Glutargin Versus Arginine for Hyperammonemia in Chronic Liver Diseases: A Randomized Controlled Trial

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Abstract: To investigate the efficacy and safety of glutargin versus arginine in the treatment of hyperammonemia in patients with chronic liver diseases, we screened consecutive patients aged 18 to 65 years with chronic liver diseases between February 2007 and October 2010 at two hepatology centers in China. The level of blood ammonia in eligible patients was at least 1.2 times the upper limit of normal. Enrolled patients were randomly assigned at a 1:1 ratio to receive either 20 g of glutargin or 20 g of arginine for 7 days. The primary outcome was the efficacy rate in lowering the levels of blood ammonia. Finally a total of 80 patients were enrolled in this study. In the intention-to-treat analysis, glutargin did not show a superior efficacy rate to arginine in lowering the levels of blood ammonia (75% in the glutargin group and 80% in the arginine group, P=0.57). The decrease in blood ammonia level was also similar in the glutargin group and the arginine group (41% versus 40%, P=0.59). Per-protocol analyses showed similar results. However, the overall symptom and sign scores decreased more in the glutargin group than in the arginine group (P=0.058 in the intention-to-treat analysis and P=0.014 in the per-protocol analysis, respectively). The incidence of adverse events was similar in the two groups.

Conclusion: Glutargin shows similar efficacy to arginine in the treatment of hyperammonemia in patients with chronic liver diseases. However, glutargin is superior to arginine in improving symptoms and signs.

Keywords: Glutargin; Arginine; Hyperammonemia; Chronic liver diseases

1. Introduction

Hyperammonemia is a metabolic disturbance characterized by elevated levels of ammonia in the blood. Increased entry of ammonia to the brain is attributed to an imbalance of neurotransmitter functions, cerebral energy failure, and brain edema, resulting in a wide range of neuropsychiatric abnormalities, such as minimal encephalopathy, which can be detected only by psychometric and electrophysiological techniques, and dangerous conditions such as coma and death [1].

Ammonia is derived from the metabolism of nitrogen-containing compounds. The vast majority of ammonia is detoxified by the liver via the urea cycle. Hyperammonemia is most frequently observed in patients with liver diseases, especially chronic liver diseases [2]. Although several factors are known to be involved in the pathogenesis of hepatic encephalopathy (HE), hyperammonemia is believed to be the main cause of HE symptoms [3,4]. Therefore, strategies to lower ammonia levels are the mainstay in the prevention and treatment of HE [5]. This involves restricting dietary protein intake, administering lactulose and non-absorbable antibiotics to reduce the production and absorption of ammonia, and targeting the process of ammonia metabolism [6,7].

Glutamate and arginine are both critical for the urea cycle and enhance the activities of urea cycle enzymes [7]. The combined use of glutamate and arginine has a greater ammonia-lowering effect than each substance used alone in rat models of acute liver failure [8]. A mixture of glutamate plus arginine also protects rats with liver cirrhosis from ammonia intoxication [9]. However, to date, there are no clinical studies on the combined use of these two substances in patients with liver diseases. Therefore, the aim
of this clinical trial was to investigate the efficacy and safety of glutargin (L-arginine L-glutamate salt) in the treatment of hyperammonemia in patients with chronic liver diseases.

2. Material and methods

2.1. Patients

We screened consecutive patients aged 18 to 65 years with chronic liver diseases between February 2007 and October 2010. The level of blood ammonia in eligible patients was at least 1.2 times the upper limit of normal. Patients with malignant tumor, and any advanced urinary, respiratory, cardiovascular, digestive, endocrine, immune, nervous system or psychiatric diseases were excluded. Other exclusion criteria included hepatic failure (prothrombin time activity less than 40% and total bilirubin more than 171 μmol/L); overt hepatic encephalopathy (grade II-IV); recent alcohol abuse; receiving medications to lower the concentration of ammonia such as glusate, L-ornithine-L-aspartate, lactulose, and lactitol; receiving nephrotoxic medications such as aminoglycosides, amphotericin B, vancomycin, and cisplatin; and taking sedatives. Other concomitant medications were permitted during the study. All enrolled patients provided written informed consent.

2.2. Study design

This randomized, single-blind, controlled clinical trial was designed by the First Affiliated Hospital of the Third Military Medical University, Chongqing, China. It was conducted at two centers: the Second Affiliated Hospital of Xi’an Jiaotong University and the Second Affiliated Hospital of Kunming Medical University. The study protocol was approved by the Ethics Committees at the First Affiliated Hospital of the Third Military Medical University and Second Affiliated Hospital of Xi’an Jiaotong University, Xi’an, China. The registration number of the study is 2006L01672. The study was conducted according to the ethical principles of the declaration of Helsinki. Data were collected by the Statistics Department of Chongqing Medical University, using EpiData software (version 3.1, EpiData Association, Odense, Denmark) [10].

Patients were randomly assigned to receive either 20 g of glutargin (Liaoning Haisco Pharmaceutical Co., Ltd) or 20 g of arginine (Shanghai First Biochemical & Pharmaceutical Co., Ltd., and Wuhan Jiuan Pharmaceutical Co., Ltd), intravenously, once daily for 7 consecutive days. A computer-generated list was provided by the Statistics Department of Chongqing Medical University, which was not involved in the conduct of the study and was delivered to each center. According to this list, drugs labeled sequentially were allocated to participants.

2.3. Outcome measurement

The primary outcome was the efficacy rate of the two drugs in lowering the levels of blood ammonia after the 7-day treatment. The secondary outcomes were the decrease in the levels of blood ammonia ((levelend − levelbase-line)/levelbase-line), the improvement in symptom and sign scores, and incidence of adverse events. Venous blood was collected and transported in an ice bath within 20 min for blood ammonia assay. Symptom and sign scores comprised the following seven items: fatigue, anorexia, nausea and vomiting, abdominal distention, flapping tremor, ankle clonus, and the number connection test. Each item was scored from 0 (the mildest) to 4 (the severest). Adverse events were monitored throughout the treatment period. If serious adverse events occurred, patients were followed after treatment.

2.4. Statistical analysis

Sample size calculation was based on our preliminary trial. In this trial, approximately 95% of patients with chronic liver diseases achieved a decrease in blood ammonia levels following treatment with glutargin. In patients treated with arginine, this rate was approximately 75%. Thus, the average efficacy rate was 85%. In order to show the superiority of glutargin over arginine with a statistical power of more than 80% at a two-sided significance level of 0.05, 40 participants per group were required (N = −2×p(1−p)(μα+μβ)/δ2, P = 0.85, α = 0.05, β = 0.2, δ = 0.20).

All participants who received at least one day of glutargin or arginine treatment were included in the intention-to-treat cohort for efficacy analysis. We also performed per-protocol analysis for participants.
who completed the 7-day treatment. Participants who received at least one day of treatment were included in the safety analysis. To avoid an effect between centers, the primary outcome was calculated using the Cochrane-Mantel-Haenszel method [11].

Continuous data are presented as means ± standard deviation or median (interquartile range, IQR). They were analyzed using the Student’s t test or Mann-Whitney U test. Dichotomous data were analyzed using the Chi-square test or Fisher’s exact test. All tests were two-tailed, with P < 0.05 considered significant. The statistical software SAS Release 9.1 (SAS Institute, Cary, NC, USA) was used for analyses.

3. Results

3.1. Study patients

We screened 135 patients, and 80 were randomly assigned to receive a study drug (Figure 1). All patients received at least one day of treatment with a study drug. Therefore, all patients were included in the intention-to-treat cohort and safety cohort.

![Flow chart of patient enrollment and screening in this study.](image)

Serum levels of alanine aminotransferase were lower in the glutargin group than in the arginine group (P = 0.05). Other baseline characteristics of the patients were similar in both groups (Table 1). After a 7-day follow-up, three patients had withdrawn in each group, leaving 37 patients in each group for the per-protocol analysis.
3.2. Blood ammonia

The intention-to-treat analysis indicated that glutargin did not yield a superior efficacy rate to arginine in lowering levels of blood ammonia (75% in the glutargin group and 80% in the arginine group, P=0.57). The per-protocol analysis showed similar results (81% in the glutargin group and 87% in the arginine group, P=0.014) (Table 2). The decrease in blood ammonia level was similar in the glutargin group and the arginine group regardless of the intention-to-treat analysis (41% versus 40%, P=0.59) or the per-protocol analysis (43% versus 40%, P=0.51) (Table 2).

### Table 2: Study outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Glutargin (n = 40)</th>
<th>Arginine (n = 40)</th>
<th>Rate difference</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Efficacy rate(^a)</td>
<td>30/37 (81%)</td>
<td>32/37 (87%)</td>
<td>-5% (-21%, 11%)</td>
<td>0.55</td>
</tr>
<tr>
<td>Efficacy rate(^b)</td>
<td>30/40 (75%)</td>
<td>32/40 (80%)</td>
<td>-5% (-22%, 12%)</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Secondary outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease in BA(^a)</td>
<td>43% (8%-62%)</td>
<td>40% (18%-48%)</td>
<td>-</td>
<td>0.51</td>
</tr>
<tr>
<td>Decrease in BA(^b)</td>
<td>41% (0.3%-62%)</td>
<td>40% (9%-48%)</td>
<td>-</td>
<td>0.59</td>
</tr>
<tr>
<td>Symptom and sign score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improvement rate(^a)</td>
<td>35/36 (97%)</td>
<td>34/38 (89%)</td>
<td>8% (-3%, 19%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Improvement rate(^b)</td>
<td>35/40 (88%)</td>
<td>34/40 (85%)</td>
<td>3% (-13%, 18%)</td>
<td>0.75</td>
</tr>
<tr>
<td>Score decrease(^a)</td>
<td>4.0 (2.0-6.0)</td>
<td>2.0 (1.0-4.3)</td>
<td>-</td>
<td>0.014</td>
</tr>
<tr>
<td>Score decrease(^b)</td>
<td>3.5 (2.0-5.8)</td>
<td>2.0 (1.0-3.8)</td>
<td>-</td>
<td>0.058</td>
</tr>
</tbody>
</table>

**Notes:**

**Abbreviations:** BA: blood ammonia
\(^a\)per-protocol analysis
\(^b\)intention-to-treat analysis

3.3. Symptoms and signs

Glutargin decreased the overall symptom and sign scores more than arginine (P=0.058 in the intention-to-treat analysis and P=0.014 in the per-protocol analysis, respectively). However, with regard
to the improvement rate in these scores, glutargin was not significantly superior to arginine (P=0.75 in the intention-to-treat analysis and P=0.20 in the per-protocol analysis, respectively) (Table 2).

3.4. Safety

The incidence of adverse events was similar in the glutargin group (5/40) and the arginine group (6/40) (Table 3). Glutargin was discontinued in two patients due to dizziness and chest tightness. Arginine was discontinued in one patient due to exacerbated abdominal distension. Recovery was achieved after drug discontinuation. Other adverse events spontaneously resolved or resolved with symptomatic treatment. No severe adverse events were observed in either group during the study.

Table 3: Adverse events

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Glutargin (n = 40)</th>
<th>Arginine (n = 40)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any event</td>
<td>5</td>
<td>6</td>
<td>0.75</td>
</tr>
<tr>
<td>Body aches</td>
<td>0</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Fever</td>
<td>0</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Rash</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Nausea</td>
<td>1</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Exacerbation of abdominal distension</td>
<td>0</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Palpitation</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Chest tightness</td>
<td>3</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>1</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Dizziness</td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

4. Discussion

Most treatments for hyperammonemia target the organs and metabolic processes involved in ammonia detoxification, including glutamate and arginine [6]. Glutamate combines with ammonia to form glutamine which is involved in the urea cycle and is excreted predominantly as urea. Arginine acts as a primer in the urea cycle. The perception of combined use of glutamate and arginine in protecting animals from ammonia intoxication is based on the biochemical consideration that urea is the main metabolite of amino acid in ureotelic animals and humans [7]. Biosynthesis of urea is started in mitochondria by the action of carbamoylphosphate synthetase I (CPS I), whose activity is allosterically stimulated by N-acetyl-l-glutamate. The synthesis of N-acetyl-l-glutamate from acetyl coenzyme A and L-glutamic acid is catalyzed by acetylglutamate synthetase which is specifically stimulated by arginine [12]. The biosynthetic capacity of acetylglutamate synthetase appears to be limited and the level of N-acetyl-l-glutamate in mitochondria seems to be barely sufficient to maintain an adequate level of activated CPS I.

To the best of our knowledge, this is the first clinical trial to compare glutargin and arginine. Although the logic of the combined use of glutamate and arginine is based on biochemical considerations and it has been proven more effective than arginine alone in lowering ammonia in rat models of liver diseases, this trial showed that glutargin has similar efficacy to arginine in the treatment of hyperammonemia in patients with chronic liver diseases. It should be noted that in liver disease models, rats were administered a sub-lethal dose of ammonium acetate, resulting in very high levels of ammonia in the blood, approximately 800 μmol/L on average [7]. Thus, the ammonia-lowering effect would be readily observed following treatment with glutaminate and/or arginine. In patients with chronic liver diseases, the levels of blood ammonia are only mildly elevated. This may dwarf the effect of study drugs and make it difficult to detect.

With regard to symptoms and signs in the alimentary and nervous systems, the findings in this trial suggested that due to additional glutamate, glutargin seems superior to arginine. Glutamate, which is involved in glycolysis, gluconeogenesis and the citric acid cycle, is a key compound in cellular metabolism. In brain cells exposed to ammonia intoxication, exogenous glutamate may relieve their energy shortage [13]. In addition, as a neurotransmitter, exogenous glutamate may also play a role in restoring the balance of neurotransmitter functions [13].

Although the overall incidence of adverse events was similar in the glutargin group and the arginine group, palpitation, dizziness, chest tightness and shortness of breath were more common in patients receiving glutargin. Attention should be paid to these adverse events in clinical practice and future studies.

We are confident of the results obtained in this trial regarding lowered blood ammonia. One reason
is that the results are quite consistent between the primary outcome (the percentage of patients whose blood ammonia levels decreased to the normal range) and the secondary outcome (the reduction in blood ammonia levels compared with baseline levels) as well as the findings from the intention-to-treat analysis and per-protocol analysis. Another reason is that unpublished results from other liver centers are also consistent with our findings.

Several specific details merit consideration in this study. Firstly, this is a randomized, single-blind, controlled clinical trial, in which only the participants were blinded and this may have introduced bias when evaluating symptoms and signs. However, during the trial, physicians evaluating symptoms and signs had no access to the involved data that could expose the allocation of the study drugs. Secondly, this trial mainly focused on hyperammonemia, and evaluation of HE was inadequate. Future studies should fully evaluate HE, not only in terms of clinical symptoms and signs, but also in terms of psychometric and electrophysiological techniques. Thirdly, although the participants were randomly allocated to study groups, the baseline alanine aminotransferase levels were not balanced between the two groups. Larger sample sizes are needed in future studies.

5. Conclusions

In summary, glutargin shows similar efficacy to arginine in the treatment of hyperammonemia in patients with chronic liver diseases. However, glutargin is superior to arginine in improving symptoms and signs.

Acknowledgements

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References