Rapid Determination of Total Bromide in Human Serum Using an Energy-Dispersive X-Ray Spectrometer

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Since the bromide preparations useful in the treatment of intractable infantile epilepsy show a tendency to accumulate in the body, they may cause chronic toxicity. To prevent this, determination of the bromide ion concentration in the serum is essential. After establishing a simple and rapid technique using energy-dispersive X-ray spectroscopy for the analysis of the serum total bromide level, we applied this technique in a clinically diagnosed epilepsy patient. The standard curve for total bromide showed linearity (r=0.999) in the range of 10–2000 μg/ml, and the lowest detection limit was 5 μg/ml. The mean recovery rate of bromide added to reference serum to yield a concentration of 50 μg/ml was 93.5% (n=5, coefficient of variation=9.1%). Analysis took only 20 min. On analysis of the serum of a 10-year-old girl whose treatment was initiated with orally administered potassium bromide 1.0 g/kg, a good correlation was found between the total bromide level obtained with energy-dispersive X-ray spectroscopic analysis and the level of bromide ions determined by ion-exchange HPLC. The determination of serum total bromide by rapid energy-dispersive X-ray spectroscopic analysis is a useful method of monitoring to prevent bromide poisoning.

Key words bromide; energy-dispersive X-ray spectroscopy; epilepsy; serum; chronic toxicity

After the 1857 report by Locock1) on the usefulness of bromide as an antiepileptic, it was used as the principal ingredient of many preparations in common use as sedatives and anticonvulsants until 1975. Since 1975, the advents of phenobarbital and phenytoin have led to the almost total disuse of bromide. However, it is known that bromide is useful for the treatment of intractable epilepsy in children, which exhibits resistance to phenobarbital and phenytoin, and the need for it is now being reassessed.2—4) When bromide is administered to humans, it is readily absorbed from the digestive tract, free bromide ions are quickly distributed through the tissues other than the brain, and in various membrane transport systems, particularly in the nervous system, exhibit an inhibitory action on membrane transport. Thus by inhibiting neurotransmission they are able to exhibit a sedative effect.4—6) The sedative action of bromide is correlated with the serum concentration of bromide ions. The range of dosages for treatment is from 500 to 1000 μg/ml, while 1500 μg/ml causes poisoning.7,8)

Bromide is mainly excreted via the kidneys, but 24—36 h after administration only 1/10—1/4 of the amount absorbed is excreted in the urine, and the half-life of serum bromide ion is as long as 12 d.9) For this reason, when bromide is administered over the long term, it accumulates in the body and can easily induce chronic poisoning, causing various symptoms that originate in the central nervous system such as lethargy and dermatological symptoms. It is known that the bromide ion concentration in the body is affected by the amount of chloride ion uptake.10—13) Thus the blood concentration of bromide ions is difficult to predict from the dose administered. Therefore appropriate treatment requires monitoring the level of bromide ions in the serum.

With energy-dispersive X-ray spectroscopy (EDX), it is generally possible to perform quick qualitative and quantitative analysis of elements with a simple pretreatment procedure and it is suitable for analysis using biological samples.14—16) EDX has been employed by Ruan et al.15) to determine the permeability of the blood-brain barrier to lead in rats; by Nasstrom et al.18) to ascertain changes in the calcium and phosphorus content of dentin after treatment with mineral corticoid in rats; and by Kodaka et al.19) to measure the calcium and phosphorus levels in calcified tissues after NaOCl treatment in cattle and horses.

In the present study, we examined the conditions for the determination of serum total bromide levels using EDX. In addition, we studied the correlation between the serum total bromide concentration and the bromide ion concentration in patients treated with potassium bromide and investigated whether serum total bromide measurements using EDX could be used for monitoring the toxicodynamics of bromide.

MATERIALS AND METHODS

Reagents and Materials The bromide ion, chloride ion, fluoride ion, nitrate ion, nitrite ion, phosphate ion, and sulfate ion standard solutions were purchased from Wako Pure Chemical Industries (Tokyo, Japan). The serum used for recovery tests was standard human serum (Sigma Chemical Co., St. Louis, MO, U.S.A.). Other solvents used were of HPLC grade and special reagent grade.

Pretreatment of Samples A 100-μl sample of serum separated by centrifugation from a specimen of whole blood was dripped onto ST-30 filter paper (Shimadzu, Kyoto, Japan) that had a paraffin circle, and drying was carried out in a drier set at 40 °C.

Equipment Conditions An energy-dispersive X-ray spectroscope (Rayny EDX-700, Shimadzu) was used. The samples dried on filter paper were affixed to a sample plate with cellophane tape, and analysis was performed in a vacuum. In qualitative analysis, confirmation of characteristic X-
rays was performed using the Kα line of bromide. The quantitative analysis of total bromide was carried out using an eight-plot standard curve of 10, 50, 100, 200, 400, 800, 1600, and 2000 μg/ml.

Recovery Experiment To 1 ml of serum, 10 μl of a standard solution of bromide ions was added and the necessary adjustments were made to obtain final concentrations of 50, 100, 500, 1000, and 2000 μg/ml of total bromide. Since a preliminary experiment had shown that human serum contained a minute amount of bromide, the measured quantity of total bromide in blank serum (without added bromide) was subtracted from each of the measured quantities of total bromide in samples with added standard bromide solutions (n=5 each), and the recovery rate was expressed as the percentage of the measured quantities of bromide in aqueous solution containing bromide ions to each corresponding concentration.

Bromide Ion Determination with HPLC Using the serum of an actual patient, we attempted to ascertain the relationship between the amount of total bromide measured by EDX and that of bromide ion measured by ion-exchange chromatography.

Pretreatment of Serum To 150 μl of serum obtained by centrifugation from whole blood, distilled water was added up to a volume of 1.5 ml, and the mixture was introduced into an ultra filtration cartridge (Centrisart 1, Sartorius GmbH, Göttingen, Germany) to remove substances with molecular weights of 10000 or greater and centrifuged at 3000 rpm for 5 min. Then HPLC was performed on 50 μl of the resulting filtrate.

Conditions for HPLC A mobile phase of p-hydroxybenzoic acid 8 mm, Bis-Tris 3.2 mm, and 50 mm boric acid was introduced into the HPLC apparatus (SCL-10A VP; Shimadzu) at a rate regulated at 1.2 ml/min, and the sample was separated on an anion-exchange column (Shim-pack, IC-A3, 4.6 mm×150 mm, Shimadzu) to which was attached a guard column (Shim-pack, IC-GA3, 4.6 mm×10 mm, Shimadzu), all in an incubator at 40 °C. An electrical conductivity detector (SPD-M10A VP; Shimadzu) was used for bromide ion detection. The amount was then determined using analytical software (Class VP; Shimadzu) by the absolute calibration method.

Case History The patient was a 10-year-old girl with allergies to phenobarbital and phenytoin who had symptomatic localization-related epilepsy. Administration of potassium bromide 1.0 g/d, which excites no allergic reaction, was initiated. During the first 132 d, the serum total bromide level was determined on seven occasions, and the results were used for regulating the dose. The bromide ion concentrations in the serum samples drawn seven times were measured using ion-exchange chromatography, and the relationship with the serum total bromide concentration was examined.

After explaining to the mother of the patient that blood sampling was necessary for both treatment and research, she gave consent.

RESULTS

Total Bromide Determination Using EDX For the standard curve for bromide prepared using EDX, a regression line of \( Y = 0.0012X - 0.0007 \) was obtained between the measured intensity (cps/UA) and concentration (μg/ml) of total bromide concentrations from 10 to 2000 μg/ml, and the correlation coefficient was \( r = 0.999 \). In addition, within this range of concentrations, it was possible to confirm characteristic X-rays using KLM markers. When the signal-to-noise ratio was taken as 5, the detection limit was 5.0 μg/ml. The EDX spectra of 100 μg/ml of standard solution of bromide ions and standard serum containing added bromide to yield a total bromide concentration of 100 μg/ml are shown in Fig. 1. The Kα and Lα lines of bromide appear at specific positions and intensities of energy shown by the markers.

The recovery rates of total bromide added to standard serum are presented in Table 1. The recovery rates of 50, 100, 500, 1000, and 2000 μg/ml were good at between 93.5 and 101.3% and there was little scattering, with a coefficient of variation of 9.1% or less. The maximum time taken to

<table>
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<th>Added (μg/ml)</th>
<th>Recovery (%)</th>
<th>C.V. (%)</th>
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<tr>
<td>2000</td>
<td>101.3</td>
<td>3.1</td>
</tr>
<tr>
<td>1000</td>
<td>98.9</td>
<td>3.6</td>
</tr>
<tr>
<td>500</td>
<td>99.1</td>
<td>4.4</td>
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<tr>
<td>100</td>
<td>96.2</td>
<td>5.3</td>
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<tr>
<td>50</td>
<td>93.5</td>
<td>9.1</td>
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C.V., coefficient of variation. a) Amounts are expressed as μg/ml of serum. b) Values are means (n=5).

Fig. 1. EDX Spectra of Standard 100 μg/ml Solution of Bromide Ions (Upper Panel) and Standard Serum with Bromide Added to Yield a Total Bromide Concentration of 100 μg/ml (Lower Panel)
complete one analysis, including the time taken to separate the serum from the sample, was 20 min.

**Bromide Ion Determination by HPLC** The standard bromide ion curve obtained through ion-exchange chromatography, within the range 0.05—200 μg/ml, yielded a regression line between area and concentration of \( Y = 27369X - 3027 \), with a correlation coefficient of \( r = 0.999 \). Samples with concentrations higher than the range of the standard curve were diluted with distilled water before being analyzed. The lower limit of determination for bromide ion solutions was 0.05 μg/ml, and when the signal-to-noise ratio was taken as 5, the detection limit was 0.01 μg/ml.

A chromatogram of the standard negative ion solution and a chromatogram using a sample that was added to obtain a standard serum with a final bromide concentration of 10 μg/ml bromide ion is shown in Fig. 2. No interference peaks can be seen, and good separation was obtained for bromide ion. The mean recovery rate was 99.6 ± 1.3% (n = 5), and one analysis, including pretreatment, took 40 min.

**Bromide Analysis in Patient Serum and Relationship between Serum Total Bromide Concentration and Bromide Ion Concentration** A graph showing the relationship between the potassium bromide dose and the serum total bromide in the 10-year-old patient is shown in Fig. 3. Administration started at a dose of 1.0 g/d, and the serum total bromide concentration on the 44th day before the morning dosing was 899 μg/ml. During this period, the patient’s epileptic seizures were well controlled, and no effect was seen on her daily activities. To maintain control at about this concentration, the dose was decreased to 0.5 g/d from day 63. However, before the morning administration on day 77, the serum total bromide was found to be 2372 μg/ml, an unexpectedly high level. Fortunately, during that time, no neurological or psychological symptoms that could be considered due to chronic poisoning were observed. From the 77th day the dose was reduced to 0.4 g/d, and on day 89 the serum total bromide concentration was 1276 μg/ml. The dosage was not changed again, and periodic analysis of the total bromide...
were conducted. The concentration remained within the range of 1032—1276 µg/ml.

We also used ion-exchange chromatography to determine the bromide ion concentrations in the same serum samples. The serum bromide ion concentrations were slightly lower than those of total bromide, but they accounted for 88.2—96.7% of total bromide levels. Between the serum bromide ion concentration and the total bromide concentration, the regression line was $Y=0.91X+17.3$, and the correlation coefficient was $r=0.998$, showing a good correlation (Fig. 4).

**DISCUSSION**

A serum bromide ion concentration of up to 500 µg/ml is considered to be the therapeutic dose range, but the dose range of 500—1000 µg/ml is thought to be associated with “possible toxicity,” 1000—2000 µg/ml with “usually serious toxicity,” 2000—3000 µg/ml with “possible coma,” and 3000 µg/ml to be “possibly fatal.”7,8) Death is rare in bromide poisoning. In acute poisoning, digestive symptoms such as nausea and vomiting may occur; in chronic poisoning, various psychological symptoms occur which include restlessness, agitation, ataxia, confusion, delusion, mental aberration, loss of muscle strength, and stupor. In addition, in 25% of patients, pustules resembling acne also appear. Since bromide tends to accumulate in the body, it is difficult to maintain the serum bromide ion concentration within the effective therapeutic range, and it is essential to control the dose level by monitoring the serum level.4,5)

In the present study, we performed analysis of the serum total bromide concentration using EDX. The standard curve using the bromide $K\alpha$ line exhibited good linearity for serum total bromide concentrations from 10 to 2000 µg/ml, and within this range of concentrations qualitative analysis was performed with ease using the EDX spectrum. The pretreatment procedures were simple and satisfactory: after the serum was centrifuged, it was dripped onto filter paper, and then the paper was dried. The mean recovery rate of bromide added to the serum in amounts in the range of 50—2000 µg/ml was 93.5% or more. The method was fast, and the time required for one analysis, including the separation of the serum from the whole blood, was no more than 20 min.

The pharmacological actions of bromide are correlated with the concentration of bromide ions in the blood.7,8) We therefore performed analysis of bromide ions by anion ion-exchange chromatography and investigated whether the results of total bromide analysis by EDX were correlated with those of bromide ion analysis. A good linear standard curve was obtained using HPLC for bromide ion concentrations between 0.05 and 200 µg/ml, and a satisfactory value of 99.6% was achieved for the mean recovery rate of 10 µg/ml of bromide ion added to the serum. However, the time taken for one analysis, including that for pretreatment, was over 40 min, which was twice that needed for EDX.

The results of analysis using these two methods for the serum of a 10-year-old girl treated with potassium bromide were: for total bromide, 899, 1032, 1061, 1219, 1271, 1276, and 2372 µg/ml; and for bromide ions in the same specimens, 833, 981, 997, 1126, 1126, 1197, and 2187 µg/ml. These two series of figures exhibit good correlation. The fact that the total serum bromide concentrations determined using EDX and the bromide ion concentrations in the same specimens were correlated was considered to indicate that the use of EDX analysis to monitor treatment with, and poisoning by, bromide is a viable possibility.

The present patient was at first administered 1.0 g/d of potassium bromide. When the serum total bromide concentration reached 899 µg/ml on the 44th day, the seizures were markedly inhibited and no symptoms of poisoning were seen. To preserve this concentration, we reduced the dose to 0.5 g/d, but 33 d later an unforeseen rise to a concentration of 2372 µg/ml was observed. No notable symptoms of toxicity were seen, but determination of the serum content permitted a judgment on dose reduction. After the dose was decreased to 0.4 g/d, the concentration reached and maintained a favorable serum level of total bromide. In this patient, there was some difficulty in controlling the pharmacological effect from the dose level of bromide, and thus a rapid technique for determining the serum level of bromide is a useful tool.

It has now become possible to monitor treatment with bromide simply and quickly and to verify the appropriateness of treatment by analyzing the total bromide concentration using EDX. Our intention is now to work on drug treatment design by calculating the pharmacokinetic variables of bromide to prevent cases of poisoning.

**REFERENCES**