Pharmacokinetic interaction between carbamazepine and neuroleptics after combined prolonged treatment in rats


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Summary. This study investigates how neuroleptics of phenothiazine or thioxanthene structure influence the pharmacokinetics of carbamazepine. Experiments were carried out on male Wistar rats. Carbamazepine and the neuroleptics were administered i.p., separately or together, for 2 weeks in the following daily doses (mg/kg): carbamazepine 15 during the 1st week of treatment and 20 during the 2nd week of treatment, promazine 10, chlorpromazine 2, perazine 10, chlorprothixene 2, flupenthixol 0.5. One hour after the last injection of carbamazepine and/or the neuroleptic, samples of blood plasma and brain were taken to determine the concentrations of carbamazepine and two of its metabolites: 10,11-epoxide and trans-10,11-diol.

The neuroleptics increased the concentration of carbamazepine in plasma and in brain, but tended to decrease (with the exception of chlorpromazine) the concentration of the epoxide and increased the concentration of trans-10,11-diol.

Metabolic in vitro studies did not show any significant differences between rats treated with carbamazepine alone and those treated with carbamazepine plus neuroleptic in the rates of the carbamazepine epoxidation, of 10,11-epoxide hydrolysis or of 1-naphthol glucuronidation.

Key words: Carbamazepine — Drug combination — Neuroleptics — Carbamazepine plasma levels — Inhibitory interaction

Introduction

Carbamazepine is a drug with a unique spectrum of clinical efficacy. This well-established drug for the treatment of epilepsy and trigeminal neuralgia has recently been shown also to have a wide spectrum of psychotropic actions: in addition to the growing evidence that it has acute antimanic and long-term prophylactic efficacy in both phases of manic-depressive illness (Ballenger and Post 1980; Elphick 1989; Kramlinger and Post 1989; Okuma et al. 1979; Post and Uhde 1985; Post et al. 1986), carbamazepine has some acute antidepressant efficacy (Post et al. 1986) and is a useful drug for the treatment of excited states in patients suffering from schizophrenic and schizoaffective disorders (Hakola and Laulumaa 1982; Okuma et al. 1989; Wetterling 1987). There are also reports of beneficial effects of carbamazepine in the treatment of schizophrenic symptoms (Dose and Emrich 1990; Herrera et al. 1987; Scher and Neppe 1989). Carbamazepine is effective in many patients who have previously not responded to traditional treatment, including lithium, tricyclic antidepressants, and/or neuroleptics.

In several studies carbamazepine was used as a sole therapeutic agent or as an adjunct to other treatments. It has been combined with lithium, neuroleptics and/or antidepressants, although the possibility of pharmacokinetic interactions has not been thoroughly investigated as yet. As such drug combinations could have a therapeutic value in the future, the problem of a pharmacokinetic interaction between them should be investigated.

Other authors as well as our own previous studies demonstrated the possibility of a pharmacokinetic interaction between carbamazepine and other psychotropics. Carbamazepine is known to produce autoinduction (Bertilsson et al. 1980; Pynnonen et al. 1980; Tybring et al. 1981) and heteroinduction of drug metabolizing enzymes (Arana et al. 1986; Hansen et al. 1971). Neuroleptics or antidepressants, depending on the substrate, can behave as inducers or inhibitors of drug metabolism (Breyer 1972; Daniel et al. 1984; Daniel and Melzacka 1986; Kakemi et al. 1971; Shand and Oates 1971). On the other hand, carbamazepine and psychotropics (dibenzoazepines, phenothiazines and thioxanthenes) have similar tricyclic structure, which suggests...
the possibility of their competing for protein-binding sites.

The interaction between carbamazepine and other psychotropic drugs may be of a very complex character, the more so as adaptive changes and a direct effect of the drugs may overlap. Thus, combined administration of carbamazepine and imipramine to rats resulted in an increase in concentration of the parent compounds and a simultaneous decrease in the concentration of their metabolites in blood plasma (Daniel and Netter 1988). The observed interaction was due to the competition of the drugs for an active centre of cytochrome P-450, and to a qualitative alteration of the enzyme by imipramine.

The addition of viloxazine to carbamazepine treatment enhanced the concentration of carbamazepine and its metabolites in the blood serum of depressed patients, which was manifested by the increase in side effects (Besser et al. 1989; Pisani et al. 1984, 1986).

On the other hand, concurrent administration of carbamazepine and haloperidol led to a reduction in plasma haloperidol levels in patients, the effect of which in some cases worsened psychotic symptoms (Arana et al. 1986; Fast et al. 1986; Kahn et al. 1990; Kidron et al. 1985).

Therefore, it seems advisable to investigate whether prolonged, concurrent administration of carbamazepine and neuroleptics with phenothiazine or thioxanthene structures results in a pharmacokinetic interaction.

In order to achieve exact dosing we have used the i.p. route. The epoxide-diol pathway, which is the main biotransformation pathway of carbamazepine (Faigle et al. 1976; Frigerio et al. 1976; Tybring et al. 1981), seems to be inhibited by neuroleptics in vivo.

**Methods**

**Chemicals.** Carbamazepine and its metabolites trans-10,11-dihydro-10,11-dihydroxy-Carbamazepine were kindly provided by Ciba-Geigy (Frankfurt, FRG). Promazine (hydrochloride) was obtained from Polfa (Jelenia Góra, Poland); perazine (hydromaleate) from Promonta (Hamburg, FRG). Flupenthixol (hydrochloride) was supplied by Hoffmann-La Roche (Grenzach-Whylen, FRG). NADP, glucose-6-phosphate, glucose-6-phosphate-dehydrogenase, UDP-glucuronic acid and bovine serum albumin were purchased from Boehringer (Mannheim, FRG); I-naphthol glucuronide and I-naphthol sulfate from Sigma (St. Louis, Mo., USA). Methanol and acetone from of a chromatographic grade, diethylether and dichloromethane were obtained from E. Merck (Darmstadt, FRG).

**Treatment of animals.** Male Wistar rats (220–270 g) were kept under standard laboratory conditions. They were fed ad libitum on standard granulated food (until 24 h before being sacrificed) and had free access to tap water. Carbamazepine and neuroleptics were administered i.p., separately or together, for 2 weeks in the following daily doses: carbamazepine (CBZ) 15 mg/kg during the 1st week of treatment and 20 mg/kg during the 2nd week of treatment, promazine (PZ) 10 mg/kg, chlorpromazine (CPZ) 2 mg/kg, perazine (PRZ) 10 mg/kg, chlorprothixene (CPTX) 2 mg/kg, flupenthixol (FPX) 0.5 mg/kg. The dose values refer to the respective salts which are mentioned above.

The chosen scheme of administration of the drugs simulates the clinical situation in respect to their combinations and their dosages. Dosages used were smaller than half of those exerting the maximum pharmacological effect. For CBZ a dose of 15 mg/kg leads to a blood level of 4 µg/ml in rats, which reaches the concentrations therapeutically accepted in man (4–10 µg/ml).

Owing to its insolubility in water, CBZ was dissolved in dimethylsulfoxide (20 mg/ml). Therefore the other animal groups also received the respective volume of dimethylsulfoxide (1 ml/kg) i.p.

**Determination of CBZ and its metabolites.** One hour after the last injection of CBZ and/or a neuroleptic the rats were decapitated, the trunk blood was collected in tubes moistened with a 30% solution of sodium citrate and the brain was rapidly excised and stored frozen in solid CO₂. The blood samples were centrifuged at 2000 g for 30 min. Samples containing 1.5 ml of plasma were stored at −15°C for 3 days. Brains were homogenized in water (1:3; w/v) and the homogenates underwent centrifugation at 2500 g for 30 min. Then 2.5 ml of each supernatant were used for the determination of CBZ and its metabolites.

CBZ and its metabolites 10,11-epoxy-CBZ and trans-10,11-dihydro-10,11-dihydroxy-CBZ were determined by a reversed-phase HPLC analysis according to Daniel and Netter (1988). They were extracted twice from alkaline solution (pH = 12) with 5 ml of a mixture of dichloromethane (1 vol.) and diethylether (3 vol.), shaking for 15 min. The sensitivity of the method allowed for quantification of CBZ in plasma as low as 0.7 nmol/ml, for 10,11-epoxy-CBZ as low as 1.5 nmol/ml, and for trans-10,11-dihydro-10,11-dihydroxy-CBZ as low as 3 nmol/ml. These figures are comparable with the results of Daniel and Netter (1988).

The detection thresholds in the brain were about three times higher and allowed for quantification of carbamazepine as low as 2.5 nmol, 10,11-epoxy-CBZ as low as 5.0 nmol and trans-10,11-dihydro-10,11-dihydroxy-CBZ as low as 10 nmol in 1 g of brain tissue.

**Preparation of liver microsomes and assessment of cytochrome P-450 and cytochrome b₅.** In order to avoid the interference of in vivo-administered drugs with in vitro experiments, livers were excised 24 h after the withdrawal of the drugs. Indeed, these microsomal preparations did not exhibit residual levels of CBZ or of its metabolites. The concentrations, if any, are below the thresholds of detection mentioned above. Microsomes were prepared according to conventional methodology by differential centrifugation in Tris/HCl buffer at pH = 7.4 (Legrum et al. 1979, modified from Netter 1960). Cytochromes P-450 and b₅ were determined according to Omura and Sato (1964) and Omura and Takesue (1970), respectively, using a Beckman DU-65 Spectrophotometer. Protein was assayed according to Lowry et al. (1951) with bovine serum albumin as standard.

**Microsomal epoxidation of CBZ.** The incubations (final volume of 1 ml) were performed in a system containing liver microsomes (2 mg of protein in 1 ml), Tris/HCl buffer (50 mM; pH = 7.4), MgCl₂ (5 mM), NADP (0.6 mM), glucose-6-phosphate (6 mM), glucose-6-phosphate dehydrogenase (2 units in 1 ml), carbamazepine (0.1 mM) at 37°C. After 2 min of preincubation the reaction was started by addition of 100 µl of CBZ in 5% ethanol solution in the buffer (the final concentration of ethanol was 0.5%). After 3 min incubation the reaction was stopped by addition of 200 µl of methanol and cooling to 0°C. The 10,11-epoxide formed during the reaction was determined by HPLC analysis. The incubations performed in the presence of neuroleptics (PZ 50 µM or CPZ 50 µM) contained pooled microsomes from five control rats.

**Microsomal hydrolysis of 10,11-epoxy-CBZ.** The incubations (final volume of 1 ml) were performed in a system containing liver microsomes (0.5 mg protein in 1 ml), Tris/HCl buffer (50 mM; pH = 7.4) and 10,11-epoxy-CBZ (0.3 mM) at 37°C for 24 h. The 10,11-epoxide was dissolved in ethanol in a way which resulted in a final concentration of the alcohol in the incubation mixture of 0.5%. The reaction was stopped by addition of 200 µl of methanol and...