<table>
<thead>
<tr>
<th>Title</th>
<th>Metabolic Correction of Plasma Aminogram by Pig or Baboon Liver Cross-Hemodialysis with an Interposed Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author(s)</td>
<td>Kamiyama, Yasuo; Ozawa, Kazue</td>
</tr>
<tr>
<td>Citation</td>
<td>日本外科宝函  (1991), 60(5): 277-288</td>
</tr>
<tr>
<td>Issue Date</td>
<td>1991-09-01</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/2433/203813">http://hdl.handle.net/2433/203813</a></td>
</tr>
<tr>
<td>Type</td>
<td>Departmental Bulletin Paper</td>
</tr>
<tr>
<td>Textversion</td>
<td>publisher</td>
</tr>
</tbody>
</table>

Kyoto University
Metabolic Correction of Plasma Aminogram by Pig or Baboon Liver Cross-Hemodialysis with an Interposed Membrane

Yasuo Kamiyama* and Kazue Ozawa**

*Department of Surgery, Kyoto City Hospital.
**Department of Surgery, Faculty of Medicine, Kyoto University.

Received for Publication, June 3, 1991.

Summary

Our previous reports have shown that the patients with both grade 4 hepatic coma and arterial blood ketone body ratio (BKBR) of over 0.25 became fully alert after treatment by pig or baboon liver cross-hemodialysis, while those with BKBR below 0.25 died of hepatic coma without increase of BKBR. The present study shows the changes in plasma amino acids (AA) in 8 patients after treatment by cross-hemodialysis and intravenous infusion of Fisher’s solution. In all patients, the ratio of tyrosine plus phenylalanine to total AA concentration decreased (p=0.012), and the AA molar ratio increased significantly (p=0.007). In unrecovered patients, total AA and branched chain AA increased after cross-hemodialysis, while in the recovered patients total AA did not increase significantly. It is suggested that this metabolic support is effective in reducing the plasma levels of aromatic AA mainly oxidized in the liver, as long as BKBR remained over 0.25.

Introduction

Serial postoperative measurements of the blood ketone body ratio in the patients with liver disease have revealed that those with BKBR over 0.7 had uneventful course and survived well even after major surgery, while those with a gradual decrease of BKBR below 0.7 had serious postoperative complication, with 85% of patients with the ratio below 0.4 died of hepatic failure complicated with multiple organ failure14-16. Recently, we have developed and used a cross-hemodialysis using perfused isolated liver for the treatment of the patients with liver failure15-17 to reduce the metabolic load imposed on the injured liver. After the liver support therapy, the patients with both BKBR from 0.4 to 0.25 and grade 4 hepatic coma (respond only to painful stimuli) showed an increase in blood ketone body ratio and recovered from coma.

On the other hand, drastic changes in blood aminograms, especially those in aromatic amino acids, have been incriminated in the pathogenesis of coma in patients with hepatic failure. The liver is known to play an important regulatory role in amino acid metabolism. Our recent studies have

Key words: Hepatic coma, Plasma aminogram, Cross-hemodialysis, Blood ketone body ratio.

索引語：肝性昏睡，血中アミノグラム，交叉透析，血中ケトン体比。

Present address: Department of Surgery, Kyoto City Hospital, 1-2 Higashi Takadacho Mibu, Nakakyo-ku Kyoto Jajan 604.
revealed that derangements in amino acids metabolism are closely related to metabolic derangements of the liver\(^{18}\). If blood aminogram is affected by metabolic derangements of the liver reflected by decrease in BKBR, the aminograms are expected to be corrected in the patients having showed an increase in BKBR after the cross-hemodialysis. Then effects of the cross-hemodialysis on aminograms were investigated. Special attentions have been paid on the changes in aminograms in the patients recovered from hepatic coma with normalization of BKBR after the treatment.

**Materials and Methods**

Eight of 11 patients treated with pig or barbon liver, cross-hemodialysis with an interposed membrane are the subjects of this study\(^{15}\). In these 8 patients with a marked decrease of blood ketone body ratio less than 0.4 and grade 4 coma, plasma amino acids were determined before and after the cross-hemodialysis (Table 1). There are 6 males and 2 females, their ages ranged between 45 and

<table>
<thead>
<tr>
<th>Table 1 PATIENTS PROFILES AND BLOOD CHEMISTRY IMMEDIATELY BEFORE CROSS-HEMODIALYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient No.</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
</tbody>
</table>

B.K.B.R. : Blood ketone body ratio
Eleven patients who had clinically normal liver function and histologically normal findings of

70, with a mean age of 55.1. Five patients with BKBR over 0.25 recovered from coma (group A). Three of these 5 recovered from coma during the hemodialysis, while the other 2 recovered from coma within 48 hours after the finishing of the cross-hemodialysis. On the other hand, 3 patients with the ratio of less than 0.25 did not recovered from coma without any increase of their blood ketone body ratios and died of hepatic failure (group B). The most frequent treatments before the hemodialysis were lactulose enema and administration of corrective amino acids (FO80)9. Blood ammonia concentrations as well as blood glucose levels were elevated immediately before the hemodialysis. In all patients, after initiation of the cross-hemodialysis, 500 ml of the synthetic amino acid solution rich in branched chain amino acid (FO80)9 was administered intravenously at the rate of 100 ml/hr.

The entire procedures of the hepatic failure treatment with the cross-dialysis have been reported previously15. The blood perfused through isolated liver was circulated from a common blood reservoir to a dialyzer, to oxygenator, and come back to the reservoir, from which it went to the isolated liver again. The isolated liver was perfused through the portal vein with 2000 ml of human blood mixed with 50 gm of low molecular weight dextran (40,000 daltons), 5,000 I.U. of heparin, and fresh frozen plasma by which the hematocrit was adjusted to 30%. Perfusion was carried out at pH 7.35 to 7.50. and PO2 of 200 to 300 mmHg at 36.5°C. The portal venous pressure was maintained at less than 15 cm H2O. Hepatic flow was kept between 0.5 and 1.0 ml/min/gm of liver. The hemodialyzer (NDU-12L, Nikkiso Co.) utilizes parallel layers of Cuprophan membrane HDF (Enka Co.) 20 μm in thickness with a total surface area of 1.2 m². This dializer allows for the transfer of substances with molecular weight up to 10,000 daltons. The blood from the reservoir and the blood from the patient are cross-dialyzed over this membrane. The blood flow rate from the patient averaged 150 to 250 ml/min.

For the assay of plasma amino acid, 2 ml of arterial blood was taken from the arterial side immediately after the beginning of the cross-hemodialysis and immediately before the finishing of the cross-hemodialysis. At the same time, for the assay of blood ketone bodies, 5 ml of arterial blood was taken using heparinized 10 ml glass syringe and mixed with 5 ml of ice-cold 10% perchloric acid at the bedside. For the assay of blood ketone bodies of the perfused liver, the hepatic vein (out flow) blood was taken. Blood glucose levels were maintained over 120 mg/dl by the infusion of 10% glucose solution at the rate of 200 ml/hr. The suspension was centrifuged at 10,000 g for 15 min at 0-4°C. The supernatant was adjusted to pH 6.0 with 69% (W/V) K2CO3 and recentrifuged for 5 min at 0-4°C. The supernatant was used for the determination of blood ketone bodies. The samples were kept at 0-4°C before the analysis which was done within 24 hours. Special attentions were paid to keep the samples at the temperature of 0-4°C during the deproteinization and pH adjustment, since acetoacetate is unstable at room temperature. 13-hydroxybutyrate and acetoacetate were measured by the method of Williamson and Mellanby23, and Mellanby and Williamson24, respectively. Amino acid determinations in plasma were carried out by a Hitachi amino acid autoanalyzer on the plasma which was deproteinized with 5% (W/V) sulfosalicylic acid.

For the statistical analysis of changes in amino acid by cross-hemodialysis, a paired t-test was used, and p value less than 0.05 was regarded as significant.

Results

Eleven patients who had clinically normal liver function and histologically normal findings of
Table 2
PLASMA AMINO ACID LEVELS IN CASES 1, 2 AND 3

<table>
<thead>
<tr>
<th></th>
<th>Patient 1 Before</th>
<th>After C.H.</th>
<th>After R.C.</th>
<th>Patient 2 Before</th>
<th>After C.H.</th>
<th>After R.C.</th>
<th>Patient 3 Before</th>
<th>After C.H.</th>
<th>After R.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>0.7</td>
<td>0.3</td>
<td>0.2</td>
<td>0.9*</td>
<td>1.5*</td>
<td>1.5*</td>
<td>1.3*</td>
<td>0.6</td>
<td>1.2*</td>
</tr>
<tr>
<td>Threonine</td>
<td>9.9</td>
<td>6.8</td>
<td>7.7</td>
<td>25.5*</td>
<td>21.0*</td>
<td>17.1</td>
<td>10.6</td>
<td>18.2*</td>
<td>19.3*</td>
</tr>
<tr>
<td>Serine</td>
<td>11.2</td>
<td>7.0</td>
<td>7.3</td>
<td>24.2*</td>
<td>15.2*</td>
<td>17.7*</td>
<td>9.6</td>
<td>12.4</td>
<td>19.2*</td>
</tr>
<tr>
<td>Asparagine</td>
<td>10.3*</td>
<td>8.2</td>
<td>6.0</td>
<td>7.2</td>
<td>–</td>
<td>6.7</td>
<td>6.7</td>
<td>6.7</td>
<td>7.2</td>
</tr>
<tr>
<td>Glutamate</td>
<td>17.6*</td>
<td>8.7</td>
<td>5.6</td>
<td>23.7*</td>
<td>67.8*</td>
<td>23.3*</td>
<td>21.8*</td>
<td>18.2*</td>
<td>18.2*</td>
</tr>
<tr>
<td>Glutamine</td>
<td>46.5*</td>
<td>26.3</td>
<td>24.8</td>
<td>57.0</td>
<td>50.9</td>
<td>41.1</td>
<td>45.3</td>
<td>100.1*</td>
<td>54.7</td>
</tr>
<tr>
<td>Proline</td>
<td>15.1*</td>
<td>9.0</td>
<td>11.1</td>
<td>57.9*</td>
<td>38.1*</td>
<td>37.9*</td>
<td>19.8*</td>
<td>77.4*</td>
<td>31.2*</td>
</tr>
<tr>
<td>Glycine</td>
<td>14.6*</td>
<td>11.3</td>
<td>12.6</td>
<td>66.4*</td>
<td>51.1*</td>
<td>32.2*</td>
<td>17.9</td>
<td>38.4*</td>
<td>32.5*</td>
</tr>
<tr>
<td>Alanine</td>
<td>34.6*</td>
<td>23.1</td>
<td>21.9</td>
<td>64.1*</td>
<td>61.4</td>
<td>48.5</td>
<td>40.7</td>
<td>108.3*</td>
<td>40.4</td>
</tr>
<tr>
<td>Valine</td>
<td>27.2*</td>
<td>13.6</td>
<td>14.0</td>
<td>44.4</td>
<td>36.7*</td>
<td>30.1*</td>
<td>12.6</td>
<td>31.7*</td>
<td>51.5*</td>
</tr>
<tr>
<td>Cystine</td>
<td>1.7</td>
<td>1.7</td>
<td>4.1</td>
<td>0.2</td>
<td>0.7</td>
<td>0.1</td>
<td>0.3</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.4</td>
<td>2.3</td>
<td>2.6</td>
<td>4.9*</td>
<td>2.7</td>
<td>4.0*</td>
<td>2.9</td>
<td>4.5*</td>
<td>4.6*</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.1</td>
<td>2.5</td>
<td>1.8</td>
<td>26.2*</td>
<td>6.3</td>
<td>12.1*</td>
<td>3.6</td>
<td>6.0</td>
<td>15.9*</td>
</tr>
<tr>
<td>Leucine</td>
<td>9.8</td>
<td>7.4</td>
<td>7.5</td>
<td>22.3*</td>
<td>19.9*</td>
<td>18.1*</td>
<td>7.6</td>
<td>10.6</td>
<td>23.2*</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>47.1*</td>
<td>9.4*</td>
<td>10.3*</td>
<td>10.9*</td>
<td>5.5</td>
<td>9.5*</td>
<td>11.4*</td>
<td>10.9*</td>
<td>12.9*</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>45.2*</td>
<td>14.7*</td>
<td>15.8*</td>
<td>11.5*</td>
<td>8.9*</td>
<td>10.2*</td>
<td>10.0*</td>
<td>8.9*</td>
<td>10.5*</td>
</tr>
<tr>
<td>Lysine</td>
<td>43.0*</td>
<td>17.8</td>
<td>18.8</td>
<td>30.6*</td>
<td>27.6*</td>
<td>29.3*</td>
<td>16.6</td>
<td>23.9*</td>
<td>26.4*</td>
</tr>
<tr>
<td>Histidine</td>
<td>8.4</td>
<td>6.4</td>
<td>7.1</td>
<td>14.7*</td>
<td>11.9*</td>
<td>10.2*</td>
<td>10.8</td>
<td>11.4</td>
<td>12.4*</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>5.6</td>
<td>5.6</td>
<td>6.3*</td>
<td>9.5*</td>
<td>4.5</td>
<td>8.0</td>
<td>3.9</td>
<td>8.6*</td>
<td>11.0*</td>
</tr>
<tr>
<td>Arginine</td>
<td>14.6*</td>
<td>14.6*</td>
<td>15.7*</td>
<td>14.9*</td>
<td>12.6</td>
<td>11.9*</td>
<td>11.9</td>
<td>13.9*</td>
<td>18.9*</td>
</tr>
<tr>
<td>Total amino acid</td>
<td>368.5*</td>
<td>194.7</td>
<td>201.2</td>
<td>521.0*</td>
<td>431.7*</td>
<td>366.3*</td>
<td>267.7</td>
<td>511.2*</td>
<td>415.3*</td>
</tr>
<tr>
<td>A.A.M.R.</td>
<td>0.43</td>
<td>0.98</td>
<td>0.89</td>
<td>4.1</td>
<td>4.4</td>
<td>4.2</td>
<td>1.1</td>
<td>2.4</td>
<td>2.3</td>
</tr>
</tbody>
</table>

C.H. cross-hemodialysis, R.C. recovery of consciousness, A.A.M.R. amino acid molar ratio (B.C.A.A./A.A.A.)
*: increased as compared with control (>mean±2S.D.).

Biopsy specimens were selected as a control. In control subjects, the blood ketone body ratio was 1.824±0.422 (mean±S.E.M. n=11) in the presence of hyperglycemia over 120 mg/dl by the infusion of 10% glucose solution at the rate of 200 ml/hour. The BKBR immediately before the cross-hemodialysis was 0.300±0.043 (mean±S.E.M.) in these 8 patients (Table 1). In group A patients, the blood ketone body ratio returned to over 0.7 within 3 days, while in group B, the ratio was 0.17±0.014 (mean±S.E.M.) after the cross-hemodialysis. Time course changes in blood ketone body ratio of the patients (No. 4 and No. 8) and the liver used are shown in Fig. 1. In No. 4 patient (group A), BKBR of the patient was increased gradually during cross-hemodialysis. The BKBR of the liver was maintained over 1.0. However, in No. 8 patients (group B), the BKBR of the patients did not change and the ratio of the liver used was decreased gradually to 0.4 during cross-hemodialysis.

Tables 2 and 3 show the concentration of each plasma amino acid of group A who recovered from coma concomitant with increase of their BKBR after the cross-hemodialysis. Before the cross-hemodialysis, all of group A patients (patients 1 to 5) had an increase in aspartate, glutamate,
Table 3
PLASMA AMINO ACID LEVEL IN CASES 4 AND 5

<table>
<thead>
<tr>
<th></th>
<th>Patient 4</th>
<th></th>
<th>Patient 5</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>C.H.</td>
<td>(μmoles/dl)</td>
<td>C.H.</td>
<td>(μmoles/dl)</td>
</tr>
<tr>
<td>Aspartate</td>
<td>0.7</td>
<td>0.2</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Threonine</td>
<td>38.0*</td>
<td>33.4*</td>
<td>27.3*</td>
<td>24.6*</td>
</tr>
<tr>
<td>Serine</td>
<td>21.6*</td>
<td>20.2*</td>
<td>22.4*</td>
<td>17.0*</td>
</tr>
<tr>
<td>Asparagine</td>
<td>15.4*</td>
<td>8.0</td>
<td>17.5*</td>
<td>14.8*</td>
</tr>
<tr>
<td>Glutamate</td>
<td>45.8*</td>
<td>29.1*</td>
<td>16.6*</td>
<td>11.7*</td>
</tr>
<tr>
<td>Glutamine</td>
<td>46.3</td>
<td>52.0</td>
<td>97.7*</td>
<td>86.5*</td>
</tr>
<tr>
<td>Proline</td>
<td>40.3*</td>
<td>59.2*</td>
<td>39.7*</td>
<td>36.0*</td>
</tr>
<tr>
<td>Glycine</td>
<td>51.2*</td>
<td>53.6*</td>
<td>33.2*</td>
<td>29.0*</td>
</tr>
<tr>
<td>Alanine</td>
<td>67.1*</td>
<td>86.5*</td>
<td>65.7*</td>
<td>69.1*</td>
</tr>
<tr>
<td>Valine</td>
<td>13.9</td>
<td>20.1</td>
<td>22.2</td>
<td>25.1</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.6</td>
<td>0.4</td>
<td>4.1</td>
<td>3.3</td>
</tr>
<tr>
<td>Methionine</td>
<td>44.3*</td>
<td>29.3*</td>
<td>20.3*</td>
<td>16.6*</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.1</td>
<td>3.2</td>
<td>5.2</td>
<td>4.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>8.3</td>
<td>4.8</td>
<td>12.3</td>
<td>14.3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>28.0*</td>
<td>12.6*</td>
<td>43.7*</td>
<td>34.7*</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>12.0*</td>
<td>4.4</td>
<td>22.9*</td>
<td>21.9*</td>
</tr>
<tr>
<td>Lysine</td>
<td>57.6*</td>
<td>43.8*</td>
<td>51.1*</td>
<td>48.1*</td>
</tr>
<tr>
<td>Histidine</td>
<td>11.4</td>
<td>10.7</td>
<td>15.8*</td>
<td>16.4*</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>3.9</td>
<td>2.5</td>
<td>8.2*</td>
<td>10.8*</td>
</tr>
<tr>
<td>Arginine</td>
<td>22.1*</td>
<td>15.6*</td>
<td>7.4</td>
<td>7.7</td>
</tr>
<tr>
<td>Total amino acid</td>
<td>533.8*</td>
<td>489.6*</td>
<td>533.7*</td>
<td>492.4*</td>
</tr>
<tr>
<td>A.A.M.R.</td>
<td>0.68</td>
<td>1.7</td>
<td>0.60</td>
<td>0.77</td>
</tr>
</tbody>
</table>

C.H. cross-hemodialysis,
A.A.M.R. amino acid molar ratio (B.C.A.A./A.A.A.)
*: increased as compared with, (>mean+2S.D.).

tyrosine and phenylalanine, as compared with control (Table 5). Since branched chain amino acids were not increased as compared with the levels in control, amino acid molar ratio \(((\text{valine} + \text{leucine} + \text{isoleucine})/ (\text{tyrosine} + \text{phenylalanine}))\) was lower than that of control.

Table 4 shows the changes in the plasma amino acid in group B, who did not recovered from coma without any increase in their blood ketone body ratio over 0.18 after the cross-hemodialysis. Before the cross-hemodialysis, they showed an increase in glutamate, phenylalanine, and tyrosine with the amino acid molar ratio less than 0.6, because they also did not accompanied with increase in the branched chain amino acids.

In group B, immediately after the cross-hemodialysis, total amino acid levels were increased accompanied with increase in aspartate, threonine, glycine, alanine, lysine, histidine, arginine and branched chain amino acids, while total amino acid was not increased in group A except No. 3 patient. The No. 3 patient whose BKBR was 0.25, showed increases both in total amino acid and branched chain amino acid after the cross-hemodialysis as observed in group B patients whose
<table>
<thead>
<tr>
<th></th>
<th>Patient 6 Before</th>
<th>Patient 6 After</th>
<th>Patient 7 Before</th>
<th>Patient 7 After</th>
<th>Patient 8 Before</th>
<th>Patient 8 After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>0.4</td>
<td>0.6</td>
<td>0.3</td>
<td>0.2</td>
<td>2.6</td>
<td>4.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>10.6</td>
<td>33.3*</td>
<td>57.6*</td>
<td>82.8*</td>
<td>54.8*</td>
<td>93.9*</td>
</tr>
<tr>
<td>Serine</td>
<td>6.9</td>
<td>22.5*</td>
<td>54.2*</td>
<td>70.3*</td>
<td>23.7*</td>
<td>57.4*</td>
</tr>
<tr>
<td>Asparagine</td>
<td>5.5</td>
<td>5.1</td>
<td>22.7*</td>
<td>17.9*</td>
<td>36.4*</td>
<td>31.2*</td>
</tr>
<tr>
<td>Glutamate</td>
<td>19.6*</td>
<td>25.8*</td>
<td>56.7*</td>
<td>31.0*</td>
<td>22.9*</td>
<td>—</td>
</tr>
<tr>
<td>Glutamine</td>
<td>43.5</td>
<td>81.1*</td>
<td>266.0*</td>
<td>309.8*</td>
<td>351.6*</td>
<td>351.7*</td>
</tr>
<tr>
<td>Proline</td>
<td>26.6</td>
<td>80.0*</td>
<td>133.3*</td>
<td>178.9*</td>
<td>150.8*</td>
<td>225.1*</td>
</tr>
<tr>
<td>Glycine</td>
<td>21.1</td>
<td>77.8*</td>
<td>109.5*</td>
<td>193.8*</td>
<td>73.6*</td>
<td>200.8*</td>
</tr>
<tr>
<td>Alanine</td>
<td>29.2</td>
<td>73.6*</td>
<td>218.5*</td>
<td>316.4*</td>
<td>190.2*</td>
<td>355.8*</td>
</tr>
<tr>
<td>Valine</td>
<td>15.5</td>
<td>61.5*</td>
<td>34.1*</td>
<td>79.1*</td>
<td>24.0*</td>
<td>85.9*</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.2</td>
<td>0.1</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>20.7*</td>
<td>22.7*</td>
<td>55.6*</td>
<td>47.2*</td>
<td>17.0*</td>
<td>23.9*</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.0</td>
<td>20.9*</td>
<td>7.7</td>
<td>22.2*</td>
<td>3.3</td>
<td>35.7*</td>
</tr>
<tr>
<td>Leucine</td>
<td>8.0</td>
<td>29.8*</td>
<td>15.8*</td>
<td>38.5*</td>
<td>16.2*</td>
<td>60.5*</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>24.7*</td>
<td>21.2*</td>
<td>48.3*</td>
<td>38.1*</td>
<td>42.3*</td>
<td>38.6*</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>29.4*</td>
<td>27.3*</td>
<td>49.4*</td>
<td>39.2*</td>
<td>48.5*</td>
<td>49.3*</td>
</tr>
<tr>
<td>Lysine</td>
<td>47.2*</td>
<td>90.2*</td>
<td>89.6*</td>
<td>103.2*</td>
<td>155.3*</td>
<td>258.9*</td>
</tr>
<tr>
<td>Histidine</td>
<td>9.0</td>
<td>18.3*</td>
<td>24.6*</td>
<td>41.4*</td>
<td>43.1*</td>
<td>64.7*</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>3.4</td>
<td>7.0*</td>
<td>5.3</td>
<td>13.8*</td>
<td>3.9</td>
<td>5.5</td>
</tr>
<tr>
<td>Arginine</td>
<td>6.8</td>
<td>18.0*</td>
<td>76.0*</td>
<td>84.5*</td>
<td>11.2</td>
<td>42.8*</td>
</tr>
<tr>
<td>Total amino acid</td>
<td>331.3*</td>
<td>716.8*</td>
<td>1325.6*</td>
<td>1706.7*</td>
<td>1271.8*</td>
<td>1985.9*</td>
</tr>
<tr>
<td>A.A.M.R.</td>
<td>0.49</td>
<td>2.3</td>
<td>0.59</td>
<td>1.8</td>
<td>0.48</td>
<td>2.1</td>
</tr>
</tbody>
</table>

C.H. cross-hemodialysis,
A.A.M.R. amino acid molar ratio (B.C.A.A./A.A.A.)
*: increased as compared with control, (>mean±2S.D.).

BKBR was less than 0.25. In all 8 patients, tyrosine was decreased. Phenylalanine was decreased in 7 patients. In all 8 patients, the ratio of tyrosine plus phenylalanine to total amino acid concentration was decreased significantly (p=0.012) after the cross-hemodialysis. Amino acid molar ratio was also significantly increased in all 8 patients immediately after the cross-hemodialysis (p=0.007). However, the increase of the amino acid molar ratio caused by the decrease in tyrosine and phenylalanine was not always accompanied with the recovery of consciousness. In all 8 patients, the ratio of alanine to total amino acid concentrations was increased immediately after the cross-hemodialysis (p=0.05). In group A, alanine level was decreased after the complete recovery.
of coma. However, in group B the plasma levels of alanine were increased markedly during the cross-hemodialysis.

Discussion

Changes in plasma amino acid concentration have been reported in patients with various pathologic conditions, such as sepsis, liver cirrhosis, acute fulminant hepatitis, chronic hepatic insufficiency and after injury. A common feature of these conditions is increased catabolism of muscle and changed liver function, which is evident even in the early stage of sepsis. Abnormal aminograms in these patients reported here are caused by the combination of injured liver function and increased catabolism of the muscle due to fasting and surgical trauma.

Before the cross-hemodialysis, tyrosine and phenylalanine were increased in the all of 8 patients. Necrosis of hepatocytes may contribute to the elevated levels of these amino acid. It is unlikely that all of these patients were complicated with severe hepatic necrosis, because serum GOT levels were less than 1000 IU/L except in case 6. It is well known that under the increased catabolism of the muscle, branched chain amino acids (valine, leucine and isoleucine) are utilized in the muscle as an energy source, while phenylalanine and tryosine accumulate in the blood, since

### Table 5

<table>
<thead>
<tr>
<th>PLASMA AMINO ACID LEVELS IN CONTROL SUBJECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Fasting (n=7)</td>
</tr>
<tr>
<td>(μmoles/dl)</td>
</tr>
<tr>
<td>Aspartate</td>
</tr>
<tr>
<td>Threonine</td>
</tr>
<tr>
<td>Serine</td>
</tr>
<tr>
<td>Asparagine</td>
</tr>
<tr>
<td>Glutamate</td>
</tr>
<tr>
<td>Glutamine</td>
</tr>
<tr>
<td>Proline</td>
</tr>
<tr>
<td>Glycine</td>
</tr>
<tr>
<td>Alanine</td>
</tr>
<tr>
<td>Valine</td>
</tr>
<tr>
<td>Cystine</td>
</tr>
<tr>
<td>Methionine</td>
</tr>
<tr>
<td>Isoleucine</td>
</tr>
<tr>
<td>Leucine</td>
</tr>
<tr>
<td>Tyrosine</td>
</tr>
<tr>
<td>Phenylalanin</td>
</tr>
<tr>
<td>Lysine</td>
</tr>
<tr>
<td>Histidine</td>
</tr>
<tr>
<td>Tryptophan</td>
</tr>
<tr>
<td>Arginine</td>
</tr>
<tr>
<td>Total amino acid</td>
</tr>
<tr>
<td>A.A.M.R.</td>
</tr>
<tr>
<td>Feeding (n=5)</td>
</tr>
<tr>
<td>(μmoles/dl)</td>
</tr>
<tr>
<td>Aspartate</td>
</tr>
<tr>
<td>Threonine</td>
</tr>
<tr>
<td>Serine</td>
</tr>
<tr>
<td>Asparagine</td>
</tr>
<tr>
<td>Glutamate</td>
</tr>
<tr>
<td>Glutamine</td>
</tr>
<tr>
<td>Proline</td>
</tr>
<tr>
<td>Glycine</td>
</tr>
<tr>
<td>Alanine</td>
</tr>
<tr>
<td>Valine</td>
</tr>
<tr>
<td>Cystine</td>
</tr>
<tr>
<td>Methionine</td>
</tr>
<tr>
<td>Isoleucine</td>
</tr>
<tr>
<td>Leucine</td>
</tr>
<tr>
<td>Tyrosine</td>
</tr>
<tr>
<td>Phenylalanin</td>
</tr>
<tr>
<td>Lysine</td>
</tr>
<tr>
<td>Histidine</td>
</tr>
<tr>
<td>Tryptophan</td>
</tr>
<tr>
<td>Arginine</td>
</tr>
<tr>
<td>Total amino acid</td>
</tr>
<tr>
<td>A.A.M.R.</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M.

A.A.M.R. amino acid molar ratio (B.C.A.A./A.A.A.)
these amino acids are not metabolized in the muscle. Under the condition that the muscle catabolism is in steady state, the extent of increase in these amino acids is considered to be correlated with the extent of the injured capacity of the liver to metabolize. Recently, for the clinical assessment of metabolic derangements of the injured liver, we have introduced and used BKBR (acetoacetate/β-hydroxybutyrate).

In liver mitochondria, acetoacetate undergoes reduction to β-hydroxybutyrate by β-hydroxybutyrate dehydrogenase localized in the mitochondrial crista. The equilibrium between the concentrations of acetoacetate and β-hydroxybutyrate reflects the free NAD⁺/NADH ratio in the mitochondria shown by the following formula: acetoacetate + NADH ⇄ β-hydroxybutyrate + NAD⁺. Thus, the free NAD⁺/NADH ratio in the mitochondria can be calculated as follows: free NAD⁺/NADH = acetoacetate/β-hydroxybutyrate × 1/K, where K indicates the equilibrium constant of β-hydroxybutyrate dehydrogenase. Since the β-hydroxybutyrate dehydrogenase activity is exceptionally high in the liver and acetoacetate and β-hydroxybutyrate are freely penetrate cell membrane, the ketone body ratio in arterial blood can reflect that in liver mitochondria.

Previous studies from our laboratory have shown that high mortality occurs in hepatectomized or obstructive-jaundiced animals, in which the hepatic energy charge maximally decreased, and that the decreased hepatic energy charge is accompanied with decrease in the blood ketone body ratio in jaundiced, and hepatectomized, or shocked animals. In all of these 8 patients, BKBR was less than normal, indicating a decreased NAD⁺/NADH ratio in their liver mitochondria. Recently, we have suggested that the reduced mitochondrial redox potential (decrease in NAD⁺/NADH ratio), coupled with enhanced muscle breakdown, results in an inhibition of amino acids to get into Krebs cycle and then the characteristic changes in free amino acid patterns which results in hepatic coma. The plasma concentrations of alanine, proline, glutamate, glutamine, phenylalanine, tyrosine, aspartate, asparagine, lysine or histidine were found to be negatively correlated with the BKBR in the patients with liver disease. Also, the amino acid molar ratio was shown to be positively correlated with the BKBR.

After the cross-hemodialysis, phenylalanine pils tyrosine was decreased in all of 8 patients. When one assume that a rate of muscle catabolism is constant during the cross-hemodialysis, the rate of metabolism of these amino acids seems to be enhanced during the cross-hemodialysis. These amino acids are thought to be metabolized by the isolated perfused liver itself used for the cross-hemodialysis.

The extent of the decrease in these amino acids was greater in group A than in group B. As shown in Fig. 1, the BKBR of No. 4 patient recovered during the cross-hemodialysis. The BKBR of the liver used was maintained over 1.0. It is suggested that in group A, the metabolic load imposed on the patient's liver was reduced and the energy metabolism of the patient's liver was improved by the cross-hemodialysis. However, in No. 8 patient, the ratio of the liver used was decreased during the cross-hemodialysis (Fig. 1). It seems that in group B, metabolic derangement of the patient is too severe to be ameliorated by the isolated liver, and that the energy metabolism of the isolated liver itself is impaired during the cross-hemodialysis with the patient having lowered BKBR. Actually, the energy charge ((ATP+0.5 ADP)/(ATP+ADP+AMP)) of the isolated liver used for group A patients was higher than that of group B (0.81 ± 0.020, n = 5, mean ± S.E.M. and 0.63 ± 0.02, n = 3, mean ± S.E.M., respectively). It is likely that the difference in the extent of decrease in these amino acids is partly influenced by the changes in the metabolic activities of the perfused liver used,
because the higher the energy charges, the higher the metabolic activity is. Thus, increased muscle breakdown as well as lowered metabolic activity of the isolated liver used could account for the small changes of tyrosine and phenylalanine after the cross-hemodialysis in group B patients. The synthetic amino acids which had been given to these patients during the cross-dialysis, were rich in branched chain amino acids, threonine, serine proline, glycine, and histidine. These amino acids are not oxidized in the muscle and released in the blood stream under accelerated muscle breakdown. After the cross-hemodialysis, these amino acids were not increased in group A patients except No. 3 patient with the BKBR of 0.25, while these amino acids were increased 2 to 4 times in group B patients. Therefore, it is suggested that in the patients with the BKBR of less than 0.25, the administrated amino acids are not utilized and account for the increase in the plasma levels of these amino acids after the cross-hemodialysis.

Alanine and glutamine are the amino acids which are synthesized de novo as the results of amino acid degradation in the muscle and released in the blood stream. Alanine is mainly used for gluconeogenesis in the liver, and glutamine is metabolized mainly in the kidney. In group A patients, these amino acids were not increased remarkably, while these was increased drastically in the group B patients after the cross-hemodialysis. These observation also suggests that in group B, proteolysis in the muscle is accelerated and the utilization of alanine by patient's liver and/or isolated liver is inhibited. It is well known that alanine released from the muscle is utilized for gluconeogenesis in the liver, which consumes ATP synthesized through mitochondrial oxidative phosphorylation. The decreased energy charge of the isolated liver and the failed liver of the group B patients as suggested by the decreased in BKBR, could also account for the marked increase of alanine after the cross-hemodialysis. By contrast, in group A immediately after the cross-hemodialysis, alanine was slightly increased or did not change. The alanine levels were decreased after the recovery of both consciousness and BKBR (patients, 1, 2 and 3). These observation could suggest that gluconeogenesis from alanine in patient's liver became more active after the recovery of both consciousness and BKBR than during the cross-hemodialysis, if the rate of alanine formation in
the muscle was constant.

Thus, there are at least two mechanisms by which the abnormal aminograms are corrected by the cross-hemodialysis. The first mechanism is a direct effect of the isolated liver which has capacity to metabolize the amino acids. The second mechanism is an indirect effect on amino acids metabolism. The cross-hemodialysis may correct the energy metabolism of the patient's liver, by which the correction of the plasma aminograms are obtained during or after the finishing of the cross-hemodialysis.

The changes in plasma amino acids are affected by many factors complicating these postoperative patients. The severity of metabolic derangement in muscle in these patients remain unanswered. It is also unclear how metabolic derangement of the liver could affect the amino acid metabolism of the muscle. However, it was found that the correction of the amino acids metabolism was possible by the use of the isolated liver having high energy levels. For further understanding the functional capacity of the isolated liver by which the BKBR and plasma aminograms are corrected, a more detailed study is now underway.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research, a Grant-in-Aid for Developmental Scientific Research and a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare.

References


和文抄録

摘出灌流肝との交叉透析による血中アミノグラムの代謝的補正

京都市立病院外科
上山 泰男
京都大学医学部外科学教室第2講座
小澤 和恵

外科的重症例、肝不全例、肝移植後症例等において肝ミトコンドリアのNAD+/NADH比の変化と血中で相関する動脈血中ケトン体比 acetooacetate/β-hydroxybutyrateを経時的に測定すると、0.7以下の症例では種々の代謝異常が発生し、合併症が発症する。0.4以下の症例は症候群を表すが、摘出灌流肝を利用した肝補助療法により、ケトン体比0.4以下の症例を除く他は未だ治療例もない。
より肝の代謝能が回復すれば、アミノ酸代謝も補正されると考えられる。

[対象・方法]肝補助療法を受けた8人の基礎的疾患は、肝硬変5例、肝炎2例、肝癌1例である。肝補助療法前には一般的治療に加えてアミノ酸輸液（フィッシュナー）の投与をしていた。抽出灌流肝による肝補助療法は現症の如く行った。抽出肝は正常人の血液で灌流した。肝血流量は0.5〜1.0 ml/min/gmに維持し、そのpH、酸素分圧はそれぞれ7.35〜7.50、200〜300 mmHg（36.5°C）に維持した。抽出肝を灌流した血液はキプロフラン膜（HDF, ENKA 社）を介して患者血液と交又透析をした。

抽出灌流肝及び患者のケトン体量測定のために抽出肝では肝静脈（out flow）側より、患者は動脈より、それぞれ経時的に採血した。アミノ酸の測定、ケトン体量測定は既報に依った。

[結果]対象患者の治療前の動脈血液中ケトン体量は0.3±0.043（n=8）であった（対照、1.824±0.0422, n=11）。肝補助療法前の中性ケトン体量が0.4以下であるが、0.25以上の5例は本治療により、0.7以上に回復したが、（A群）、治療前、0.25以下の症例では治療後も0.17±0.014であり、全例肝不全で死亡した。

(B群)両群の肝補助療法前の生化学データはケトン体量以外には差異は認めなかった。肝補助療法前、両群ともtyrosine, phenylalanineの増加に対応した分枝鎖アミノ酸の増加がなく、アミノ酸モチル比((valine + leucine + isoleucine)/ (tyrosine + phenylalanine))は正常より低かった。治療前よりA群ではB群と異なり、アミノ酸総量が増加していた。

補助肝のみを灌流した時の肝静脈中ケトン体量は高い。A群では患者との交又透析により、補助肝のケトン体量の上昇が認められた。B群では交又透析により、補助肝のケトン体量は低下し、患者のケトン体量の上昇は見られなかった。アミノ酸代謝をみると、両群において(tyrosine+phenylalanine)量のアミノ酸総量に対する割合は、有意（P=0.012）に減少し、アミノ酸モチル比は有意（P=0.007）に上昇した。しかしアミノ酸モチル比の上昇は必ずしも意識の回復を伴わないかった。B群では総アミノ酸量、分枝鎖アミノ酸量が、肝補助療法後も上昇した。A群では、肝補助療法終了時増加していたalanineは意識の回復した時点では正常化していた。

[考案]抽出肝の高いケトン体量が、B群との交又透析により低下したのは、患者のmetabolic loadが膜を通して抽出肝に及んだものと考えられる。B群では1回のみの交又透析では患者肝のエネルギー代謝の態定にはおおよばなかったと考えられる。しかし、tyrosine, phenylalanineは両群において低下した。これらは膜を通過して抽出肝にて代謝されたと考えられる。肝でATP供給下で新たに利用されるalanineは肝補助療法中でなく意識が回復し、ケトン体量が上昇した時点で正常化している（A群）。これは、高いRedox stateを有する抽出肝により患者のRedox stateが回復し、患者肝のエネルギー代謝が改善された結果であることを示唆している。従って、抽出灌流肝によるアミノ酸代謝の補正の機能としては、抽出灌流肝自体による直接的なアミノ酸代謝の促進こと、肝補助療法により患者肝のエネルギー代謝が回復した結果、そのアミノ酸代謝機能が回復する機構とが想定される。肝不全時の筋肉でのアミノ酸代謝の病態や筋肉での代謝障害のこれらの症例の血中アミノグラムにどのような影響を与えるかについては今後の検討を要する。