DOES CARBAMAZEPINE-EPOXIDE CONJUGATE WITH GLUTATHIONE?

On an Existing but Almost Forgotten Possibility

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SUMMARY

The anticonvulsant carbamazepine is widely used to treat affective disorders and behavioural disorders in non-epileptic children. We report an elevated plasma level of carbamazepine-10,11-epoxide in a cystinuric child after daily medication with 300 mg carbamazepine while the serum level of carbamazepine was in the therapeutic range. The concentrations of carbamazepine and its epoxide derivative were determined by HPLC. The formation of a glutathione conjugate of carbamazepine-10,11-epoxide is raised as a hypothesis.

KEY WORDS

carbamazepine, carbamazepine-10,11-epoxide, glutathione, cystinuria

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INTRODUCTION

Carbamazepine (CBZ) is a widely used anticonvulsant drug in the treatment of most forms of epilepsy /1-3/. Indications for CBZ also include trigeminal and other cranial neuralgias, and peripheral neuralgias /1-3/. Beside neurological indications, CBZ is used to treat psychiatric disorders such as affective disorders and behavioural disorders in non-epileptic children /1-4/.

CBZ is considered as a drug with a low frequency of unwanted side-effects, which have been observed mainly in older patients /1,2/. CBZ induces its own metabolism /1-3/; it is metabolized by a number of pathways, including 10,11-oxidation via the epoxide-diol pathway, hydroxylation of the aromatic ring, and formation of 9-hydroxy-CBZ through an as yet unknown mechanism /1-3/. N-Glucuronidation of the side-chain has also been described /1,2/.

In this report we suggest that a glutathione conjugate of the epoxide might also be an important intermediate of carbamazepine metabolism. Our suggestion is based on the case of a child treated with CBZ who showed a toxic level of carbamazepine-10,11-epoxide (CBZ-E) even after a low dose of CBZ.

PATIENT REPORT

An 11 year-old girl was referred to our department for behavioural problems, in particular for auto- and hetero-aggression. She had a previous psychiatric history.

Her father was an alcoholic, and her mother suffered from nephrolithiasis. In the parents' families, there was a history of alcoholism, hypertension and non-insulin dependent diabetes mellitus. Our patient was the only child of unrelated parents and was born from the first pregnancy of her 21 year-old mother after an eventful pregnancy of 40 weeks (body weight 3100 g, Apgar 8-9). Because of vomiting, her mother had been hospitalized three times during the first half of pregnancy and treated with vitamin B_6 plus haloperidol (Haloperidol®, Richter-Janssen-Cilag, Budapest, Hungary) and promethazine (Pipolphen®, Egis, Budapest, Hungary). She had a normal delivery.

At the age of 10 months, luxatio coxae congenita l.s. was diagnosed and the child was operated on when she was 2 years old. Psychomotor development was retarded and her behaviour was

autistic. From the age of 4.5 years she was controlled for autism by specialists. Cystinuria was diagnosed at the age of 9 years and since then she has been subjected to a diet.

On admission to our department the behaviour of the child was mainly characterized by auto- and hetero-agression, restlessness, screaming and continuous rolling of the head. At admittance she was already on antipsychotic and anxiolytic therapy. Changes were introduced into the doses of her medication. However, the behaviour of the child did not improve but worsened, mainly showing signs of an affective disorder.

Administration of carbamazepine (Neurotop retard®, Gerot Pharmazeutika, Vienna, Austria) was introduced. On the morning of the 12th day after admittance, 150 mg carbamazepine was given (10 mg/kg body weight). On the next day, the daily amount of carbamazepine was elevated to 300 mg/day, given as two equal doses in the morning and in the evening. Since there was no significant change in the clinical picture, the antipsychotic drug was replaced with sulpirid (Depral®, Alkaloida, Tiszavasvári, Hungary). On the 18th day after admission, blood was drawn to test the level of carbamazepine.

The levels of CBZ and CBZ-E were 6.9 mg/l and 6.4 mg/l, respectively, showing an elevated blood concentration of the epoxide. Therefore, on the 20th day after admission, carbamazepine was omitted. Eight days later, the carbamazepine level was controlled and neither CBZ nor CBZ-E were present in the plasma at detectable concentration.

MATERIALS AND METHODS

CBZ and CBZ-E were generous gifts from CIBA (Basle, Switzerland). Extralut®, KH_2PO_4, dichloromethane and acetonitrile, and octane-sulphuric acid sodium salt were from Merck, Darmstadt (Germany), Reanal, Budapest (Hungary), Carlo-Erba (Italy) and Sigma-Aldrich, Heidelberg (Germany), respectively.

Determination of CBZ and CBZ-E

Sample preparation. The concentrations of CBZ and CBZ-E were measured by a HPLC technique with modifications of the published methods /5,6/. In brief, after 10 min incubation at room temperature

the samples were spun down at 2000 rev/min at 4°C and 0.5 ml of the supernatant was loaded onto an Extralut® phase separator column (250 mg packed column material) equilibrated with 0.2 ml KH_2PO_4 (0.05 mM) before use. After 15 min equilibration the columns were washed six times with 2 ml dichloromethane and the eluted organic phase was evaporated with a gentle stream of nitrogen at 38°C. The concentrated material was then dissolved in 0.2 ml of mobile phase (see below) and an aliquot (10 µl) was loaded onto the HPLC column.

HPLC analysis. Analyses were performed on a reverse phase C8 column (Chrompack, Chromsep RP 8, 150 mm x 4; i.d. 5 µm) connected to a Pharmacia LKB 2248 pump, and linked to a 2141 variable wavelength UV detector with two channels, a 2157 auto-sampler and a Spark Holland Mistral column thermostat (setting 22°C). Mobile phase was 70/30% v/v of buffer (0.01 M KH_2PO_4 and 5 mM octane-sulphuric acid sodium salt, pH 6.86) and acetonitrile. Flow rate was 1 ml/min and detection was performed at wavelengths 210 and 230 nm.

Quantification of CBZ and CBZ-E. Quantification was based on the determination of peak areas by using known concentrations of CBZ and CBZ-E as internal standards. The peak height data were analyzed by PC-PE Nelson software. Detection limits for CBZ and CBZ-E were 0.1 mg/l and 0.05 mg/l, respectively. In our laboratory the therapeutic ranges for CBZ and CBZ-E are 4-12 mg/l and 0.5-3 mg/l, respectively. In our patients treated with a combination of carbamazepine and neuroleptics, the CBZ-E/CBZ ratio is 16.03±6.28% (mean±S.E.M.) (n=200).

DISCUSSION

The most important pathway in CBZ metabolism is the formation of CBZ-E, which is further converted by epoxide hydrolase into the dihydroxy-dihydro form of CBZ /1-3/. This latter compound is believed to be excreted in the urine as an unconjugated compound /1,2/. Although the percentage contribution of the different routes of elimination of CBZ is not exactly determined, it is estimated that about one-two thirds of the administered dose is recovered in urine as an unconjugated derivative of the parent molecule /1,2/. However, beside hydration by epoxide hydrolase for the elimination of epoxides, there is another possibility, conjugation with glutathione including

non-enzymatic and glutathione S-transferase catalyzed reactions /7/. Glutathione S-transferases are a large family of powerful enzymes capable of conjugating hydrophobic electrophylic compounds with reduced glutathione /7/. One benefit of glutathione conjugation is that the conjugate itself is likely to be secreted into the bile and excreted in the faeces /7/.

Glutathione is known to play an essential role in cellular defence against reactive agents such as kinons, phenols and free radicals /8/. Hence, any disturbance of glutathione metabolism would affect cellular function, and a decrease in cellular glutathione levels would sensitizes cells to toxic attack /8/. Unfortunately, the literature has few data concerning the effect of CBZ on tissue glutathione concentrations, and observations published to date are either contradictory or insufficient in this regard /9-11/. In rat brain, the coadministration of valproic acid and carbamazepine led to a dramatic decrease of glutathione content in one study, whereas in another study they had no effect on glutathione /9,10/. In rat liver carbamazepine depressed glutatione level by about 40% when it was administered at a dose of 100 mg/ kg body weight, referred to as a high dose /11/.

In our patient, a cystinuric girl, the administration of CBZ led to a toxic level of serum CBZ-E. Cystinuria is an inborn error of metabolism characterized by elevated urinary excretion of cystine, arginine, lysine and ornithine /12/. Since cystine is a dimeric form of cysteine, cysteine metabolism is also disturbed and thus cystinuria also indicates a disorder of cysteine metabolism. As glutathione synthesis is dependent on cysteine, glutathione metabolism may also be affected /13/.

Although urinary thioethers of CBZ metabolites have been detected in rats /14/, to our knowledge no glutathione adduct of CBZ-E has yet been identified in humans. The fact that glutathione adducts of CBZ-E have not yet been documented does not argue against the feasibility of this reaction in humans.

In children aged 8-15 years (n=7) who were on multiple doses of carbamazepine, a value of 14.3±2.9 mg/kg body weight/day total dose resulted in a CBZ-E/CBZ ratio of 23±8% when carbamazepine monotherapy was administered as a tablet /15/. In our patient, the CBZ-E/CBZ ratio was 93%. Since the plasma level of the parent molecule was in the normal therapeutic range, overdose of CBZ can be excluded as the cause of the toxic level of CBZ-E. One possible explanation for

the elevation of the CBZ-E concentration in this cystinuric girl is the lack of or an impaired rate of glutathione conjugation with epoxide. Our observation directs attention to the possible conjugation of CBZ-E with glutathione in humans, resulting in a potentially important therapeutic implication: namely, that during CBZ therapy, particular attention should be paid to those patients who might have any disturbance of glutathione metabolism (e.g. diabetes mellitus, inborn errors of metabolism).

REFERENCES

1. Bertilsson L, Tomson T. Clinical pharmacokinetics and pharmacological effects of carbamazepine and carbamazepine-10,11-epoxide. Clin Pharmacokinet 1986; 11: 177-198.
2. Sillanpaa M. Carbamazepine: Pharmacology and clinical uses. Acta Neurol Scand 1981; 64 (Suppl 88): 11-202.
3. Spina E, Pisani F, Perucca E. Clinically significant pharmacokinetic drug interactions with carbamazepine. Clin Pharmacokinet 1996; 31: 198-214.
4. Ballanger JC. The clinical use of carbamazepine in affective disorders. J Clin Psychiatry 1988; 49 (Suppl): 13-19.
5. 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.
6. Farinotti R, Mahuzier G. Simultaneous determination of six anticonvulsants in serum by high performance liquid chromatography. J Liq Chromatogr 1979; 2: 345-364.
7. deBethizy JD, Hayes JR. Metabolism: a determinant of toxicity. In: Hayes AW, ed. Principles and Methods of Toxicology, 2nd Ed. New York: Raven Press, 1988; 29-66.
8. Meister A. Glutathione metabolism and its selective modification. J Biol Chem 1988; 263: 17205-17208.
9. Attaguile G, Caruso A, Cutuli VMC, di Giacomo C, Ruuso A, de Bernardis E, Amico-Roxas M. Influence of sodium valproate and carbamazepine on GSH levels in rat cerebral cortex. Drugs Exp Clin Res 1992; 18: 465-467.
10. 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.
11. Yesilaltay AK, Ersoy Ö, Omurtag GZ, Yurdun T. Effects of carbamazepine on hepatic glutathione level in rats and determination of carbamazepine and its epoxide metabolite in plasma by HPLC. Drug Metab Drug Interact 1998; 14: 251-258.
12. Gitomer WL, Pak CYC. Recent advances in the biochemical and molecular biological basis of cystinuria. J Urol 1996; 156: 1907-1912.

13. Stipanuk MH, Coloso RM, Garcia RAG, Banks MF. Cysteine concentration regulates cysteine metabolism to glutathione, sulfate and taurine in rat hepatocytes. J Nutr 1992; 122: 420-427.
14. 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. Pharm Toxicol 1988; 62: 3-6.
15. Lanchote VL, Bonato PS, Campos GM, Rodridues I. Factors influencing plasma concentrations of carbamazepine and carbamazepine-10,11-epoxide in epileptic children and adults. Ther Drug Monit 1995; 17: 47-52.