9-HYDROXYMETHYL-10-CARBAMOYLACRIDAN IN HUMAN SERUM IS ONE OF THE MAJOR METABOLITES OF CARBAMAZEPINE

Sciji ETO, a Noriko TANAKA, a Hiroshi NODA, a * and Atsuko NODA b

Department of Hospital Pharmacy, School of Medicine, University of Occupational and Environmental Health, Japan (Sangyo Ika—Daigaku), a 1–1 Iseigaoka, Yahatanishi—ku, Kitakyushu 807, Japan, and Faculty of Pharmaceutical Sciences, Kyushu University, a 3–1–1 Maidashi, Higashi—ku, Fukuoka 812, Japan

In sera of 29 patients under the chronic administration of carbamazepine (CBZ, dose: 10.9 ± 4.3 mg/kg/day), a ring—contracted metabolite of CBZ was detected at a relatively higher concentration of 0.57 ± 0.30 μg/ml (mean ± S.D.) by high—performance liquid chromatographic assay. The structure of the metabolite was identified as 9—hydroxymethyl—10—carbamoylacridan (HMCA) by the liquid chromatography—mass spectral examination, and also by comparison with the authentic sample. Although HMCA was already reported in a few papers as a minor metabolite of CBZ in human urine, any reliable report for the determination of HMCA in human blood has never been published. Our results show that HMCA is one of the major metabolites of CBZ.

KEY WORDS carbamazepine metabolite; ring contraction; acridan derivative; human serum; HPLC; mobile phase

During the investigation of the high—performance liquid chromatographic separation of the chiral and achiral drugs and their metabolites in serum samples taken from the epileptic patients receiving carbamazepine (CBZ), we have noticed that an "unknown peak" with a considerably higher intensity in a chromatogram was always detected. 11 In the present study, we report that the "unknown peak" was attributed to 9—hydroxymethyl—10—carbamoylacridan (HMCA), a ring—contracted metabolite of CBZ. This is the first clear report of a determination of HMCA in human blood as a major metabolite of CBZ.

MATERIALS AND METHODS

Reagents 10,11—dihydro—10,11—epoxycarbamazepine (CBZ—epoxide) and 10,11—dihydro—10,11—trans—dihydroxy—carbamazepine (CBZ—diol) were kindly provided by Dr. F. A. Sedlacek of Ciba—Geigy (Basel, Switzerland). HMCA was prepared according to the method of Bellucci et al., 11 and was also given from Dr. Sedlacek. CBZ and 5—ethyl—5—p—tolylbarbituric acid used as an internal standard (I.S.) were purchased from Tokyo Kasei (Tokyo, Japan) and Aldrich Chem. Co. (Milwaukee, WI, USA), respectively. β—glucuronidase Type VII—A (from Escherichia coli) was obtained from Sigma (St. Louis, MO, USA). Other chemicals were of reagent grade, and the solvents for elution were of HPLC grade. Extrelut—1 was obtained from E. Merck (Darmstadt, Germany).

Apparatus and Chromatographic Conditions A Shimadzu LC—6AD HPLC system (Kyoto, Japan) equipped with an integrator (Shimadzu C—R4A) and a UV detector (Shimadzu SPD—6AV) with a Shimadzu Model SCL—6B system controller was employed. The columns, ODS—80Ts (150 × 4.6 mm I.D., TOSOH, Japan); the column temperature; ambient (ca. 26 °C) for HPLC and 40 °C for LC/MS; the mobile phase, a mixture of acetonitrile/methanol/water (19:16:65 by volume); the flow rate, 0.8 ml/min; the monitoring wave length, 210 nm. A liquid chromatography—mass spectrometer (JMS—LX1000, JEOL, Tokyo, Japan) was employed for characterization of the "unknown peak" observed in the chromatograms of serum samples of epileptic patients received CBZ.

Extraction Procedures 0.25 ml of 75 mM phosphate buffer solution (pH 6.8) containing 200 units of β—glucuronidase was added to 0.25 ml of serum taken from an epileptic patient receiving drug therapy. After incubation at 37 °C for 30 min, 0.1 ml of the I.S. solution was added, and then 0.5 ml of the mixture was poured into Extrelut—1 column. After 10 min, the column was eluted with 3 ml of ethyl acetate. The eluate was dried up under a gentle stream of nitrogen, and the residue was dissolved in 50 μl of methanol. An aliquot (10 μl) was injected into the chromatograph.

RESULTS AND DISCUSSION

We always noticed the presence of an "unknown peak" with a relatively higher intensity beside the peak of CBZ—epoxide in the chromatograms of HPLC each time the components in the serum samples from the epileptic patients were assayed for the investigation of the separation of chiral and achiral antiepileptics including CBZ (for

*a To whom correspondence should be addressed.
Fig. 1 The Characterization of HMCA on Liquid Chromatography–Mass Spectrometric Analysis
(A) Total ion monitoring ranging from \( m/z \) 235 to 275.
- peak-1, CBZ-diol; peak-2, CBZ-epoxide;
- peak-3, unknown peak; peak-4, CBZ.
(B) FAB mass spectrum of the unknown peak.
(C) Mass chromatogram at \( m/z \) 255.

9-Hydroxymethyl-10-carbamoylacridan (HMCA)

Fig. 2. Chromatogram of the Extract of Serum Sample Taken from an Epileptic Patient Treated with CBZ Alone in a Daily Dose of 250mg for a Long Period
Mobile phase
(A) : acetonitrile/methanol/water (19:16:65 by volume)
(B) : acetonitrile/water (30:70 by volume)
example, an unidentified peak between the peaks CBZ–epo and PHT shown in Fig. 3B in our previous paper). The chemical structure of the "unknown peak" metabolite was examined by liquid chromatography–mass spectrometry.

Fig. 1A is the total ion monitoring chromatogram (TIM) ranging from m/z 235 to 275, in which a peak at the retention time 14.4 min is the "unknown peak". The FAB mass spectrum of the "unknown peak" showed the parent peak at m/z 255 (molecular ion peak, [M+1] + ) and the characteristic peaks at m/z 180 ([M+1] + –CH₂OH-CO-NH₂) and 347 ([M+1] + + glycerol) (Fig. 1B). From the mass–chromatogram of the molecular ion (m/z 255), it is evident that m/z 255 was produced only from the "unknown peak" metabolite (Fig. 1C). Thus the molecular weight of the "unknown peak" metabolite, 254, corresponds to a one oxygen atom-deficient structure of CBZ–diol (M.W. 270), and possesses a hydroxymethyl group without elimination of carbamoyl group from the precursors such as CBZ–diol. It could therefore be presumed that the "unknown peak" metabolite was an aridivan derivative, 9-hydroxymethyl-10-carbamoylacidan (HMCA) which was possibly formed by the reduction of the Pinacol rearrangement product of CBZ–diol. The fragmentation pattern of the authentic sample of HMCA (see the section REAGENTS IN MATERIAL AND METHODS) was in accord with the "unknown peak" metabolite.

CBZ–epoxide or CBZ–diol was added to the mixed solution of drug–free serum and an equal amount of phosphate buffer solution (pH 6.8) with or without β-glucuronidase, and each solution was incubated for 2 h at 37 °C. The possibility of the rearrangement to HMCA from these metabolites during the sample preparation was eliminated, since any trace of HMCA could not be detected from the reaction mixtures.

By the application of the HPLC conditions described above to the assay of serum samples from the other 29 patients who received CBZ alone (dose: 10.9 ± 4.3 mg/kg/day), HMCA was detected from all patients’ samples without exception at a relatively higher concentration of 0.57 ± 0.30 μg/ml (mean ± S.D.). This mean concentration is comparable to 50.2 ± 17.5% (mean ± S.D.) of that of CBZ–epoxide. Some patients showed HMCA levels similar extent to those of CBZ–epoxide. From these observations, it is clear that HMCA is one of the major metabolites of CBZ in man.

CBZ is one of the typical antiepileptic drugs, of which the therapeutic monitoring is essential for individualization of dosage regimen. For this purpose, two major oxidized metabolites in serum, CBZ–epoxide and CBZ–diol, are usually assayed along with CBZ itself. Most of the papers regarding the determination of CBZ and its metabolites by HPLC recommended employing ODS columns, the mixtures of acetonitrile/methanol/water as mobile phases, and 30% or more of the concentrations of acetonitrile in most cases. Under these conditions, both peaks of CBZ–epoxide and HMCA overlap each other (Fig. 2B), and the intensity of CBZ–epoxide is overestimated, that makes incorrect results leading erroneous pharmacokinetic analysis. The mobile phase employed by us is a 19:16:65 mixture of acetonitrile/methanol/water which separates HMCA distinctly from CBZ–epoxide to get an exact conclusion (Fig. 2A).

Two reports showed that HMCA was excreted in urine as a minor metabolite. However, distinct discussions on the assay conditions were not described. On the other hand, the presence of HMCA in human blood has never been reported as yet, except a paper by Westenberg et al., in which they only stated the detection of trace amounts of HMCA in plasma without any presentation of data as well as discussion. Recently, Bernus et al determined small amounts of HMCA excreted in urine under appropriate conditions of HPLC, however, HMCA in blood was also never mentioned.

Reinvestigation of the determination of the serum concentrations of CBZ and its metabolites under an effective condition such as ours presented here is recommended.

REFERENCES


(Received March 20, 1995; accepted May 1, 1995)