Effect of a Glutamine-Supplemented Diet on Response to Methicillin-Resistant *Staphylococcus aureus* Infection in Mice

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Summary

The following study was undertaken to determine whether dietary supplementation with glutamine can be used to modulate the immune response following challenge with methicillin-resistant *Staphylococcus aureus* (MRSA) organisms in mice. Thirty BALB/c female mice were randomized into 3 groups: group A (n = 10) were fed 20% casein diet (control), whereas the mice in Groups B (n = 10) and C (n = 10) were given 20% casein diet supplemented with 2 and 4% glutamine, respectively. The diets were made isonitrogenous by glycine and alanine supplementation. On the 10th day on these treatments, each mouse was challenged intravenously with $2 \times 10^8$ colony-forming units (CFU)/ml of MRSA organisms and mortality was noted for 20 days. The survival rate in Group A (20%) tended to be lower than the rates in Group B (40%), and Group C (70%). CFU values of spleen and kidney of the surviving mice 20 days post challenge were not different among the three groups ($p < 0.05$). The present results suggest that dietary glutamine supplementation may be effective as a nutritional immunomodulator for the recovery from MRSA infection.

Key Words MRSA, glutamine, infection, mouse

Infections with opportunistic pathogens represent one of the major problems in the management of immunocompromised hosts, especially in surgical, trauma, burn, cancer patients and in the case of patients heavily treated with radiotherapy or chemotherapy (1). Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) have emerged as a frequent cause of nosocomial infections world-wide (2). Most of the strains are virulent and can produce a fatal generalized disease. The morbidity, mortality, and costs associated with treatment and prevention of
MRSA Infections are substantial (3), and thus any therapy that would prevent infection or enhance the host defense mechanisms would be beneficial.

Glutamine has been classified as a nonessential or nutritionally dispensable amino acid (4). As a result of this classification, it has been eliminated from total parenteral nutritional formulas and most enteral formulas include only small quantities or are devoid of glutamine. More recently, glutamine has been shown to improve immunologic, physiologic, and metabolic functions in laboratory and clinical studies. Supplemental dietary glutamine has been shown to act as a vehicle for the transfer of nitrogen between tissues (5), a regulator of protein synthesis (6), an essential precursor for nucleic acid biosynthesis in all cells (7), enhances lymphocyte responses to mitogens (8), decreases bacteremia and endotoxemia, improves nitrogen balance and survival in methotrexate and 5-fluorouracil-induced enterocolitis in rats (9, 10). For these reasons, the present study was undertaken to see the effect of glutamine supplementation on response to MRSA challenge.

Materials and methods. Thirty BALB/c female mice, 4 weeks old, were randomized into 3 groups and maintained daily throughout a 30-day period on the following diets: Group A mice were fed 20% casein diet (control), whereas Groups B and C mice were fed 20% casein diet supplemented with 2 and 4% glutamine, respectively. The diets were isonitrogenous by glycine and alanine supplementation. Table 1 shows the composition of the diets used. The mice had access to food and water ad libitum. From 10 to 11 a.m. every morning, the animals were weighed and food and water were renewed.

Table 1. Composition of experimental diets.

<table>
<thead>
<tr>
<th></th>
<th>Control (g/kg)</th>
<th>2% glutamine (g/kg)</th>
<th>4% glutamine (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200.0</td>
<td>200.0</td>
<td>200.0</td>
</tr>
<tr>
<td>Glutamine</td>
<td>0.0</td>
<td>20.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>15.8</td>
<td>7.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>31.1</td>
<td>15.8</td>
<td>0.0</td>
</tr>
<tr>
<td>α-Starch</td>
<td>415.4</td>
<td>417.6</td>
<td>420.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>207.7</td>
<td>208.7</td>
<td>210.0</td>
</tr>
<tr>
<td>Mineral1</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Vitamin2</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Oil</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>

1 Obtained from Oriental Yeast Co., Tokyo. The composition was as follows: (mg/kg) CaHPO₄·2H₂O, 7,280; KH₂PO₄, 12,860; NaH₂PO₄, 4,680; NaCl, 2,330; Ca-lactate, 17,550; Fe-citrate, 1,590; MgSO₄, 3,590; ZnCO₃, 55; MnSO₄·4·6H₂O, 60; CuSO₄·5H₂O, 15; KI, 5. 2 Obtained from Oriental Yeast Co., Tokyo. The composition was as follows: (mg/kg) thiamin-HCl, 12; riboflavin, 40; pyridoxine HCl, 8; vitamin B-12, 50; ascorbic acid, 300; d-biotin, 50; niacin, 60; inositol, 60; choline chloride, 2,000; tocopheryl acetate, 50; menadione, 52; and (in IU) retinyl acetate, 5,000; ergocalciferol, 1,000.
EFFICIENCY OF GLUTAMINE ON MRSA

Fig. 1. Survival rates of BALB/c female mice fed 20% casein diet (control) (Group A ■; n=10), 20% casein diet supplemented with 2% glutamine (Group B ○; n=10), and 20% casein diet supplemented with 4% glutamine (Group C ▲; n=10). On the 10th day, mice in each group were challenged intravenously with MRSA strain 8985N and mortality was noted for 20 days. Values with the same letter are not significantly different (p<0.05).

Staphylococcus aureus 8985N (donated by Prof. T. Imamura of our faculty) used in this study was isolated from a clinical specimen, identified by standard taxonomic criteria, checked for purity by standard bacteriologic methods and confirmed to be methicillin-resistant. The maintenance, culture, and preparation of the bacteria for inoculation were the same as described previously (11). On the 10th day, mice in each dietary group were administered 0.3 ml (6.7×10^8 colony-forming units (CFU)/ml) of the bacterial suspension through the tail vein and monitored for mortality.

All the surviving mice were killed 20 days post inoculation by cervical dislocation. The spleen and kidney were removed aseptically and homogenized using a Dounce homogenizer in 3 ml of sterile saline. After preparations of serial 10-fold dilutions, the organs were assayed for the presence of viable Staphylococcus aureus organisms on mannitol salt agar by the plate dilution method (12).

The data were analyzed by chi-square test and Student’s t-test; when p values were equal to or less than 0.05, the difference was considered significant.

Results. Figure 1 shows the percent survival in each dietary group. Twenty days post challenge, 20% (2 out of 10) of the Group A (control) mice were alive, whereas 40 (4 out of 10) and 70% (7 out of 10) in Groups B and C were alive, respectively. Although the survival rate in Group B tended to be higher than that of Group A, there was no difference statistically. Significant difference was observed between Groups A and C (p<0.05).

Table 2 shows the body, spleen, and kidney weights and the recovery of MRSA organisms in the organs 20 days post challenge. There was no significant difference between glutamine-supplemented groups (Groups B and C) and the control group...
Table 2. Body, spleen, and kidney weights and the recovery of viable MRSA organisms of the organs in surviving mice fed 20% casein diet (Group A) and 20% casein diet supplemented with 2 (Group B) and 4% (Group C) glutamine, respectively.1

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of mice</td>
<td>2</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>15.00±0.61</td>
<td>14.34±1.33</td>
<td>14.67±2.43</td>
</tr>
<tr>
<td>Spleen weight (mg)</td>
<td>130±20</td>
<td>165±40</td>
<td>190±40*</td>
</tr>
<tr>
<td>Spleen CFU ($\times 10^5$)</td>
<td>0.03±0.03</td>
<td>8.77±11.40</td>
<td>0.82±1.36</td>
</tr>
<tr>
<td>Kidney weight (mg)</td>
<td>255±15</td>
<td>245±20</td>
<td>245±30</td>
</tr>
<tr>
<td>Kidney CFU ($\times 10^7$)</td>
<td>5.35±5.35</td>
<td>3.78±3.67</td>
<td>4.08±6.15</td>
</tr>
</tbody>
</table>

1The results are M±SD. *Indicates significant difference from the value of Group A ($p<0.05$).

(Group A) in body weight gain, kidney weight, spleen and kidney CFU; except, the Group C mice had significantly higher splenic weight (190±40 mg) as compared to the mice in Group A (130±20 mg). However, the increased splenic weight was not accompanied by a significant reduction of CFU recovery of MRSA organisms in the organs.

Discussion. Our results suggest that dietary glutamine may be used to an advantage to improve survival following challenge with MRSA infections. The supplementation resulted in an enhancement of the survival rate. The effect was more evident in the 4% dietary group as against the 2% dietary group.

In the food amino acid composition table, glutamine and asparagine are not shown (13), because they are oxidized to glutamic acid and aspartic acid, respectively, by acid hydrolysis for the amino acid analysis. The calculated nitrogen concentration of the amino acid mixture prepared following the pattern shown in the table is 12.7%. However, the nitrogen concentration of casein as determined by the Kjeldahl method is 15.7% (conversion ratio 6.38) (13). The difference may be attributed to the elimination of glutamine and asparagine in the mixture. If one replaces all the glutamic acid and aspartic acid by glutamine and asparagine, respectively, the nitrogen concentration of the amino acid mixture will be 15.2%, which is still slightly lower than that of casein (15.7%), indicating that casein contains glutamine and asparagine rather than their acidic forms. From the amino acid composition table, the concentration of glutamic acid is about 18% (13). Since the molecular weights of glutamic acid (147.13) and glutamine (146.15) are similar, glutamine concentration in casein may also be about 18%. Hence in the 20% casein diet, the glutamine concentration is about 3.6%. In our studies, 4% dietary glutamine supplementation offered a better response than the 2% supplemented group. This indicates that the total glutamine concentration which amounts to 7.6% (3.6% in casein plus 4% supplementation) was more effective than 5.6% (3.6% in casein plus 2% supplementation). In our experiment, only two levels of supplementation were evaluated and as such cannot specify at what level...
the glutamine supplementation could offer significant protection following induction of sepsis; however, from our studies it is evident that it may exceed 7.6%.

Glutamine has been categorized as a nutritionally dispensable amino acid (4). Since this classification implies that glutamine can be synthesized endogenously in adequate quantities from other amino acids and precursors, it has not been considered necessary to include glutamine in nutritional formulas. However, growing evidence shows that glutamine supplementation regulates various steps of the metabolic physiologic, and immunologic functions (5–9). Whereas much attention has been focused on the use of glutamine in the management of septic or immunocompromised patients, relatively few studies have attempted conclusively to define the optimal dosage required for efficient metabolic, physiologic, and immunologic functions. Karner et al. (14) showed that supplementation of total parenteral nutrition (TPN) with 0.37 g glutamine or glycyrglutamine/kg body weight (equivalent to about 10% glutamine in TPN) in dogs after surgery was not sufficient to restore glutamine deficiency of the skeletal muscle in the depleted state. Hammarqvist et al. (15) documented that supplementation of TPN with 0.285 g glutamine/kg body weight per day for 3 days (equivalent to about 7% glutamine in TPN) improved nitrogen balance and protein synthesis in catabolic patients. Ardawi (16), and Klimberg et al. (17) showed that 2 and 3% glutamine-enriched TPN improved nitrogen balance, mucosal morphometrics, and survival in septic and whole abdominal radiation-treated rats for 4 and 8 days, respectively. Our studies also demonstrated that 5.6% dietary glutamine (3.6% in casein plus 2% supplementation) was not adequate in offering protection to the mice. These and other studies suggest that the optimal dosage of glutamine needed to improve or maximize the metabolic, physiologic, and immunologic functions is not clear or well defined. Further studies will prove useful in determining the precise dose required for maintenance and regulation of the physiologic, metabolic, and immunologic functions. Such studies are currently in progress in our laboratory.

In summary, the present result suggest that glutamine may be used to an advantage to enhance the immune system of the mice following MRSA challenge.

REFERENCES


