Successful Treatment of Severe Hyperammonemia Using Sodium Phenylacetate Powder Prepared in Hospital Pharmacy

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In order to treat a hyperammonemic patient with adult-onset type-II citrullinemia (CTLN2), sodium phenylacetate powder was prepared from chemical reagent grade phenylacetic acid in Gunma University Hospital. After purification by recrystallization, phenylacetic acid was neutralized with sodium carbonate and dried at 70 °C under reduced pressure. A solution of the prepared powder produced a single peak of \( m/z = 181.0 \) (M+Na⁺) in electrospray-ionization-MS spectrogram. The content of phenylacetate was 74% of theoretical value, suggesting the existence of water of crystallization. The content of phenylacetate remained constant for 5 months under dark conditions at room temperature. The prepared sodium phenylacetate powder was orally administered to a 16-year-old patient with CTLN2 at a dosage of 12 g/d. The serum ammonia concentration of the patient, who did not show adequate response to intravenous arginine or oral sodium benzoic acid, decreased remarkably to less than 100 \( \mu \)mol/d. Sodium phenylacetate powder should be an essential drug for the treatment of hyperammonemia caused by an inborn error of the urea cycle.

Key words phenylacetate; hyperammonemia; pharmacy

In the human body, the urea cycle is the major pathway for the disposal of waste nitrogen, and inborn errors of this pathway, including type-II citrullinemia (CTLN2) which shows a liver-specific decrease of argininosuccinate synthetase, may reduce the nitrogen flux. As a result, there is an accumulation of ammonia and disordered metabolism of other amino acids. Episodic hyperammonemia is observed in these patients, often resulting in coma and death. Serum ammonia can be reduced by administration of sodium benzoate and phenylacetate, since these compounds can produce the respective amino acid acylation products, hippurate or phenylacetylglutamine, resulting in an increase in urinary nitrogen. 1) Although the efficacy of phenylacetate for the treatment of hyperammonemia has been well established,2–7) the use of phenylacetate or benzoic acid for the treatment of hyperammonemia is not approved in Japan. Benzoic acid is sometimes prescribed as an unlabeled use drug, while phenylacetate was rarely used because of legal controls. Additionally, as phenylacetic acid has an unpleasant smell, it must be changed to sodium salt for clinical use, because sodium phenylacetate is not commercially available in Japan. We report the case of a hyperammonemic patient who was successfully treated with sodium phenylacetate powder, which was prepared at the manufacturing section of the Department of Pharmacy in Gunma University Hospital. Clinical details of the patient have been reported elsewhere.8)

All reagents except for phenylacetic acid were purchased from commercial sources and were used without further purification. Chemical reagent grade phenylacetic acid was purchased from Wako Pure Chemical Industries (Osaka, Japan). Since the reagent appeared to be light-yellow color, the phenylacetic acid was recrystallized to remove impurities. Fifty grams of phenylacetic acid were dissolved in 700 ml of distilled water at 70 °C, and the resulting lower yellow layer was discarded. The clear colorless layer was stored overnight at 4 °C for eduction, then filtered using a 0.45 µm membrane filter and washed twice with cold water. The purified powder was dried in a desiccator at reduced pressure for 1 d, then desiccated again with diphosphorus pentaoxide for 1 d.

The procedure described above was repeated five times and the resultant phenylacetic acid powder (191.5 g) was dissolved in distilled water. Then, sodium carbonate (74.5 g) was added to the solution at room temperature. The final concentration of phenylacetic acid and sodium carbonate was adjusted to be 2 and 1 M, respectively. After gentle mixing at 40 °C for 2 h, the solution stored at room temperature for one night. The mixture was then evaporated at 95 °C under the reduced pressure to obtain sodium phenylacetate powder (222.2 g).

To verify the ingredients of the powder, MS analysis was performed. The powder was dissolved in 50% methanol containing 0.5% acetic acid, and was infused at a rate of 10 µl/min. Time-of-flight MS equipment used was a Mariner (Applied Biosystems, U.S.A.), and the analytical conditions included a spray tip potential 4100 V, nozzle potential 140 V, quadrupole RF voltage 900 V, quadrupole temperature 140 °C, mode positive. A single peak of \( m/z = 181.0 \) (M+Na⁺) in electrospray-ionization-MS spectrogram was obtained (Fig. 1).

The content of phenylacetate was determined using HPLC. The prepared powder or purchased chemical reagent were dissolved into a mixture of 20 mM potassium dihydrogenphosphate : acetonitril (80 : 20) and subjected to HPLC, and the peak areas were compared. The HPLC instrument used was the Waters 2690 (U.S.A.) equipped with a Waters 2487 UV detector set at 230 nm. The column was a Symmetry ODS stainless column (4.6 mm×150 mm; Waters) main-

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Fig. 1. Electrospray-ionization MS Spectrogram of Sodium Phenylacetate

The MS conditions are described in the text.

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tained at 40 °C. The mobile phase was a 20 mM potassium dihydrogenphosphate : acetonitril (80 : 20) pumped at a rate of 1 ml/min. The content of sodium phenylacetate was 74% of the theoretical value (Table 1), suggesting the existence of water of crystallization. Since obtained powder may be a mixture of acid form and sodium salt, chemical element analysis should be performed to determine the content of each form. However, we did not perform such a test because total amount of both forms is clinically important and the unpleasant smell of obtained powder is endurable.

To investigate the stability of the preparation, the powder was stored at room temperature in dark conditions. The content of the phenylacetate after 5 months was 106.7±6.2% (n=5) of initial value.

A 16-year-old boy was admitted to the Department of Pediatrics in Gunma University Hospital. He demonstrated signs and symptoms of hepatic encephalopathy and his serum ammonia level elevated, but jaundice, ascites or splenohepatomegaly were not observed. The laboratory test results included total bilirubin of 2.1 mg/dl, alanine aminotransferase of 371 IU/l, cholinesterase of 3500 IU/l, ammonia of 530 µg/dl, citrulline of 814.5 nmol/ml and arginine of 188.3 nmol/ml. The diagnosis of CTLN2 was made based upon gene analysis showing a homozygote for IVS11+1G→A mutation in SLC25A13 gene.8) Total parenteral nutrition of 1800 kcal/d (15 g protein/d) and intravenous arginine 7.7 g/d were initiated. Serum ammonia decreased to 100 µg/dl and the patient’s consciousness became clear. On hospital day 13, serum ammonia level was elevated to 237 µg/dl, then dosage of arginine increased to 15 g/d and administration of sodium benzoate 4.8 g/d was commenced. Temporal decrease of ammonia level was observed due to the sodium benzoate, but the patient’s consciousness remained unclear. Following Pharmaceutical Committee in Gunma University Hospital approval, oral administration of sodium phenylacetate powder 12 g/d ter in die was initiated on hospital day 44. Serum ammonia levels were markedly reduced to 15—50 µg/dl within a week.

To access the most effective concentration of phenylacetate in the plasma, a blood sample was taken before drug administration and at 0.5, 1, 2, 3 and 4 h after administration on hospital day 90. Blood was immediately centrifuged to obtain plasma. To 0.4 ml of plasma, 0.4 ml of 1 M phosphoric acid and 0.1 ml of p-nitrobenzoic acid in methanol as an internal standard were added. The mixture was shaken with 2.5 ml of dichloromethane for 10 min and centrifuged for 5 min. Two milliliters of organic phase were transferred to another tube, and evaporated at reduced pressure at room temperature. The dried residue was dissolved in a mobile phase and subjected to HPLC.

The HPLC conditions were the same as described above. The detection limit of this assay was 5 µg/ml of phenylacetate and reproducibility was high with the coefficient of variation of less than 5%. The trough plasma concentration of phenylacetate was 16.8 µg/ml, and reached to a maximum concentration of 93.2 µg at 1 h after drug administration (Fig. 3).

The serum ammonia level was maintained under 100 µg/ml during phenylacetate therapy. The patient experienced a progressive improvement in physiological status, and underwent a liver transplantation on hospital day 118.

This is the report of a case of citrullinemia with argini-

<table>
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<th>Lot No.</th>
<th>Content (%)</th>
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<td>4</td>
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Mean±S.D. 73.8±6.1

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![Fig. 1](image1.png)

*Fig. 1.* Plasma Phenylacetate Concentration Profile after Oral Administration

The patient received 4 g of sodium phenylacetate powder 3 times a day. The abscissa represents the time after oral dose of 4 g in the morning.
nosuccinate synthetase deficiency treated with oral sodium phenylacetate. In our case, the efficacy of benzoic acid, which is easily available as an antiseptic, was limited. Sodium phenylacetate therapy markedly improved the hyperammonemia and was likely essential for this patient. Though the efficacy of phenylacetate is well established, it is not commercially available as a pharmaceutical preparation in Japan. Since phenylacetic acid can be obtained as a chemical reagent, change of preparation form is an alternative to avoid an unpleasant smell or taste, or to make dispensing easier, but it remains to be considered. We prepared the sodium phenylacetate powder and attempted to accurately ensure the quality of the preparation. The prepared powder may be clinically significant in the treatment of hyperammonemia with urea cycle abnormality.

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REFERENCES