EFFECTS OF CARBAMAZEPINE ON HEPATIC GLUTATHIONE LEVEL IN RATS AND DETERMINATION OF CARBAMAZEPINE AND ITS EPOXIDE METABOLITE IN PLASMA BY HPLC

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SUMMARY

We investigated whether carbamazepine, which is known to be metabolized to an electrophilic epoxide derivative in the body, causes any decrease, analogous to the action of epoxides, of hepatic glutathione (GSH) level in rats. Carbamazepine was administered to rats and liver GSH levels were determined spectrophotometrically. Neither a single low nor repeated low doses (30 mg/kg) of carbamazepine (CBZ) produced a statistically significant difference in GSH levels relative to controls. A single high dose of CBZ (100 mg/kg) produced a large and significant decrease relative to control (GSH level 3.82 ± 0.64 vs 6.54 ± 0.45 μmol GSH/g liver). CBZ and its metabolite carbamazepine-10,11-epoxide were determined in plasma by HPLC after the high dose of carbamazepine administration. The concentrations of carbamazepine and carbamazepine-10,11-epoxide were 18.9±2.9 μg/ml and 10.7 ± 2.8 μg/ml, respectively.

KEY WORDS

carbamazepine, carbamazepine-10,11-epoxide, glutathione, liver, HPLC

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INTRODUCTION

Carbamazepine (CBZ) was approved in the United States for use as an antiepileptic agent in 1974. CBZ is related chemically to the tricyclic antidepressants. It is a derivative of iminostilbene with a carbamyl group at the 5 position; this moiety is essential for potent antiepileptic activity. Peak concentrations of CBZ in plasma are observed 2 to 6 hours after oral ingestion, and binding to plasma proteins occurs to the extent of about 80% /1/. CBZ is metabolised to the 10,11-epoxide (CBZ-E) /2/ (Fig. 1), and this compound also has anticonvulsant activity. The half-life of the parent compound in plasma after chronic administration is between 13 and 17 hours. The half-life of the 10,11-epoxide is probably shorter than that of the parent compound, ranging from 5 to 8 hours. Less than 1% of CBZ is recovered in the urine as the parent compound or the epoxide /1/. CBZ exerts a useful therapeutic effect at plasma concentrations of 2-5 μg/ml; plasma levels higher than 12 μg/ml are often associated with adverse effects /3,4/.

Electrophilic substances formed in the liver can be detoxified in the body by conjugation with the hepatic nucleophil glutathione (GSH). Since the capacity of hepatic GSH is limited, depletion may occur in the GSH level after conjugation reactions. This may lead electrophiles to react with nucleophilic macromolecules in the cell resulting in toxic effects, such as loss of cell viability and mutagenic/carcinogenic effects. Regnaud *et al.* have reported that conjugation with GSH is an important detoxification mechanism for CBZ and the major portion of its excreted metabolites are thioethers /5/. Runci *et al.* reported a dramatic decrease in cerebral GSH level after the simultaneous administration of valproic acid and CBZ /6/, but Attaguile *et al.* found no modification in brain GSH levels after CBZ and sodium valproate administration in rats /7/. Madden *et al.* identified dihydrohydroxy-SG adducts of CBZ in the bile of rats administered CBZ intravenously;

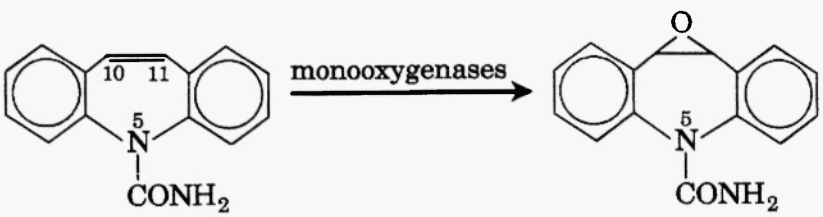

Fig.1: Carbamazepine and its active metabolite carbamazepine-10,11-epoxide.

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these were the products of thioether conjugation of GSH with arene oxides /8/. They provided evidence for the generation of a reactive arene oxide species from CBZ and this has the potential to initiate cellular damage if not adequately detoxified via conjugation with glutathione.

The aim of this study was to evaluate GSH depletion in the liver of CBZ-treated rats and to determine CBZ and its active metabolite CBZ-E in plasma. Because both these compounds contribute to both therapeutic and adverse effects /3/, and because there are inter- and intra-individual variations of their plasma concentrations /9,10/, it seems to be necessary to measure the plasma concentrations of the parent drug, CBZ, and its active metabolite, CBZ-10,11-epoxide. Many different methods have been used for the estimation of CBZ and its epoxide, based on UV spectrophotometry /11,12/, gas chromatography /13,14/, enzyme-multiplied immunoassay technique (EMIT) /10,15/ and high performance liquid chromatography (HPLC) /3,9,10, 16-21/.

MATERIALS AND METHODS

Hepatic GSH levels of Wistar albino male rats were determined by means of a spectrophotometric method /12/. In order to determine CBZ and CBZ-E in plasma HPLC was used /16/.

Chemicals

5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) (Sigma D-8130) and other chemicals were from Merck. Carbamazepine-10,11-epoxide was a gift from Novartis (Basel, Switzerland). All solvents used were HPLC grade. Water used for the preparation of standards was glass-distilled in the laboratory.

Procedure for GSH determination

Wistar albino male rats (200-300 g) were used in this study. CBZ was dissolved in propylene glycol and injected i.p. to test animals; the solution was freshly prepared for each use. CBZ was administered in two dose levels to test animals: low (30 mg/kg, single; and repeated, 3 days, two times a day in order to observe the possible effect of enzyme induction) and high (100 mg/kg, single). Animals were killed three

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hours after drug administration (in repeated drug administration studies after the last administration). The liver was removed, washed with water, gently blotted and weighed. Tissue homogenization and subsequent GSH determination were performed according to Kaplowitz /12/. Briefly, the tissue was homogenized with two parts (v/w) 0.1 M sodium phosphate (pH 7.4) and 0.25 M sucrose buffer and the homogenate was then immediately spun at 4000 rpm for 15 min in a J-21 B Beckman centrifuge at 4 C to yield supernatant A. To 2.0 ml aliquots of supernatant A, 0.5 ml of 25% trichloroaceticacid was added. The mixture was allowed to stand for 30 min at 4°C followed by centrifugation at 3000 rpm for 15 min at 4 C to yield supernatant B. To each cuvette was added 2.0 ml of 0.5 M sodium phosphate buffer (pH 8.0) and 0.03 ml of DTNB (stock solution 4 mg/ml in 0.1 M sodium phosphate buffer, pH 7.0, with the addition of 1.5 mg/ml of sodium bicarbonate). To the sample cuvette was added 0.1 ml of supernatant B and to the reference cuvette was added 0.1 ml of buffer-trichloroaceticacid mixture (4:1 as above). Samples were mixed and after precisely 2 minutes, absorbance was read at 412 nm. All samples were assayed in duplicate with less than 2% variation. Absorbance of the samples was checked against a standard curve constructed with freshly prepared glutathione solutions (0.05-5.0 mM) treated in the same manner as the tissue samples.

Procedure for CBZ and its metabolite CBZ-E

4 ml of blood was withdrawn from high dose CBZ (100 mg/kg) administrated rats into heparinized tubes and the plasma was separated by centrifugation. 0.4 ml of plasma was extracted with 5 ml of dichloromethane by vortexing for 20 sec in a 10-ml glass centrifuge tube. The mixture was then centrifuged at 2500 rpm for 5 min and the top (aqueous) layer removed by aspiration. The solvent layer was then carefully transferred to a separate 15-ml conical tube and evaporated under a gentle stream of oxygen-free nitrogen. The residue was dissolved in 0.2 ml of acetonitrile:water (30:70) and vortexed for 15 sec. An aliquot (5 µl) was injected into the HPLC apparatus and analyzed in triplicate. The areas of the peaks were used for quantification.

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Preparation of standard stock solution

CBZ and CBZ-E were dissolved in methanol at a concentration of 400 µg/ml. The stock solutions were then used to prepare the solution of 4 µg/ml in water. The working solutions were kept at 4°C and were stable for at least 4 weeks.

HPLC

The HPLC system consisted of a Model 510 pump and a Model 481 variable-wavelength UV detector (Waters Assoc., Milford, MA, USA) , and a Unicam 4880 chromatography data handling system. 10 µm µBondapak C_{18} (Waters), 3.9 x 300 mm I.D. analytical column protected by a guard column filled with the same material, and mobile phase acetonitrile:water (30:70, v/v) were used. The mobile phase consisted of a freshly prepared mixture of acetonitrile and bidistilled water, which was filtered through a Millipore filter type HV (pore size, 0.45 µm; Waters Assoc.) and degassed ultrasonically before use. The volume of sample injected was 5 µl (Model 7725 Rheodyne injector).

The following operating conditions were used: wavelength, 210 nm; flow rate, 1.0 ml/min; chart speed, 0.25 cm/min; column temperature, ambient. The standard curves of CBZ (t_R=12.6 min) and CBZ-E (t_R=7.4 min) were linear in the concentration ranges studied. The linear regression line for CBZ was y = 6.357x - 4.44 (r = 0.9998), and for the epoxide y = 7.545x - 1.68 (r = 0.9999).

RESULTS AND DISCUSSION

Neither a single low dose nor repeated low doses of CBZ produced a statistically significant difference in GSH levels relative to controls. A single high dose CBZ administration produced a statistically significant decrease in the hepatic GSH level relative to controls (t-values: single low dose: 0.482; repeated low dose: 0.389; high dose: 11.944; t-test value calculated from the high dose group >4.221 [t 0.1%, 13]) (Table 1).

Carbamazepine and its metabolite carbamazepine-10,11-epoxide concentrations were determined in the plasma of the high dose CBZ administered animals by HPLC. This method allows a simple and rapid extraction of the compound requiring no further purification. The

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concentrations of CBZ and CBZ-E were 18.9 ± 2.9 μg/ml and 10.7± 2.8 μg/ml, respectively.

TABLE 1

Hepatic glutathione content and its depletion after carbamazepine administration in rats

Group	n	μmol GSH/g liver	Decrease (%)
Control	10	6.54±0.45	
Single low dose	5	6.43±0.28	1.7
Repeated low dose	5	6.62±0.12	-1.2
High dose	5	3.82±0.64	41.6

Electrophilic substances and electrophilic metabolites formed in the liver may react with nucleophilic macromolecules or may undergo conjugation with the hepatic nucleophil GSH, and can be detoxified by this pathway. This depletes the hepatic labile GSH pool and makes the cell more vulnerable to electrophilic substances. The reported occurrence of CBZ-associated adverse effects such as hepatocellular and cholestatic jaundice /1/ and granulamatous hepatitis /22,23/, may result from such mechanisms in the liver.

In this study, significant decreases in hepatic GSH level were observed after the high dose CBZ administration. The decrease in the hepatic GSH pool that we observed could occur due to the conjugation of the GSH in the labile pool with the electrophilic metabolite(s) of CBZ. In addition, CBZ and its active metabolite CBZ-E were identified and determined in plasma, and this determination seems to us to be necessary during the treatment of epileptic patients, especially with high doses of carbamazepine, due to the possibility of the occurrence of adverse effects and inter-individual variations.

REFERENCES

1. Rall TW, Schleifer LS. Drugs effective in the therapy of the epilepsies. In: Gilman GA, Goodman LS, Gilman A, eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics, 6th Ed. New York: Macmillan Publishing Co Inc, 1980; 448-474.

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Download Date | 6/16/12 6:30 AM

2. Frigerio A, Pantarotto C. Epoxide-diol pathway in metabolism of tricyclic drugs. J Pharm Pharmac 1976; 28. 665-666.
3. Astier A, Maury M, Barbizet J. Simultaneous, rapid high performance liquid chromatographic microanalysis of plasma carbamazepine and its 10,11-epoxide metabolite. J Chromatogr 1979; 164: 235-240.
4. Benet LZ, Sheiner LB. Design and optimization of dosage regimens; pharmacokinetic data. In: Gilman GA, Goodman LS, Gilman A, eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics, 6th Ed. New York: Macmillan Publishing Co Inc, 1980; 1690.
5. Regnaud L, Sirois G, Chakrabarti S. Effect of four-day treatment with carbamazepine at different dose levels on microsomal enzyme induction, drug metabolism and drug toxicity. Pharmacol Toxicol 1988; 62: 3-6.
6. Runci FM, Micheli L, Fiaschi AI, Giorgi G. Valproic acid and carbamazepine dramatically decrease the glutathione cerebral levels. Pharmacol Res 1990; 22 (Suppl 2): 437.
7. Attaguile G, Caruso A, Cutuli VMC, Di Giacomo C, Russo A, De Bernardis E, Amico-Roxas M. Influence of sodium valproate and carbamazepine on GSH levels in rat cerebral cortex. Drug Exp Clin Res 1992; 18: 465-467.
8. Madden S, Maggs J, Park BK. Bioactivation of carbamazepine in the rat: in vivo evidence for the formation of reactive arene oxide(s). Drug Metab Dispos 1996; 24: 469-479.
9. Chan K. Simultaneous determination of carbamazepine and its epoxide metabolite in plasma and urine by high performance liquid chromatography. J Chromatogr 1985; 342: 341-347.
10. Elyas AA, Ratnaraj N, Goldberg VD, Lascelles PT. Routine monitoring of carbamazepine and carbamazepine-10,11-epoxide in plasma by high performance liquid chromatography using 10-methoxycarbamazepine as internal standard. J Chromatogr 1982; 231: 93-101.
11. Hundt HKL, Clark EC. Thin-layer chromatographic method for determining carbamazepine and two of its metabolites in serum. J Chromatogr 1975; 107: 149-154.
12. Kaplowitz N. Interaction of azathioprine and glutathione in the liver of the rat. J Pharmacol Exp Ther 1977; 200: 479-486.
13. Ranise A, Benssi E, Besio G. Rapid gas chromatographic method for the determination of carbamazepine and unrearranged carbamazepine-10,11-epoxide in human plasma. J Chromatogr 1981; 222: 120-124.
14. Chambers RE. Simultaneous determination by gas-liquid chromatography of carbamazepine and carbamazepine-10,11-epoxide in plasma. J Chromatogr 1978; 154: 272-274.
15. Svirbely J. Toxicology and therapeutic drug monitoring (TDM). In: Kaplan LA, Pesce AJ, eds. Clinical Chemistry, Theory, Analysis and Correlation. St. Louis, MO: The C.V. Mosby Co. 1984; 1327-1343.
16. Westenberg HGM, De Zeeuw RA. Rapid and sensitive liquid chromatographic determination of carbamazepine suitable for use in monitoring multiple-drug anticonvulsant therapy. J Chromatogr 1976; 118: 217-224.

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17. MacKichan JJ. Simultaneous liquid chromatographic analysis for carbamazepine and carbamazepine 10,11-epoxide in plasma and saliva by use of double internal standardization. J Chromatogr 1980; 181: 373-383.
18. Bonato PS, Lanchote VL, De Carvalho D, Ache P. Measurement of carbamazepine and its main biotransformation products in plasma by HPLC. J Anal Toxicol 1992; 16: 88-92.
19. Eichelbaum M, Bertilsson L. Determination of carbamazepine and its epoxide metabolite in plasma by high-speed liquid chromatography. J Chromatogr 1975; 103: 135-140.
20. Juergens UJ. Simultaneous determination of zonisamide and nine other antiepileptic drugs and metabolites in serum - a comparison of microbore and conventional high-performance liquid chromatography. J Chromatogr 1987; 385: 233-240.
21. Menge GP, Dubois JP, Bauer G. Simultaneous determination of carbamazepine, oxcarbazepine and their main metabolites in plasma by liquid chromatography. J Chromatogr 1987; 414: 477-483.
22. Levy M, Goodman MW, VanDyne BJ, Sumner HW. Granulomatous hepatitis secondary to carbamazepine. Ann Intern Med 1981; 95: 64-65.
23. Davies-Jones GAB. Anticonvulsants. In: Dukes MNG, ed. Meyler's Side Effects of Drugs, 11th Ed. Amsterdam: Elsevier Science Publishers B.V., 1988; 128.

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