Effect of single oral dose of sodium benzoate on ureagenesis in healthy men and two patients with late onset citrullinaemia

K. Kubota and T. Ishizaki

Division of Clinical Pharmacology, Clinical Research Institute, National Medical Center, Toyama, Shinjuku-ku, Tokyo, Japan

Received: February 3, 1993/Accepted in revised form: July 28, 1993

Summary. Although sodium benzoate therapy is beneficial in patients with inborn error of urea cycle, it has been suggested that an accumulation of benzoyl-CoA would inhibit ureagenesis. In this study, we examined several aspects of ureagenesis after the single oral dosing of sodium benzoate in six healthy subjects who participated in the study previously reported and in two patients with citrullinaemia.

Urea nitrogen appearance (UNA) did not change after the doses of 40, 80 and 160 mg·kg⁻¹ of sodium benzoate in the 6 healthy subjects. Sum of UNA and urinary hippurate nitrogen (UHN) increased with increasing the dose. In the 2 patients with late-onset citrullinaemia who benefit from a partially functional urea cycle, no apparent inhibitory effects on the UNA were observed after the administration of 80 mg/kg of sodium benzoate.

The precursors of urea (ammonia, glutamate, glutamine and α-amino nitrogen) did not increase after the benzoate administration in the 6 normal subjects as well as in the 2 patients with citrullinaemia.

Key words: Benzoate, Citrullinaemia; hyperammonaemia, ureagenesis

Benzoic acid is transformed into hippuric acid, in the two reaction sequence below [1, 2]:

\[
\text{Benzoic acid} + \text{ATP} + \text{CoA} \rightarrow \text{Benzoyl-CoA} + \text{PPi (Step 1)}
\]

\[
\text{Benzoyl-CoA} + \text{Glycine} \rightarrow \text{Hippuric acid} + \text{CoA (Step 2)}
\]

Benzoate therapy has been reported to be beneficial in hyperammonaemic patients with various inborn errors of urea cycle [3–5]. However, it is not yet fully known whether protection against ammonia toxicity could be offered by benzoate in patients who benefit from a partially functional urea cycle. Benzoate may decrease the total nitrogen excretion by its possible inhibitory effect on ureagenesis [6]. In addition, sodium benzoate has been reported to potentiate ammonia toxicity by ammonium acetate in mice [7] and rats [8]. According to Cyr et al [9], toxic effects associated with sodium benzoate are considered to be caused by an accumulation of benzoyl-CoA.

On the other hand, only a few minor adverse-effects (e.g., nausea, vomiting, diarrhoea, anorexia, headache, tinnitus, vertigo and giddiness) may occur with benzoate in normal subjects and patients with inborn error of urea cycle [10–12]. Letarte et al [13] and Takeda et al [14] have reported that a long-term oral administration of sodium benzoate was effective in reducing the frequency and severity of hyperammonaemic attacks in patients with partial ornithine carbamoyl transferase deficiency.

In the study previously reported [15], we described the pharmacokinetics of benzoic and hippuric acids after the single oral administration of the three doses of benzoate to the normal subjects. In the present paper, urea nitrogen appearance and blood levels of precursors of urea (as well as hippurate) measured in the above study are reported to ask if sodium benzoate would show an in vivo inhibitory effect on the ureagenesis and elimination of waste nitrogen. We also provide the results obtained after the single and long-term administration of benzoate in two patients with late-onset citrullinaemia, a unique variant which is known to occur almost exclusively among Japanese and is characterized by a mild deficiency of hepatic arginosuccinate synthetase.

Material and methods

Study subjects

Six healthy male volunteers participated in the study after giving the informed consent as described previously [15].

Patient 1 is a 31 years-old male. Since his early childhood, he favored large amounts of soybeans and peanuts as observed commonly in late-onset citrullinaemia patients [16]. Otherwise, he was well till aged 18 years when vomiting, drowsiness, dysarthria and gait disturbance developed, particularly in the nighttime. Five months before the admission, valproate therapy (600 mg per day) was started after an episode of unconsciousness and convulsion. Thereafter, progressive weakness and drowsiness developed, and he was admitted to the hospital. His plasma citrulline concentration ranged from 12.6 to 26.9 μmol·dl⁻¹ (normal, 3.0 to 4.7 μmol·dl⁻¹) and urinary citrulline excretion from 26.4 to 39.4 μmol per day (normal <2 μmol per day) without sodium benzoate administration after
the admission. Plasma ammonia levels measured without sodium benzoate therapy were 81 μmol l−1 (137 μg dl−1) before breakfast but 181 μmol l−1 (307 μg dl−1) at 21.00 h after evening meal. Argininosuccinate synthetase activity in the liver, obtained by an open liver biopsy, was 7.6% of the average value in normal controls, whereas carbamoylphosphate synthetase, ornithine transcarbamylase, arginase and arginosuccinate and arginase were within normal limits.

Patient 2, a 33 years old male, was admitted after his first episode of unconsciousness. He also favored large amounts of soybeans and peanuts. His plasma citrulline concentrations ranged from 7.0 to 37.6 μmol dl−1 and urinary citrulline excretion from 20.0 to 1540 μmol per day. He had the amino acid pattern reported to be unique in patients with late-onset citrullinemia [17]. Although the biopsied liver specimen was not available, late-onset citrullinemia was a possible diagnosis in this patient.

**Table 1.** Urea Nitrogen Appearance (UNA) and Urinary Hippurate Nitrogen (UHN). UUN urinary urea nitrogen excretion rate. Means (SEM)

<table>
<thead>
<tr>
<th>Dose of sodium benzoate (mg · kg⁻¹)</th>
<th>0 (control)</th>
<th>40</th>
<th>80</th>
<th>160</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UNA</strong>&lt;sub&gt;a&lt;/sub&gt; (mg N/kg/h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1.5 h</td>
<td>7.32 (0.45)</td>
<td>7.75 (1.07)</td>
<td>8.18 (1.31)</td>
<td>7.74 (0.61)</td>
</tr>
<tr>
<td>[7.36 (0.46)]</td>
<td>[7.77 (1.06)]</td>
<td>[8.19 (1.31)]</td>
<td>[7.75 (0.61)]</td>
<td></td>
</tr>
<tr>
<td><strong>UNA</strong>&lt;sub&gt;b&lt;/sub&gt; (mg N/kg/h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1.5 h</td>
<td>4.64 (0.65)</td>
<td>4.24 (0.90)</td>
<td>5.09 (0.89)</td>
<td>5.37 (1.78)</td>
</tr>
<tr>
<td>[4.65 (0.64)]</td>
<td>[4.53 (1.02)]</td>
<td>[6.71 (0.87)]</td>
<td>[6.81 (1.84)]</td>
<td></td>
</tr>
<tr>
<td>1.5-3 h</td>
<td>4.88 (0.80)</td>
<td>5.54 (0.51)</td>
<td>6.19 (0.62)</td>
<td>5.15 (0.10)</td>
</tr>
<tr>
<td>[4.89 (0.79)]</td>
<td>[6.20 (0.58)]</td>
<td>[8.09 (0.58)]</td>
<td>[6.94 (1.04)]</td>
<td></td>
</tr>
<tr>
<td>3-4.5 h</td>
<td>4.95 (0.69)</td>
<td>5.84 (0.39)</td>
<td>5.06 (0.60)</td>
<td>6.29 (1.10)</td>
</tr>
<tr>
<td>[4.96 (0.69)]</td>
<td>[5.95 (0.40)]</td>
<td>[8.16 (1.10)]</td>
<td>[6.81 (1.10)]</td>
<td></td>
</tr>
<tr>
<td>4.5-6 h</td>
<td>4.64 (0.66)</td>
<td>4.75 (0.52)</td>
<td>4.57 (0.82)</td>
<td>5.20 (0.79)</td>
</tr>
<tr>
<td>[4.66 (0.66)]</td>
<td>[4.79 (0.53)]</td>
<td>[4.92 (0.88)]</td>
<td>[6.51 (0.78)]</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Values in the brackets represent those of [UUN + UHN]<br>
<sup>b</sup> Values in the brackets represent those of [UNA + UHN]. Difference in the mean values for [UNA + UHN] among four doses is significant (P < 0.01, ANOVA), whereas differences in the mean values for UNA is not significant.

**Drug used and its administration schedules**

To each of the healthy volunteers, 40, 80 and 160 mg · kg⁻¹ of sodium benzoate dissolved in 300 ml of water and 300 ml of water as the control baseline were orally administered as described previously [15]. They ate a constant diet containing 1.5 g · kg⁻¹ per day of protein for 3 days before the study. To patients 1 and 2.0 (i.e., 300 ml water) and 80 mg · kg⁻¹ of sodium benzoate dissolved in the 300 ml water were orally given. Each dose was given after an overnight fast at least 1 week apart. The patients ate a constant diet containing the same amount of protein (0.8 and 1.1 g · kg⁻¹ per day in Patients 1 and 2, respectively) throughout the study. All subjects were fasted for 6 h after the dosing, but with a free access to tap water. Patient 2 received sodium benzoate at a dose of 280 mg · kg⁻¹ per day of sodium benzoate for 6 months.

**Sample collection**

Blood samples were collected at 0 (predose), 0.5, 1, 1.5, 2, 3, 4.5 and 6 h postdose in normal subjects, and at 0, 1.5, 3, 4.5 and 6 h postdose in patients with late-onset citrullinemia. Urine samples were collected at −1.5 to 0 (baseline), 0 to 1.5, 1.5 to 3, 3 to 4.5 and 4.5 to 6 h postdose in all subjects.

**Assay procedures and calculation of urea nitrogen appearance (UNA)**

Plasma ammonia levels were determined according to Kurahashi et al [18] (coefficients of variation, CV < 5% for between-day assay). Serum and urinary urea nitrogen were determined by the urease-glutamate dehydrogenase method [19] (CV < 1.5%). Plasma amino nitrogen levels were determined according to Goodwin [20] (CV < 2.2%). Amino acid analysis in serum was performed with a JLC-200A amino acid analyzer (JEOL, Tokyo, Japan). Urea nitrogen appearance (UNA) or net urea nitrogen production was calculated according to Walser et al [21] as:

\[
\text{UNA} = \frac{(\text{BUN}_f - \text{BUN}_i) \times \text{BW} \times 0.60 + (\text{BW}_f - \text{BW}_i)}{\text{BW}_1}\]

where i and f indicate the initial and final values for the period of measurements, respectively; BUN is blood (serum) urea nitrogen; BW is body weight; 0.60 is the fraction of BW representing urea compartment and UNe is urea nitrogen excreted in urine.

**Statistical analysis**

All values are reported as the mean (SEM). ANOVA followed by the least significant difference (LSD) test was used where appropriate. The P values < 0.05 were considered statistically significant.

**Results**

Nausea and lightheadedness developed in 0, 1, 4 and 5 volunteers after the administration of 0 (control), 40, 80 and 160 mg · kg⁻¹ of sodium benzoate, respectively. After the largest dose (160 mg · kg⁻¹), two subjects had tinnitus and three had visual disturbances (scotomata and red vision). These unwanted effects usually developed at 0.5 h postdose and lasted for 1 to 2 h. None had those untoward effects for more than 3 h.

There were no statistically significant differences in baseline or predose serum glycine, glutamine and glutamate.