Multiple Organ Diseases

Changes of arterial blood ketone body ratio following hypoperfusion in old and adult rats

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Objective To evaluate the sensitivity of arterial ketone body ratio as an indicator for multiple organ failure.

Materials and methods The experimental model of multiple organ failure was made in adult and old rats by hypoperfusion-induced hemorrhagic shock. After blood sampling, the arterial acetoacetate, β-hydroxybutyrate, total ketone body, ALT, AST, BUN, creatinine at 2, 4, 8 hr in hypoperfusion were examined to compare the differences of ketone body ratio and organ failure between adult and old rats. Hepatic and mitochondrial metabolism were assessed by comparing ketone body ratios (AcAc/β-OHB) and free NAD+/NADH ratios. Results Ketone body ratio in old rats at 2, 4, 8 hr after the induction of hemorrhagic shock decreased from 0.68 to 0.31, 0.27 and 0.22, respectively. In adult rats, it decreased from 1.12 to 0.17, 0.12 and 0.09, respectively. Changes of ketone body ratio in the adult group were larger than in the elderly group (P < 0.001). The development of multiple organ failure is associated with the time of hemorrhagic shock development. Conclusions There was a different ketone body ratio between multiple organ failure in the elderly (MOFE) and multiple organ failure (MOF) in general adults. Ketone body ratio is a better indicator than ALT and AST in reflecting hepatic function in the early status of MOF. (J Geriatr Cardiol 2004;1(2):125-128.)

Key Words multiple organ failure in the elderly; arterial ketone body ratio; hypoperfusion; hemorrhagic shock; rat

Introduction

Multiple organ failure, a syndrome caused by various etiologic factors, has received much attention for its high mortality. Studies of hepatic energy metabolism during hemorrhagic shock have proved that hepatic mitochondrial phosphorylative activity is an important factor in deciding the severity of hepatic failure. Changes in the energy charge [ATP + 1/2 ADP/(ATP + ADP + AMP)] are correlated with the changes of the hepatic mitochondrial redox potential. The mitochondrial free NAD+/NADH ratio reflects the oxidoreduction state, NAD+/NADH = AcAc/β-OHB × 1/K (for β-hydroxybutyrate dehydrogenase, K = 4.93 × 10^2). Liver is the principal organ in synthesizing ketone body acetoacetate (AcAc) and β-hydroxybutyrate (β-OHB).

Arterial blood ketone body ratio (AcAc/β-OHB) may reflect the hepatic mitochondrial oxidoreduction state and the hepatic energy status in hemorrhagic shock.

In the present study, the time course of arterial blood ketone body ratio in old and adult rats following hypoperfusion-induced hemorrhagic shock was investigated. The relationship between ketone body ratio and the degree of organ failure with age, as well as the sensitivity of ketone body ratio compared with ALT (SGPT) and AST (SGOT), was investigated.

Materials and methods

Wistar strain rats were divided into two groups, 2.5-3 year-old (old) rats (weighing 517.5 ± 125.6 g) and 4.5 month-old (adult) rats (weighing 236 ± 30.7 g). There was no restriction regarding male or female rats. Old and adult rats were divided into experimental and control groups randomly. According to the method described by Yamamoto et al. with modification, the rats were anesthetized with 30 mg of pentobarbital sodium per kg of body weight intraperitoneally and fixed on a surgical board in supine position. A total of 1125 U/kg of heparin
was administered into the cervical vein. The cervical artery was cannulated with a catheter, which was filled with 1 mg% heparin balanced saline before performance and connected with a blood pressure measurement and a blood reservoir. The mean arterial blood pressure fell to 5.3 kPa (40 mmHg, 1 kPa = 7.5 mmHg) by withdrawing blood slowly by a syringe, and it was maintained at that level by intermittent removal or by returning the blood through the blood reservoir. The arterial blood samples were collected from the cannula without suction at 2, 4, and 8 hr respectively after hypoperfusion-induced hemorrhagic shock. Control rats were fixed on surgical boards, heparinized, and cannulated in the same way. Arterial blood was analyzed because it specifically reflects changes in hepatic ketone body metabolism as opposed to venous blood, which provides a measure of total body metabolism. After the respiration and blood pressure were confirmed to be stable, blood samples were taken. The blood samples were centrifuged at 8000 rpm for 5 min immediately after their collection. The plasma acetocetate (AcAc) and total ketone body (TKB) were determined by diazonium and enzymatic methods described by Harano et al. with kits from Japan (KBN, Sanwa Kagaku Co.). The difference between TKB and AcAc was the value of β-OHB. Ketone body ratio was calculated as the ratio of AcAc/β-OHB. ALT, AST, BUN, and Cr were assayed in routine clinic lab tests. Data were analyzed by Student’s t test. P < 0.05 was considered to be significant. All results are expressed as mean ± SD.

Results

Relationship between hemorrhagic shock time and the severity of organ failure

Table 1 shows the changes of ALT, AST, BUN, and Cr in arterial blood after hemorrhagic shock for 2, 4, and 8 hr. Combined with the histological evidences of heart, lungs, liver, kidneys and intestine, as well as blood gas analysis and amino acids assay, it can be deduced that the rats developed the multiple organ failure (MOF) after hypoperfusion-induced hemorrhagic shock. The degree of MOF in old rats was more serious than in adult rats. Along with the increase of hemorrhagic shock the organs failed progressively.

Comparison of ketone body ratio between old and adult rats

Table 2 shows changes of ketone body ratio and redox status in old and adult rats subjected to hemorrhagic shock. At 2 hr after hemorrhagic shock, levels of the ketone body concentration, ketone body ratio and NAD⁺/NADH ratio in both groups changed maximally. TKB increased 148% (old rats) and 140% (adult rats). Ketone body ratio decreased 54% (old) and 85% (adult). Levels of TKB, AcAc and β-OHB in old rats were significantly higher than those in adult rats. However, the decreases of ketone body ratio and NAD⁺/NADH ratio were more significant in adult rats than in old rats. The marked decrease of ketone body ratio in adult rats was coincidental with the high mortality in adult rats with hemorrhagic shock.

Comparison of ketone body ratio and the hepatic function status between old and adult rats

Figs. 1 and 2 show the relationship between ketone body ratio and the concentrations of ALT and AST under hemorrhagic shock for 2, 4, and 8 hr in the old and adult rats respectively. Fig. 1 shows that the decrease of ketone body ratio was more rapid than the increase of ALT/AST within the first 2 hr. There was negative correlation between ketone body ratio and ALT/AST from 2-8 hr (r = -0.9895 and -0.9890, respectively). Similarly, Fig. 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (hr)</th>
<th>n</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>BUN (mmol/L)</th>
<th>Cr (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old</td>
<td>Control</td>
<td>14</td>
<td>58.2 ± 9.5</td>
<td>89.0 ± 12.7</td>
<td>6.6 ± 0.6</td>
<td>42.4 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
<td>74.7 ± 10.3</td>
<td>127.7 ± 14.2</td>
<td>7.4 ± 0.1</td>
<td>105.2 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>8</td>
<td>130.3 ± 15.5</td>
<td>211.6 ± 20.6</td>
<td>8.1 ± 0.7</td>
<td>105.2 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8</td>
<td>250.5 ± 28.9</td>
<td>395.9 ± 40.9</td>
<td>12.2 ± 0.1</td>
<td>115.8 ± 5.3</td>
</tr>
<tr>
<td>Adult</td>
<td>Control</td>
<td>7</td>
<td>35.1 ± 3.3</td>
<td>56.1 ± 2.7</td>
<td>8.0 ± 0.5</td>
<td>55.7 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9</td>
<td>53.6 ± 8.4</td>
<td>114.3 ± 11.5</td>
<td>11.0 ± 0.8</td>
<td>89.3 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6</td>
<td>108.5 ± 5.5</td>
<td>136.8 ± 27.3</td>
<td>14.1 ± 1.2</td>
<td>101.7 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7</td>
<td>137.9 ± 21.6</td>
<td>361.8 ± 44.9</td>
<td>22.0 ± 1.1</td>
<td>102.5 ± 96.3</td>
</tr>
</tbody>
</table>

* P < 0.01, ** P < 0.001, compared with the control in the same group; * P < 0.01, ** P < 0.001, compared with the old group.
Table 2. Changes of ketone body ratio and the redox state in the old and adult rats during hemorrhagic shock (M ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (hr)</th>
<th>n</th>
<th>TKB (µmol/L)</th>
<th>AcAc (µmol/L)</th>
<th>β-OHB (µmol/L)</th>
<th>AcAc/β-OHB</th>
<th>NAD+/NADH</th>
<th>Survival/Total(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>8</td>
<td>351 ± 42</td>
<td>143 ± 16</td>
<td>208 ± 28</td>
<td>0.68 ± 0.08</td>
<td>13.8 ± 1.62</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11</td>
<td>870 ± 103**</td>
<td>192 ± 21**</td>
<td>678 ± 85**</td>
<td>0.31 ± 0.05**</td>
<td>6.29 ± 1.01**</td>
<td>11/12</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9</td>
<td>1058 ± 101**</td>
<td>202 ± 34**</td>
<td>856 ± 101**</td>
<td>0.27 ± 0.06**</td>
<td>5.48 ± 1.22**</td>
<td>9/13</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>11</td>
<td>1402 ± 151**</td>
<td>226 ± 51**</td>
<td>1172 ± 139**</td>
<td>0.22 ± 0.04**</td>
<td>4.46 ± 0.81**</td>
<td>11/25</td>
</tr>
<tr>
<td>Adult</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>9</td>
<td>142 ± 14ΔΔ</td>
<td>67 ± 9ΔΔ</td>
<td>75 ± 14ΔΔ</td>
<td>1.12 ± 0.33ΔΔ</td>
<td>22.7 ± 6.69ΔΔ</td>
<td>9/9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
<td>341 ± 24**ΔΔ</td>
<td>73 ± 12ΔΔ</td>
<td>268 ± 31**ΔΔ</td>
<td>0.17 ± 0.02**ΔΔ</td>
<td>3.45 ± 0.40ΔΔ</td>
<td>7/8</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7</td>
<td>546 ± 82**ΔΔ</td>
<td>68 ± 10ΔΔ</td>
<td>478 ± 74**ΔΔ</td>
<td>0.12 ± 0.02**ΔΔ</td>
<td>2.43 ± 0.40ΔΔ</td>
<td>7/16</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3</td>
<td>577 ± 103**ΔΔ</td>
<td>65 ± 10ΔΔ</td>
<td>512 ± 102**ΔΔ</td>
<td>0.09 ± 0.01**ΔΔ</td>
<td>1.82 ± 0.20ΔΔ</td>
<td>3/12</td>
</tr>
</tbody>
</table>

* P < 0.001 compared with the control in the same group; ** P < 0.001 compared with the old group.

presents the negative correlation of ketone body ratio and ALT/AST in adult rats during hemorrhagic shock between 2-8 hr (r = -0.9996 and -0.8339, respectively). The decrease of ketone body ratio was even more significant than the increase of ALT/AST within the first 2 hr.

**Fig. 1. Changes of ketone body ratio and ALT, AST in old rats during hemorrhagic shock**

**Fig. 2. Changes of ketone body ratio and ALT, AST in adult rats during hemorrhagic shock**

**Discussion**

In the present study, we performed a dynamic observation up to 8 hr to analyze the effect of hemorrhagic shock on MOF. When the rats were subjected to hemorrhagic shock induced by hypoperfusion for 2 hr, the levels of the arterial blood ketone body and ketone body ratio changed dramatically. After 4 hr, some organ functions and indexes showed severe multiple organ failure. Based on the histological analysis and biochemical examination, more serious signs of MOF took place in old rats than in adult rats. Nevertheless, the mortality of adult rats was higher than that of old rats (although there was no statistical significance for those small samples). The decreases of AcAc/β-OHB and NAD+/NADH in the adult group were more rapid. Tables 1 and 2 show the differences of various indexes between the controls in the old group and the adult group (P < 0.001). These phenomena may be caused by the differences of age and the organ function status in the two groups. The old rats with low organ functions had been in a compensatory status for quite a long time. It is common that the course of the disease in elderly people could be prolonged relatively. On the contrary, the energy metabolism in younger people is more active. Once the organ function and the energy metabolism are abnormal, series of responses emerge suddenly and the body system itself will be incapable of self-regulation. Wang SW12-14 initially described multiple organ failure in the elderly (MOFE) as a new clinical syndrome, which is different from MOF caused by trauma occurring most often in young and mid-aged persons. MOFE evolves from multiple organ chronic diseases on the basis of multiple organ dysfunctions in elderly people. Clinically, the probability of success in rescuing MOFE patients was higher than that of adult MOF patients. Such a phenomenon was confirmed by our animal experiments. It means that the characteristic of MOFE is more complex than that of MOF. From Tables 1 and 2, we found that the net increase of ALT and AST during 2, 4, 8 hr intervals were directly proportional to the time of hemorrhagic shock (r = 0.9988 and 0.9997, respectively). It suggests that the changes of ALT and AST levels gradually occur after a
period of time. In contrast, ketone body ratio decreased at 2 hr > 4 hr > 8 hr. Ketone body ratio changed markedly within a short period of time. The change of AcAc/β-OHB within 2 hr was more significant than those of ALT and AST following hemorrhagic shock. We think that ketone body ratio can reflect liver damage and the energy metabolism more rapidly at an early stage.

There were distinct changes in ketone body ratio in the animal models of both MOF induced by hypoperfusion and single organ failure induced by galactosamine. Changes of the arterial ketone body ratio are mainly dependent upon the activity of β-OHB in the liver and the mitochondrial free NAD+/NADH ratio. When the liver is damaged, the impairment of the membrane structure and function of cytosol and mitochondria are unavoidable. The decrease of oxidative phosphorylation attributes to a restricted mitochondrial reoxidation of NADH. The accumulation of NADH in mitochondria depresses the activity of the tricarboxylic acid cycle by the inhibiting the dehydrogenase reaction. Subsequently, the supplement of the energy is seriously deficient. In revealing the mechanism of changes of the arterial ketone body ratio as an indicator of hepatic function deterioration, Satoh et al reported that the overproduction of nitric oxide (NO) radical plays an important role in causing fatal metabolic disorders in patients with postoperative sepsis and leads to a decrease in ketone body ratio and ATP content due to the inhibition of mitochondrial electron transport. Recent findings suggest that apoptosis may contribute to the development of MOF after trauma/hemorrhage. Further insight into the pathogenesis of MOFE would give opportunities for developing clinically useful therapeutical approaches.

References