Objective  We report a risk of worsening of encephalopathy by glycerol infusion when this osmotic agent is used for the treatment of brain edema in patients with adult-onset type II citrullinemia (CTLN2) caused by citrin deficiency.

Patients and Methods  We performed a retrospective investigation of 3 patients with CTLN2 treated for brain edema at our institute: a 31-year-old male patient and a 40-year-old female patient received treatment for encephalopathy-related brain edema with 10% glycerol infusion and 20% D-mannitol, and a 40-year-old male patient received only 20% D-mannitol infusion. In addition, we also performed a retrospective study in 11 CTLN2 patients reported previously (8 patients treated with 10% glycerol, 2 treated with 10% glycerol and 20% mannitol, and 1 treated with 20% mannitol).

Results  The 12 patients treated with 10% glycerol, including 2 of our patients, died due to rapidly deteriorating encephalopathy and brain edema. On the other hand, the 2 patients who received only 20% D-mannitol, including one of our patients, recovered with the disappearance of brain edema.

Conclusion  In CTLN2 patients, glycerol infusion seems to be associated with exacerbation of encephalopathy itself and only mannitol should be used for the treatment of brain edema in patients with this disorder. (Internal Medicine 44: 188–195, 2005)

Key words: type II citrullinemia, citrin, glycerol, mannitol, brain edema, cytosolic NADH

Introduction  Citrullinemia is an autosomal recessive disease characterized by highly elevated plasma levels of citrulline and ammonia due to a deficiency of argininosuccinate synthetase (ASS) (1). This disorder can be classified into three types: neonatal/infantile (types I and III, CTLN1) and adult (type II, CTLN2) (2). Most cases of CTLN2 have been reported in Japan, and the causative gene of this disorder, SLC25A13, which encodes a calcium-binding mitochondrial solute transporter (citrin), was recently identified (3, 4). Patients with CTLN2 present various neurological and psychotic symptoms that closely resemble those of hepatic encephalopathy (5). Before application of liver transplantation as a curative treatment (5–8), most patients died within a few years after onset. Severe brain edema due to encephalopathy is a direct cause of death in patients with CTLN2, and there have been only a few reports of successful recovery of patients from serious encephalopathy with brain edema by medication (9, 10). In general, glycerol and/or mannitol have been administered intravenously as osmotic agents for the management of brain edema. However, many patients with CTLN2 died of brain edema soon after administration of glycerol (11–19).

Here, we report the results of treatment of brain edema in three patients with CTLN2: two patients treated with glycerol and mannitol died due to rapidly expanding brain edema, while the remaining one patient recovered with mannitol infusion. Together with the results in the previously reported patients treated with glycerol and/or mannitol, we investigate the risk of glycerol infusion in CTLN2 patients.
Case Reports

During the past 3 years, three patients with CTLN2 were treated at our hospital with 10% glycerol and/or 20% D-mannitol for encephalopathy-related brain edema. Clinical data for these 3 patients are summarized in Table 1: a definite diagnosis of CTLN2 was made by DNA analysis of the SLC25A13 gene (3) and/or demonstration of selective reduction in hepatic ASS activity with elevated plasma concentrations of ammonia, citrulline, and arginine (2), and elevated serum levels of pancreatic secretory trypsin inhibitor (PSTI) (20).

### Patient 1

The patient was a 31-year-old Japanese man whose clinical course was reported elsewhere (5). Briefly, one night in April 1997 (when he was 25 years old) he suddenly woke up, shrieked, and started banging his head violently against a wall. The next morning he became normal but he experienced a similar attack one month later. He was admitted to a local hospital where highly increased plasma concentra-

### Table 1. Clinical and Laboratory Summary of Three CTLN2 Patients

<table>
<thead>
<tr>
<th>Case (sex)</th>
<th>1 (M)</th>
<th>2 (F)</th>
<th>3 (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>31</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>NH₃ (µg/dl)</td>
<td>137</td>
<td>187</td>
<td>166</td>
</tr>
<tr>
<td>Citrulline (nmol/ml)</td>
<td>794.6</td>
<td>373.4</td>
<td>186.9</td>
</tr>
<tr>
<td>Arginine (nmol/ml)</td>
<td>119.7</td>
<td>258.2</td>
<td>175.0</td>
</tr>
<tr>
<td>PSTI (ng/ml)</td>
<td>120</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>ASS activity (U/mg protein)</td>
<td>0.0030</td>
<td>0.0053</td>
<td>0.0013</td>
</tr>
<tr>
<td>SLC25A13 gene</td>
<td>I/II</td>
<td>II/II</td>
<td>II/?</td>
</tr>
</tbody>
</table>


![Image of serial CT images of the brain in patient 1 (A) and patient 2 (B).](image)

**Figure 1.** Serial CT images of the brain in patient 1 (A) and patient 2 (B). In both patients 1 and 2, brain edema was rapidly exacerbated one or two days after administration of glycerol. Plasma ammonia level: normal <66 µg/dl. NE: not examined.
tions of ammonia (359 μg/dl; normal <66 μg/dl) and citrulline (794.6 nmol/ml; normal <50 nmol/ml) were detected. He was referred to our hospital and was diagnosed as having CTLN2 by urea cycle enzyme assay (Table 1). Intravenous infusion of arginine and branched-chain amino acids (BCAA) was started, with restriction of oral protein intake (40 g/day), and oral administration of lactulose (90 ml/day) and kanamycin (1.5 g/day) was added. However, attacks of disturbed consciousness recurred once or twice a month. In November 2001, oral administration of L-arginine (15 g/day) (19) was started instead of intravenous infusion of BCAA and arginine, and the frequency of disturbed consciousness attacks decreased gradually. Although his physical condition had been stable until October 2002, he began to suffer awkward gait. He was admitted to our hospital again on January 14, 2003. On neurological examination, he was alert but showed slight weakness in both lower legs with ankle clonus and hyperactive deep tendon reflexes. His plasma ammonia level was within the normal limits (33 μg/dl) on admission. Although he was thought to have spastic paraplegia, magnetic resonance images (MRIs) of the brain and whole spinal cord were not informative. While investigating the causes of spastic paraplegia, the patient again showed disturbed consciousness on January 30, 2003. Brain CT showed low-density areas in the bilateral frontal lobes (Fig. 1A). While his plasma ammonia level was not markedly elevated (137 μg/dl), intravenous infusion of BCAA (1,000 ml/day) and 10% glycerol drip (600 ml/day) were started. His serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were mildly elevated to 82 U/l (normal <37 U/l), 77 IU/l (normal <45 U/l), and 281 U/l (normal <220 U/l), respectively. The next day, he had repeated convulsions and fell into a deep coma. Brain CT revealed widely expanded low-density areas throughout the whole brain and severe brain edema (Fig. 1A). His plasma ammonia level was markedly elevated to 316 μg/dl, although there were no remarkable changes in serum levels of AST (71 U/l), ALT (75 U/l), or LDH (269 U/l). Plasma lactate level was highly elevated to 40 mg/dl (normal: 3–17 mg/dl). In addition to 10% glycerol drip (600 ml/day), 20% D-mannitol drip (500 ml/day) was added, but his condition did not improve and he died of severe brain edema on January 31, 2003. On postmortem macroscopic examination, his brain had a soft and edematous appearance with tentorial herniation. Microscopic examination revealed severe edematous change with marked vascular congestion in an extensive area of white matter, with no necrotic changes, and well-preserved cortical structures.
Patient 2

The patient was a 40-year-old Japanese woman whose detailed clinical course was reported elsewhere (21). Briefly, at age 39 in October 2000, she developed disturbance of consciousness accompanied by abnormal behavior. She was alert when she visited our hospital. Laboratory examinations revealed markedly increased concentrations of plasma ammonia (187 μg/dl), citrulline (373.4 nmol/ml), and arginine (258.2 nmol/ml: normal <130 nmol/ml). Serum concentrations of α-fetoprotein (460.5 ng/ml; normal <20 ng/ml) and protein induced by vitamin K absence-II (466 IU/ml; normal <40 IU/ml) were also elevated. Abdominal CT revealed a solid mass lesion in segment 6 of the liver, suggesting the presence of hepatocellular carcinoma (HCC). She was diagnosed as having CTLN2 by DNA analysis (Table 1). Transarterial embolization (TAE) of the HCC was performed in January 2001, followed by surgical resection of the liver, including the tumor in August 2001. After the operation, the plasma ammonia concentration fluctuated between 200 and 300 μg/dl, and hepatic encephalopathy occurred occasionally, even when she had been hospitalized on a strict regimen of conservative treatment consisting of intravenous infusion of hyperalimentation fluid with BCAA (1,000 ml/day) and a low protein diet (40 g/day) with oral administration of lactulose (90 ml/day) and kanamycin (1.5 g/day). The long-term use of intravenous hyperalimentation and protein-restricted nutrition induced liver dysfunction with severe fatty liver. From September 2000, L-arginine (15 g/day) was added orally and protein intake was increased slightly to 60 g/day to ameliorate her poor nutritional state. She still experienced disturbed consciousness for a duration of one or two days twice a month and her general condition gradually deteriorated. On December 20, 2001, disturbed consciousness recurred and her plasma ammonia level was slightly elevated to 145 μg/dl. On December 23, the patient fell into a semi-coma and her plasma ammonia level was elevated to 234 μg/dl. Laboratory examination revealed elevation of serum levels of transaminases (AST: 60 U/l and ALT: 221 U/l). Brain CT demonstrated moderate brain edema (Fig. 1B). Intravenous administration of 10% glycerol (600 ml/day) was begun and plasma exchange was carried out. The next day, her consciousness level deteriorated further and brain CT revealed exacerbated brain edema. Prothrombin time was mildly prolonged to 15.8 seconds (normal 10–12 seconds) but activated partial thromboplastin time was within normal limits (27.7 seconds). Her serum levels of transaminases were 38 U/l (AST) and 73 U/l (ALT). The amount of 10% glycerol drip was increased up to 800 ml/day, 20% D-mannitol drip was added (500 ml/day) to prevent the development of further brain edema, and plasma exchange was again carried out. However, her physical condition worsened rapidly and she experienced repeated convulsions. On December 25, she finally fell into a deep coma, and brain edema became more remarkable on CT (Fig. 1B). Despite the extensive treatment against encephalopathy-related brain edema, she died on December 27, 2001.

Patient 3

This patient was a 40-year-old Japanese man with a history of repeated generalized convulsions and disturbed consciousness. He began to suffer from auditory hallucinations and display occasional abnormal behaviors at age 25 in 1987 and was thought to have schizophrenia. He was frequently admitted to a local psychiatric hospital. In 1998, at the age of 36, he occasionally fell into a delirious state, accompanied by a flapping tremor, but these episodes were usually relieved within a few days. Elevated plasma ammonia level of 163 μg/dl was detected at that time. Since then, he sometimes experienced similar attacks and had been in the psychiatric hospital since November 2000. From the beginning of August 2003, his consciousness level deteriorated gradually and oral major and minor tranquilizers (haloperidol 9 mg/day, chlorpromazine hydrochloride 150 mg/day, lorazepam 3 mg/day, and nitrazepam 5 mg/day) were stopped. However, he had become bedridden by the end of August. On September 9, 2003 (at the age of 40), he was referred to our hospital because of prolonged unconsciousness. On examination, he was in a catatonic state, accompanied by severe rigidity in all limbs and hyperactive deep tendon reflexes. The following day, he experienced a generalized tonic seizure and intravenous anticonvulsant therapy (diphenylhydantoin:
250 mg/day) was started. Laboratory data indicated raised plasma concentrations of ammonia (166 μg/dl), citrulline (186.9 nmol/ml), and arginine (175.0 nmol/ml), and serum PSTI level was also elevated (30 ng/ml). Therefore, he was thought to have CTLN2 and intravenous administration of BCAA (500 ml/day) was begun. Subsequently, his unconsciousness gradually improved and a low protein diet (50 g/day) and lactulose (60 ml/day) and kanamycin (1.5 g/day) were started from September 19. However, on October 7, he suddenly became comatose and plasma concentration of ammonia increased markedly to 757 μg/dl (Fig. 2). Brain CT on the same day revealed that sulci of the cerebrum became obviously unclear, indicating the presence of brain edema (Fig. 3), and intravenous administration of D-mannitol (600 ml/day) was started. Plasma lactate and pyruvate concentrations on October 8 were 39.8 mg/dl and 0.2 mg/dl (normal: 0.3–0.9 mg/dl), respectively. After this treatment, his unconsciousness state gradually improved and his plasma ammonia level decreased to 98 μg/dl on October 9; oral diet was begun on October 15. Although he again became comatose on October 23 and November 2, his condition recovered with similar administration of only D-mannitol. Brain CT on October 31, 2003 revealed apparent improvement of brain edema (Fig. 3). On November 10, he was discharged from our hospital and returned to the previous psychiatric hospital.

**Discussion**

CTLN2 is characterized by a liver-specific deficiency of ASS protein with normal kinetic properties (2). Several mutations in the causative citrin gene have been identified among patients with CTLN2 (3, 22–24). Citrin, which consists of 675 amino acid residues, is now known to be a mitochondrial solute transporter (3). Palmieri et al (25) reported that citrin is an isoform of aspartate glutamate carrier (AGC) on the mitochondrial inner membrane (Fig. 4). AGC is an important component of the malate aspartate shuttle (MAS), which plays a major role in transferring cytosolic NADH into the mitochondria in the liver (4, 24, 25) (Fig. 4). Therefore, citrin (liver-type mitochondrial AGC) deficiency blocks the function of MAS, which may increase the cytosolic NADH/NAD⁺ ratio (4, 24). Two of our CTLN2 patients who were treated with glycerol died of rapidly progressive brain edema (patients 1 and 2), while the remaining patient was rescued with only administration of mannitol (patient 3). Glycerol and/or mannitol were used previously for treatment of brain edema in many patients with CTLN2 (Table 2) (9–19). Among these cases, eight patients were treated with glycerol, two with both glycerol and mannitol, and one with only mannitol (9–19). Of those with administration of glycerol, all died of severe brain edema with extensive necrosis of brain soon after treatment, with the exception of a 40-year-old female patient reported by Oshiro et al
this patient was treated by both administration of glycerol and continuous ambulatory peritoneal dialysis to remove the extra fluid from the brain. On the other hand, Takashima et al (10) reported successful treatment of a 21-year-old female patient with mannitol along with intravenous arginine L-glutamate and oral L-carnitine.

Glycerol and D-mannitol have been widely used as osmotic agents for the treatment of brain edema to reduce the intracranial pressure by reducing the brain water content and/or increasing cerebrospinal fluid absorption (26). As shown in Fig. 5, intravenously administrated glycerol can be metabolized to glycerol 3-phosphate (G3P) by glycerol kinase in the liver in addition to its function as an osmotic diuretic. G3P is metabolized to pyruvate via dihydroxyacetonephosphate by cytosolic G3P dehydrogenase. Thus, glycerol metabolism facilitates increases in the cytosolic NADH/NAD⁺ ratio under conditions of citrin deficiency (24): Williamson et al (27) reported that after intramuscular administration of glycerol, the cytosolic NADH/NAD⁺ ratio increased in the liver of starved rats. Furthermore, on the basis of liver perfusion study in citrin-knockout mice (28), Moriyama reported that the lactate/pyruvate ratio within hepatocytes increased by approximately twice as much as in wild-type mice in 15 minutes after perfusion of 5 mM glycerol (29). In addition, in Asian countries, including Japan, glycerol solution usually contains 5% fructose to prevent

Table 2. CTLN2 Patients Treated by Osmotic Agents

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (Sex)</th>
<th>Treatments for brain edema</th>
<th>Prognosis</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>21 (M)</td>
<td>Glycerol</td>
<td>Worsened</td>
<td>Shoda et al (11)</td>
</tr>
<tr>
<td>2</td>
<td>46 (M)</td>
<td>Glycerol+HD</td>
<td>Worsened</td>
<td>Yamamoto et al (12)</td>
</tr>
<tr>
<td>3</td>
<td>35 (M)</td>
<td>Glycerol</td>
<td>Worsened</td>
<td>Ikeda et al (13)</td>
</tr>
<tr>
<td>4</td>
<td>24 (M)</td>
<td>Glycerol</td>
<td>Worsened</td>
<td>Shindo et al (14)</td>
</tr>
<tr>
<td>5</td>
<td>29 (M)</td>
<td>Glycerol+PP</td>
<td>Worsened</td>
<td>Yuki et al (15)</td>
</tr>
<tr>
<td>6</td>
<td>41 (M)</td>
<td>Glycerol+PP</td>
<td>Worsened</td>
<td>Ishii et al (16)</td>
</tr>
<tr>
<td>7</td>
<td>40 (F)</td>
<td>Glycerol+CAPD</td>
<td>Recovered</td>
<td>Oshiro et al (9)</td>
</tr>
<tr>
<td>8</td>
<td>37 (M)</td>
<td>Glycerol</td>
<td>Worsened</td>
<td>Imamura et al (19)</td>
</tr>
<tr>
<td>1</td>
<td>48 (M)</td>
<td>Glycerol+Mannitol</td>
<td>Worsened</td>
<td>Ishikawa et al (17)</td>
</tr>
<tr>
<td>2</td>
<td>29 (M)</td>
<td>Glycerol+Mannitol</td>
<td>Worsened</td>
<td>Hoshi et al (18)</td>
</tr>
<tr>
<td>3</td>
<td>31 (M)</td>
<td>Glycerol+Mannitol</td>
<td>Worsened</td>
<td>This study (patient 1)</td>
</tr>
<tr>
<td>4</td>
<td>40 (F)</td>
<td>Glycerol+Mannitol+PP</td>
<td>Worsened</td>
<td>This study (patient 2)</td>
</tr>
<tr>
<td>1</td>
<td>21 (F)</td>
<td>Mannitol</td>
<td>Recovered</td>
<td>Takashima et al (10)</td>
</tr>
<tr>
<td>2</td>
<td>40 (M)</td>
<td>Mannitol</td>
<td>Recovered</td>
<td>This study (patient 3)</td>
</tr>
</tbody>
</table>


Figure 5. Glycerol metabolism in the hepatocytes.
hemolysis, hemoglobinuria, and related renal dysfunction (30, 31). Fructose is mainly metabolized to fructose 1-phosphate, glyceraldehyde, and glyceraldehyde 3-phosphate by fructokinase and aldolase B in the liver (Fig. 6). Cytosolic NADH may also be generated when glyceraldehyde 3-phosphate is metabolized to 1,3-bisphosphoglycerate in glycolysis (Fig. 6). On the other hand, mannitol undergoes little metabolism in the living body, and accordingly, intravenous administration of mannitol does not influence cytosolic NADH production even under conditions of citrin deficiency. The increased cytosolic NADH/NAD$^+$ ratio inhibits aerobic glycolysis (4, 24) as lactate is overproduced from pyruvate to reduce the cytosolic NADH level (Figs. 5, 6). In citrin-knockout mice, the lactate/pyruvate ratio is significantly increased in the hepatocytes (28); in our patient 3, the plasma lactate level was highly elevated, as compared to that of pyruvate. Furthermore, increased cytosolic NADH facilitates the activity of aspartate aminotransferase to synthesize oxaloacetate with consumption of cytosolic aspartate, resulting in exacerbation of ASS insufficiency in the hepatocytes of CTLN2 patients (19). In contrast, to reduce the cytosolic NADH level under conditions of citrin deficiency, the malate citrate shuttle may be activated (4, 24). As this system also plays a role in fatty acid synthesis, fat accumulation in the hepatocytes can be further accelerated (4, 24). Increased cytosolic NADH/NAD$^+$ ratio might cause a great deal of damage to the remnant hepatocytes and further impair metabolism of ammonia and other toxic agents in the liver. Especially, an increase in ammonia concentration in the cerebrospinal fluid may cause an acute unbalance in brain osmolarity secondary to the glial accumulation of glutamine produced from ammonia metabolism in the central nervous system (15, 32). In our patient 1, plasma ammonia level was elevated after administration of glycerol, which may have led to worsening of brain edema. Therefore, we concluded that in CTLN2 patients, glycerol infusion with possible elevation of cytosolic NADH/NAD$^+$ ratio could be associated with exacerbation of the disease itself and that use of mannitol alone is recommended as an alternative for the treatment of encephalopathy-related brain edema in patients with citrin deficiency.

**References**

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