + PMZ	0.423	0.500	0.153
PMZ + CBZ	0.357	0.211	1.095

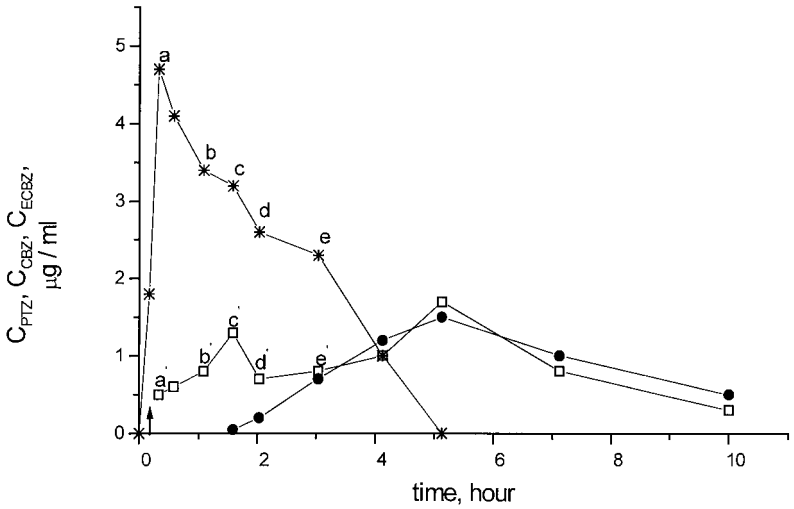

Figure 9. Plasma concentration–time curves of PMZ in the rabbit after oral administration of (1) 100 mg PMZ; (2) 100 mg CBZ + 100 mg PMZ; (3) 100 mg PMZ + 100 mg CBZ.

the initial stages of elimination. The order of elimination is zero in all cases. In contrast to CBZ, the multiple peaks of PMZ are not observed by simultaneous administration.

The change of character of the kinetic curve of PMZ at the presence of CBZ is induced by simultaneous influence of these drugs on the processes of biotransformation in the liver. As mentioned above, the competition between these drugs and periodic saturation of liver for their biotransformation takes place at simultaneous presence of CBZ and PMZ (see above).

The periodic and simultaneous saturation of liver for biotransformation of both PMZ and CBZ will be considered by us in detail for the case of (b) PMZ + CBZ (Fig. 9). The elimination of PMZ proceeds step-wise (Fig. 9, curve 3), but the multiple peaks of CBZ are observed on the segment of elimination of PMZ (Fig. 9, curve 1).

The elimination of PMZ on the segment ab coincides with the increasing of concentration of CBZ in plasma (segment a′ b′). Further, the rate of elimination of PMZ decreases (segment bc) and simultaneously the concentration of CBZ in plasma is sharply increased (segment b′ c′). This points to saturation of liver for biotransformation of both CBZ and PMZ. After the liver 'gathers force' and carries out an intensified elimination of PMZ and CBZ, the rate of elimination of PMZ increases drastically (segment cd) and the first descending branch of CBZ appears (segment c′ d′). After this, the decreasing rate of elimination of PMZ is followed (segment de) and the second ascending branch of CBZ (segment d′ e′) arises, ie saturation of liver is present again. Thus, the periodic and simultaneous decrease of biotransformation power of the liver regarding CBZ and PMZ occurs by simultaneous administration of these drugs.

The existence of sharp and prolonged segments of elimination of PMZ is focused at the simultaneous administration: this segment is observed at the beginning of elimination in the case of CBZ + PMZ, but at the finish of elimination in the case of PMZ + CBZ (Fig. 9, curves 2 and 3). As is known, CBZ is the strong inductor of microsomal liver enzymes (Eadie and Tyrer, 1983; Markova and Nezhentsev, 1994; Levy and Pitlick, 1982). It is possible that, in the case of CBZ + PMZ, ie when CBZ enters in the organism of rabbit in the first instance, it had time to induce the liver for biotransformation of PMZ, which is reflected by the presence of sharp segment of elimination of PMZ at the outset of the process (Fig. 7, curve 3). However, in the case of PMZ + CBZ, where CBZ enters after PMZ, the induction of liver enzymes occurs later and the basic elimination of PMZ takes place at the end of the process (Fig. 8, curve 3).

CONCLUSIONS

Promethazine, which is characterized by a considerable presystem metabolism, somehow prepares the liver for the binding of carbamazepine and the liver captures carbamazepine by its transferal through the liver in the presence of promethazine. This results in the strong suppression of CBZ in plasma and appearance of multiple peaks of CBZ by simultaneous administration of these drugs.

Carbamazepine influences significantly the pharmacokinetics of promethazine: the rate of terminal elimination of promethazine is altered in the presence of carbamazepine, and prolonged segments of rapid elimination of promethazine arise. These changes in the kinetics of promethazine may be caused by induction of microsomal liver enzymes of liver via carbamazepine.

The comparison of kinetics of carbamazepine and promethazine by simultaneous administration, as well as in the cases of administration of these drugs separately, indicates the presence of competition in the elimination of carbamazepine and promethazine and also the periodic saturation of the liver for the elimination of both drugs.

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