β-Hydroxybutyrate, a Cerebral Function Improving Agent, Protects Rat Brain Against Ischemic Damage Caused by Permanent and Transient Focal Cerebral Ischemia

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ABSTRACT—In our previous study, β-hydroxybutyrate (BHB) was found to prolong survival time and to inhibit cerebral edema by improving energy metabolism in the hypoxia, anoxia and global cerebral ischemia models. In this study, the cerebroprotective effect of BHB was examined in rats with permanent (p)-occlusion and transient (t)-occlusion of middle cerebral artery (MCA). BHB (30 mg·kg⁻¹·h⁻¹) was continuously administered through the femoral vein. In rats with p-MCA occlusion, BHB significantly reduced infarct area at 24 h after the occlusion, but not at 72 h after the occlusion. In rats with 2-h t-MCA occlusion followed by 22-h reperfusion, BHB significantly reduced cerebral infarct area, edema formation, lipid peroxidation and neurological deficits. Moreover, in the t-MCA occlusion model, delayed administration of BHB started at 1 h after the initiation of the MCA occlusion also significantly reduced cerebral infarct area. Taking together the results obtained in our previous study into account, these results indicate that BHB decreased cerebral edema formation and infarct area by improving of the cerebral energy metabolism during ischemia and by inhibition of lipid peroxidation after reperfusion.

Keywords: β-Hydroxybutyrate, Permanent ischemia, Transient ischemia, Middle cerebral artery occlusion, Cerebroprotection

In the previous study, we have shown β-hydroxybutyrate (BHB), one of the ketone bodies, demonstrated cerebral protective activity in experimental screening models for evaluating ischemic brain damage. BHB (50 mg·kg⁻¹·h⁻¹) prolonged the survival time against N₂ gas-induced hypoxia, KCN-induced anoxia and decapitation-induced complete ischemia (1). BHB at a dose of 30 mg·kg⁻¹·h⁻¹ also inhibited cerebral edema formation, maintained high tissue ATP level, and reduced lactate accumulation without affecting cerebral blood flow in rats subjected to incomplete global cerebral ischemia by bilateral common carotid artery ligation (1). BHB was reported to suppress lactic acidemia and hyperglycemia via alleviation of glycolysis during hemorrhagic shock in rats (2). BHB is converted to acetyl-CoA through pathways other than glycolysis before entering the tricarboxylic acid. These results suggest that preferential utilization of BHB rather than glucose as an energy substrate might reduce the deleterious accumulation of lactate during ischemia.

Cerebral edema and infarction are serious clinical complications in acute human cerebrovascular diseases. Recently, several cerebral ischemic models similar to human cerebrovascular diseases have been developed using rats subjected to middle cerebral artery (MCA) occlusion. Tamura et al. developed the electrocauterization model of MCA occlusion in rats (3), and this model has widely been used to investigate therapeutic approach in permanent cerebral ischemia because of its advantages to produce limited cerebral infarction and to be chronically available. There is another model, the intraluminal suture model in rats, in which a nylon suture is inserted into the common (4) or external (5) carotid artery to reach the origin of the MCA. This model is also widely used and permits reperfusion of the occluded MCA by withdrawing the suture.

The purpose of this study was to evaluate the cerebroprotective effect of BHB in rats with permanent and transient MCA occlusion described above. In all studies, BHB was
examined at an intravenous dose of 30 mg·kg⁻¹·h⁻¹, which was the dose demonstrating the maximum effect in the previous report (1).

MATERIALS AND METHODS

Reagents

BHB was synthesized in the research laboratories of Shimizu Pharmaceutical Co., Ltd. (Shizuoka). All other chemicals and enzymes used in this study were obtained from Wako Pure Chemicals (Osaka) and of reagent grade.

Administration of solutions

BHB was prepared at a concentration of 2.0% as an isotonic solution and intravenously administered to an animal at a rate of 1.5 mL·kg⁻¹·h⁻¹, corresponding to 30 mg·kg⁻¹·h⁻¹ of BHB. Saline was administered as the vehicle control at the same rate.

Animals

Male Wistar rats weighing 280 to 300 g (8-week-old) were obtained from Japan Charles River (Yokohama) were housed in a group of 3 per cage, were given water ad libitum, and fed a commercial diet (MF; Oriental Yeast, Tokyo), and acclimated to standard controlled environment, and fed a commercial diet (MF; Oriental Yeast, Tokyo), and acclimated to standard controlled environment. They were used in experiments following adjustment to these conditions for at least 7 days and were fasted overnight before the experiments, but had free access to water. All animals received humane care in compliance with the Guiding Principles for the Care and Use of Laboratory Animals formulated by The Japanese Pharmacological Society.

General surgical operation technique

The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p). The right femoral artery was catheterized with the polyethylene tube (PE-50) for the measurement of mean arterial blood pressure (MABP) and blood sampling. The left femoral vein was catheterized with the polyethylene tube for continuous administration of BHB or saline. One day after the surgery, the animals were reanesthetized with 3.5% halothane in a mixture of 30% O₂ and 70% N₂O using a face mask followed by 1.5 – 2.0% halothane during the operation. The body temperature was monitored by a rectal probe and maintained at 37.5 ± 0.5°C during the operation with a heating blanket regulated by an animal blanket system (MK-900; Muromachi, Tokyo). Body temperature, MABP and arterial blood gases (pH, PaO₂, PaCO₂) were measured just before and immediately after the induction of ischemia and before sacrifice using an Acid-Base Analyzer (ABL-30; Radiometer, Copenhagen, Denmark).

Permanent ischemia model

Permanent MCA (p-MCA) occlusion was executed using the electrocauterization method developed by Tamura et al. (3) with a minor modification by Matsui et al. (6). Briefly, the skin incision was made in the right temporoparietal region of the head between the eye and ear, and a small craniectomy, 3 to 4 mm in diameter, was drilled at a site superior and lateral to the right foramen ovale to expose the right MCA. Then, the dura was opened by an incision with a 27-gauge needle. The MCA was occluded by a stainless miniclip and electrocauterized at the main trunk of the MCA proximal to the lenticulostriate arteries. The intravenous infusion of BHB started at immediately after the electrocauterization and continued until sacrifice. Twenty-four or seventy-two hours after the p-MCA occlusion, the rats were sacrificed by an overdose of sodium pentobarbital for the measurement of infarct area. Rats were then perfused transcardially with physiological saline and 4% paraformaldehyde (4°C at a pressure of 100 mmHg). Brains were removed and fixed in 4% paraformaldehyde for 24 h, and coronal section (2-mm-thick) was prepared using a rat brain slicer (Muromachi). After standard histological processing and embedding in paraffin, 7-μm-thick sections were prepared and stained with hematoxylin and eosin. The imaging of the coronal sections was taken with a CCD camera (XC-003; Sony, Tokyo), and the infarct area was quantified with an image analyzer (Luxex-FS; Nireco, Tokyo).

Transient ischemia model

Transient MCA (t-MCA) occlusion was conducted as previously described (5). Briefly, a midline incision was made in the neck and the right carotid artery bifurcation was exposed. The branches of the external carotid artery were dissected. The external carotid artery was incised and a nylon suture (0.28 mm in diameter) was inserted via this incision to a distance of 21 mm into the internal carotid artery. In sham-operated rats, a 15-mm-long nylon suture, which could not occlude the MCA for its short length, was inserted into the internal carotid artery. Two hours after the MCA occlusion, rats were reanesthetized with halothane, and reperfusion was performed by withdrawal of the nylon suture. Twenty-two hours after the reperfusion, neurological deficits of each rat were evaluated in a blind fashion. Neurological deficits were evaluated as described by Zea Longa et al. (5): Score 0: no neurological deficit, Score 1: failure to extend left forepaw fully, Score 2: circling to the left, Score 3: falling to the left, and Score 4: do not walk spontaneously and deep coma. Then, rats were sacrificed by an overdose of sodium pentobarbital for the following assessment.
To measure the infarct area, brains were cut at 2-mm-thick through the infarct area using the brain slicer. Coronal sections were stained with 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) in physiological saline at 37 °C for 20 min and then fixed by 10% phosphate-buffered formalin. The imaging of the coronal sections was taken with the CCD camera and the infarct area was quantified with the image analyzer as done for the p-MCA occlusion model.

For measuring cerebral water and sodium contents, the brain was dissected into the cerebral cortex and striatum. Cerebral water content was measured by the dry-wet method and expressed as percentage of the wet brain weight. Sodium content was assayed by the atomic absorption spectroscopy method and expressed as mEq/kg brain.

For the measurement of thiobarbituric acid reaction substance (TBARS) as an index of lipid peroxide, the brain tissues were dissected into cerebral cortex and striatum. Each 0.1 mL of homogenized specimen was mixed with 0.2 mL of 8.1% sodium, 0.2 mL of 0.7% 2-thiobarbituric acid and 1.8 mL of acetic acid. After boiling for 60 min, the reaction mixture was immediately cooled in ice. TBARS was extracted and measured fluorometrically (Ex, 515 nm; Em, 553 nm). TBARS was estimated as a malondialdehyde (MDA) formation and tissue MDA concentrations were expressed as nmol/mg protein. Protein content was measured by the Bradford method using chicken egg albumin as the standard.

**Statistical analyses**

All data were expressed as the mean ± S.E.M. Statistical analysis was performed with the unpaired Student’s t-test and Dunnett’s multiple test for comparison between two

### Table 1. Physiological parameters in rats with p-MCA occlusion

<table>
<thead>
<tr>
<th></th>
<th>Before MCA occlusion</th>
<th>After MCA occlusion</th>
<th>24 h after MCA occlusion</th>
<th>72 h after MCA occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>12</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>Saline</td>
<td>109.2 ± 14.4</td>
<td>104.3 ± 8.9</td>
<td>109.8 ± 14.7</td>
</tr>
<tr>
<td></td>
<td>BHB</td>
<td>115.8 ± 15.1</td>
<td>107.1 ± 10.1</td>
<td>112.7 ± 10.9</td>
</tr>
<tr>
<td>pH</td>
<td>Saline</td>
<td>7.45 ± 0.07</td>
<td>7.45 ± 0.06</td>
<td>7.46 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>BHB</td>
<td>7.41 ± 0.03</td>
<td>7.51 ± 0.04</td>
<td>7.49 ± 0.07</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>Saline</td>
<td>36.0 ± 3.2</td>
<td>35.1 ± 2.9</td>
<td>29.2 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>BHB</td>
<td>37.1 ± 4.1</td>
<td>35.4 ± 5.5</td>
<td>33.7 ± 4.6</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>Saline</td>
<td>113.8 ± 10.4</td>
<td>102.5 ± 14.8</td>
<td>95.4 ± 8.8</td>
</tr>
<tr>
<td></td>
<td>BHB</td>
<td>123.1 ± 5.5</td>
<td>104.9 ± 10.0</td>
<td>91.0 ± 10.1</td>
</tr>
<tr>
<td>RT (°C)</td>
<td>Saline</td>
<td>38.1 ± 0.8</td>
<td>36.8 ± 0.1</td>
<td>37.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>BHB</td>
<td>38.6 ± 0.4</td>
<td>36.4 ± 0.2</td>
<td>37.1 ± 0.2</td>
</tr>
</tbody>
</table>

MBP: mean arterial blood pressure, RT: rectal temperature.

### Table 2. Physiological parameters in rats with t-MCA occlusion

<table>
<thead>
<tr>
<th></th>
<th>Before MCA occlusion</th>
<th>After MCA occlusion</th>
<th>2 h after MCA occlusion</th>
<th>22 h after Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>Saline</td>
<td>117.6 ± 13.8</td>
<td>110.6 ± 3.9</td>
<td>111.6 ± 7.7</td>
</tr>
<tr>
<td></td>
<td>BHB</td>
<td>116.0 ± 7.9</td>
<td>117.5 ± 7.2</td>
<td>111.5 ± 5.4</td>
</tr>
<tr>
<td>pH</td>
<td>Saline</td>
<td>7.43 ± 0.03</td>
<td>7.42 ± 0.02</td>
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<tr>
<td></td>
<td>BHB</td>
<td>7.47 ± 0.01</td>
<td>7.44 ± 0.02</td>
<td>7.47 ± 0.01</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>Saline</td>
<td>35.6 ± 3.5</td>
<td>37.0 ± 5.1</td>
<td>35.6 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>BHB</td>
<td>36.1 ± 2.4</td>
<td>37.9 ± 3.4</td>
<td>36.1 ± 2.4</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>Saline</td>
<td>97.2 ± 8.0</td>
<td>106.6 ± 14.0</td>
<td>97.2 ± 8.0</td>
</tr>
<tr>
<td></td>
<td>BHB</td>
<td>91.7 ± 3.6</td>
<td>97.3 ± 10.8</td>
<td>91.7 ± 3.6</td>
</tr>
<tr>
<td>RT (°C)</td>
<td>Saline</td>
<td>38.2 ± 0.1</td>
<td>37.3 ± 0.1</td>
<td>39.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>BHB</td>
<td>38.1 ± 0.1</td>
<td>37.4 ± 0.1</td>
<td>39.4 ± 0.2</td>
</tr>
</tbody>
</table>

MBP: mean arterial blood pressure, RT: rectal temperature.
RESULTS

Physiological parameters

Physiological parameters in rats with p-MCA or t-MCA occlusion are summarized in Tables 1 and 2. In all rats treated with BHB or saline, abnormal behaviors, depression of respiration and hypothermia were not observed. All parameters were not statistically different between saline and BHB-treated rats.

p-MCA occlusion

Continuous intravenous infusion of BHB started immediately after the p-MCA occlusion. The infarct area at 24 h after the p-MCA occlusion was significantly \( P < 0.05 \) reduced by BHB treatment in all coronal sections (Fig. 1). The infarct area in sections at 1.7, −0.3, −2.3 and −4.3 mm of distance from the bregma were 20.19 ± 0.84%, 21.50 ± 0.77%, 14.11 ± 0.86% and 5.12 ± 1.0% for saline-treated rats, and 15.68 ± 0.89%, 15.96 ± 1.42%, 11.19 ± 0.56% and 2.12 ± 0.39% for BHB-treated rats, respectively, showing statistical significance \( P < 0.05 \). Seventy-two hours after the p-MCA occlusion, however, BHB no more exhibited reduction in infarct area, although extensive infarct areas were obtained in saline-treated rats in comparison to that at 24 h after the p-MCA occlusion (Fig. 2).

The infarct areas in the 4 coronal sections were 27.40 ± 2.80%, 23.67 ± 3.63%, 19.99 ± 1.56% and 12.32 ± 3.52% for saline-treated rats, and 25.32 ± 2.83%, 23.25 ± 4.59%, 18.34 ± 2.67% and 11.10 ± 1.65% for BHB-treated rats, respectively.

t-MCA occlusion

Continuous intravenous infusion of BHB started immediately after the initiation of the MCA occlusion. The infarct area was determined at 22 h after the reperfusion, and a typical photograph of the TTC-stained coronal section at −0.3 mm distance from the bregma is shown in Fig. 3. The right cerebral cortex and the striatum were not stained with TTC, demonstrating damaged areas (infarction). The infarct area was significantly \( P < 0.01 \) reduced by BHB.

Fig. 1. Effect of BHB on cerebral infarct area after the p-MCA occlusion in rats. Infusion of saline or BHB (30 mg·kg\(^{-1}\)·h\(^{-1}\)) was started immediately after MCA occlusion. Brains were obtained at 24 h after p-MCA occlusion. Cerebral infarct area was determined by staining with hematoxylin and eosin. The data are indicated as the mean ± S.E.M. and significant differences from saline-treated are marked *\( P < 0.05 \). The number in parentheses shows the number of rats used.

Fig. 2. Effect of BHB on cerebral infarct area after the p-MCA occlusion in rats. Infusion of saline or BHB (30 mg·kg\(^{-1}\)·h\(^{-1}\)) was started immediately after MCA occlusion. Brains were obtained at 72 h after the p-MCA occlusion. Cerebral infarct area was determined by staining with hematoxylin and eosin. The data are indicated as the mean ± S.E.M. The number in parentheses shows the number of rats used.

Fig. 3. Typical photographs of infarct area show TTC-stained coronal sections at the −0.3 mm distance from bregma after the t-MCA occlusion. A; Saline- or B; 30 mg·kg\(^{-1}\)·h\(^{-1}\) BHB-treatment in rats with t-MCA occlusion. Scale bar = 10 mm.
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The infarct area in sections at 1.7, 0.3, 2.3 and 4.3 mm of distance from the bregma were 40.68 ± 2.48%, 37.60 ± 4.93%, 37.61 ± 2.56% and 30.68 ± 3.23% for saline-treated rats, and 29.57 ± 2.72%, 23.38 ± 2.74%, 20.78 ± 3.87% and 19.70 ± 3.62% for BHB-treated rats, respectively, showing statistical significance (P < 0.05 or P < 0.01). In the present t-MCA occlusion model, the BHB significantly (P < 0.05) improved the neurological deficit when compared to saline (Fig. 5). The mean scores in the neurological deficit test were 3.5 ± 0.3 and 2.0 ± 0.5 treatment for saline- and BHB-treated rats, respectively. Furthermore, 1-h delayed administration of BHB after the initiation of the MCA occlusion also significantly reduced infarct area as compared to saline-treated rats (Fig. 6). The infarct area of each region for BHB-treated rats as 28.6 ± 2.8% (P < 0.01), 30.9 ± 2.0% (P < 0.05), 24.2 ± 1.2% (P < 0.01), and 17.3 ± 1.4%, respectively.

Cerebral water contents at 22 h after the reperfusion are shown in Fig. 7. In sham-operated rats, cerebral water contents in the cortex and the striatum were 79.94 ± 0.60% and 78.72 ± 0.57%, respectively. With t-MCA occlusion, cerebral water contents of saline-treated rats rose to 86.98 ± 0.40% and 84.93 ± 0.85% in the cortex and striatum, respectively, being significantly (P < 0.05) higher than those of sham-operated rats. BHB showed significant (P < 0.05) suppression of increased cerebral water contents with values of 85.10 ± 0.40% (cortex) and 82.34 ± 0.87% (striatum). As shown in Fig. 8, sodium contents of saline-treated rats at 22 h after the reperfusion were 407.67 ± 6.56 mEq/kg (cortex) and 647.23 ± 61.82 mEq/kg (striatum), being

Fig. 4. Effects of BHB on cerebral infarct area after the t-MCA occlusion in rats. Infusion of saline or BHB (30 mg·kg⁻¹·h⁻¹) was started immediately after the MCA occlusion. Coronal sections were obtained at 22 h after the reperfusion of 2-h MCA occlusion. The data are indicated as the mean ± S.E.M. and significant differences from saline-treated are marked *P < 0.05, **P < 0.01. The number in parentheses shows the number of rats used.

Fig. 5. Ameliorative effect of BHB on neurological deficits as evidenced by posture reflex score after the t-MCA occlusion in rats. Infusion of saline or BHB (30 mg·kg⁻¹·h⁻¹) was started immediately after the MCA occlusion. Posture reflex score was measured in a blind fashion at 22 h after 2-h MCA occlusion and the scores were evaluated according to the following criteria: Score 0: no neurological deficit, Score 1: failure to extend the left forepaw fully, Score 2: circling to the left, Score 3: falling to the left, and Score 4: no spontaneous walking. The data are indicated as the mean ± S.E.M. and significant differences from saline-treated are marked *P < 0.05. The number in parentheses shows the number of rats used.

Fig. 6. Ameliorative effect of delayed administration of BHB on cerebral infarct area after the t-MCA occlusion in rats. Infusion of saline or BHB (30 mg·kg⁻¹·h⁻¹) was started at 1 h after the initiation of the MCA occlusion. Brains were obtained at 22 h after the reperfusion of 2-h MCA occlusion. Cerebral infarct area was determined by staining with TTC. The data are indicated as the mean ± S.E.M. and significant differences from saline-treated are marked *P < 0.05, **P < 0.01. The number in parentheses shows the number of rats used.
Protective Effect of BHB on Brain Damage

significantly higher ($P<0.01$) than those of sham-operated rats (cortex: 344.53 ± 16.63 mEq/kg, striatum: 326.23 ± 14.09 mEq/kg). BHB significantly ($P<0.01$) decreased sodium content elevated in the striatum region to 441.09 ± 30.41 mEq/kg, but failed in the cortex region (384.91 ± 10.37 mEq/kg).

MDA concentration in the cerebral cortex and the striatum at 22 h after the reperfusion was shown in Fig. 9. MDA concentrations were 6.00 ± 1.41 nmol/mg protein (cortex) and 4.58 ± 0.91 nmol/mg protein (striatum) for saline-treated rats and 3.53 ± 0.41 nmol/mg protein (cortex) and 2.70 ± 0.32 nmol/mg protein for BHB-treated rats. BHB showed an apparent tendency to reduce MDA concentration, but not statistically significantly compared to saline.

DISCUSSION

Our previous study have revealed that BHB exhibited protective effects on the brain under several abnormal conditions such as hypoxia induced by exposure to N2 gas and anoxia induced by the injection of KCN in experimental animals (1). BHB also inhibited cerebral edema formation and maintained high cerebral tissue ATP levels in rats subjected to incomplete cerebral ischemia by bilateral carotid artery ligation (BLCL) (1). These beneficial actions of BHB are thought to be attributable to its preferential use as an energy substrate rather than glucose, which is well known to aggravate ischemic brain damage under such conditions.

In the present study, BHB was further examined in experimental models of cerebral ischemia in which permanent and transient cerebral ischemia similar to human diseases are realized in limited area of the brain irrigated by the MCA. These two different states of blood irrigation in
the ischemic brain, complete ischemia and reperfusion, are also concomitantly or independently observed in stroke patients (7). This, in turn, indicates that any kinds of drug intervention should be evaluated in each experimental animal model, which can possibly realize such states. BHB given by intravenous infusion proved effective in the p-MCA occlusion model, in which the protective effect of BHB can be explained by substituting for glucose as an energy substrate, as described in the previous paper (1). However, administration of BHB at 72 h after the p-MCA occlusion was no longer effective on the infarct area. It has been reported that under the condition of permanent ischemia, the threshold of the disturbance of energy metabolism gradually increases dependent on the duration of the blood flow reduction, resulting in enlargement of cerebral infarct area (8, 9). We have previously reported that BHB did not influence the regional cerebral blood flow in a BLCL model (1). Therefore, it seems that only improving the energy metabolism can not reduce the infarct area if the brain is exposed to ischemia for a long period. On the other hand, in rats with t-MCA occlusion, cerebral infarction at 22 h after reperfusion of 2-h MCA occlusion was reduced by BHB treatment as well as inhibiting cerebral edema formation and neurological deficits. The protective effect by BHB was still observed even when it was administered at 1 h after the initiation of the MCA occlusion. The protective effect of BHB in t-MCA occlusion would be partly due to alleviation of oxygen radical production after reperfusion, as shown by the tendency to reduce MDA production, in addition to protecting brain during cerebral ischemia, in which BHB retained cerebral ATP levels after BLCL (1). Since rectal temperature and other physiological parameters were not affected by BHB in both t-MCA and p-MCA occlusion models, the protective effect of BHB is thought to be direct, rather than operating through nonspecific effects such as hypothermia.

As for other drugs effective in both ischemic states, allopurinol, a xanthine oxidase inhibitor, demonstrated its protective effect on ischemic brain in both p-MCA (10) and t-MCA (11) occlusion models. This implies that some mechanism to generate oxygen radicals still works and aggravates ischemic damage in permanent ischemia caused by occluding cerebral arteries. Allopurinol reduced infarct volume of cortical tissue more effectively than caudate tissue (11), suggesting that a collateral blood supply existed in the cortical area where the branches of MCA have extensive anastomoses with distal branches of the anterior and posterior cerebral arteries (12). During the period of ischemia, the depletion of tissue ATP due to the restriction of its production results in an elevated concentration of AMP. The AMP is catabolized to adenosine, inosine, and finally to hypoxanthine, which is then oxidized by xanthine oxidase to generate a superoxide anion (13). Allopurinol inhibits xanthine oxidase and further diminishes oxygen radical produced via this pathway, finally leading to alleviation of ischemic damage. In addition to ATP-preservative effects of BHB during ischemic episode, BHB also demonstrated the tendency to decrease oxygen radical formation as evidenced by reducing MDA. These indicate that a part of protective effects of BHB on ischemic brain in p-MCA occlusion and BLCL models would be in consequence of decreasing oxygen radical production. The α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)-receptor antagonists, YM872 (14, 15) and YM90K (16), have also been reportedly known to reduce infarct size after the MCA occlusion in rats with and without reperfusion, but the effect of BHB on AMPA receptors and on excitatory amino acid neurons is unknown.

BHB is metabolized to acetyl-CoA before entering the tricarboxylic acid (TCA) cycle through pathways other than glycolysis (17). Glucose as a primary energy substrate of brain is firstly metabolized to pyruvate through the glycolytic pathway under the normal condition and then enters the TCA cycle. However, the activated anaerobic glycolytic pathway during ischemia induces brain damage as a consequence of lactate accumulation in cerebral tissues (18, 19). Our previous study indicated that ATP reduction and deleterious lactate accumulation in brain tissues under BLCL were attenuated by BHB (1). Moreover, inhibition of lactate production by BHB may lead to 1) an increase in acetyl-CoA, resulting in inhibiting pyruvate oxidation through the feedback inhibition of the pyruvate dehydrogenase complex (20 – 22), and to 2) a reduction in glycolytic rate by inhibiting phosphofructokinase activity (23).

Taken together, the present study shows that BHB decreased cerebral edema and infarct area mainly through improving the energy metabolism during the ischemia and partly through inhibiting lipid peroxidation after the reperfusion. However, the protective effect by BHB was not observed at 72 h after the p-MCA occlusion, thereby suggesting that BHB would have potential value for prolonging the therapeutic time window in the early phase of cerebral ischemia. Although nutritional support for the patients with acute cerebrovascular diseases is a complex problem, it may adversely affect cerebral ischemia. These data indicate that preferential metabolism of BHB rather than glucose as energy substrate may reduce the deleterious accumulation of lactate during ischemia and support the potential clinical utility of BHB as nutritional support in treating acute cerebrovascular diseases of humans.

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