EFFECT OF A NEW HYPEROSMOTIC AGENT, NIK-242 INJECTION, ON BRAIN WATER CONTENT, METABOLITES AND CEREBRAL BLOOD FLOW IN CEREBRAL ISCHEMIA IN THE SPONTANEOUSLY HYPERTENSIVE RAT

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We examined the effects of a new hyperosmotic agent (NIK-242inj.) on brain edema, energy metabolites and regional cerebral blood flow (r-CBF) during acute cerebral ischemia. Cerebral ischemia was induced by bilateral common carotid artery ligation (BLCL) using spontaneously hypertensive rats (SHR). The experimental animals were divided into 4 groups, A:20% NIK-242inj., B:20% mannitol, C:10% glycerol in 5% fructose, D:normal saline. All the animals were administered the agent or saline intravenously beginning at 1h after BLCL and continuing for 2h for a total dose of 6.8 ml/kg body weight. Brain water content and metabolites (ATP, lactate, pyruvate) were determined 3h after BLCL. Regional cerebral blood flow (r-CBF) in thalamus was also measured by the hydrogen clearance technique. The brain water content in the NIK-242inj. group was significantly lower than that of saline group. The concentration of brain ATP in the NIK-242inj. group remained higher than those of saline group. Accumulation of lactate in the NIK-242inj. group was less than in the mannitol and saline groups. The lactate/pyruvate ratio of the NIK-242inj. group was significantly lower than that of saline and mannitol groups. At 3h after BLCL, the reduction of r-CBF in the NIK-242inj. group was smaller than that of saline group. The present study suggests that NIK-242inj. as well as glycerol could ameliorate brain edema, disruption of brain energy metabolism and reduction of r-CBF in acutely induced cerebral ischemia.

Brain edema is one of the most serious complications in cerebral infarction! Therefore, it is very important to reduce brain edema in stroke patients. In recent years hyperosmotic solutions have been extensively tested as treatments for increased intracranial pressure and brain edema. Mannitol and glycerol, which are water soluble alcohols, have been used for that purpose. The hyperosmotic agent, NIK-242inj. (1, 2, 3, 4-butaneetetrol), which we used in this experiment, is a new tetravalent alcohol (Fig. 1).

The present study was designed to evaluate the effect of NIK-242inj. on brain edema, energy metabolites and regional cerebral blood flow in ischemia. Furthermore, we made a comparison between NIK-242inj. and other 2 hyperosmotic solutions (mannitol and glycerol) using the animal model of bilateral common carotid artery ligation in spontaneously hypertensive rats.

Key words:
Hyperosmotic agent
Cerebral ischemia
Brain edema
Brain metabolism
Cerebral blood flow

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1246 Japanese Circulation Journal Vol. 55, December 1991
NIK-242 (Erythritol)

\[
\text{CH}_2\text{OH} \\
| \\
\text{H—C—OH} \\
| \\
\text{H—C—OH} \\
| \\
\text{CH}_2\text{OH}
\]

MW : 122.12

Fig.1 Chemical structure of NIK-242 inj.

MATERIALS AND METHODS

Preparation of animals
The experiments were performed on 16 week old male stroke-resistant spontaneously hypertensive rats (SHR) weighing about 300 g. The animals were anesthetized with 1.5% halothane in O\textsubscript{2}. One femoral artery was cannulated for blood pressure recording and for blood gas analysis. One femoral vein was also cannulated for fluid infusion. Both common carotid arteries were exposed and separated from the vagosympathetic trunks. After discontinuation of halothane, brain ischemia was induced by bilateral common carotid artery ligation (BLCL).

Administration of solutions
The experimental animals were divided into 4 groups. Group A was administered a solution of 20% NIK-242 inj.; Group B, 20% mannitol; Group C, 10% glyceral in 5% fructose; Group D, the same volume of saline. An infusion pump was used to administer the agent or saline intravenously beginning at 1h after BLCL and continuing for 2h for a total dose of 6.8 ml/kg body weight. Each group included 7 animals.

Brain water content and metabolites
Three hours after BLCL, rats were sacrificed by microwave radiation (Toshiba Microwave Applicator, 5.0 kw output for 1.5 sec) to simultaneously inactive enzymes. Immediately after irradiation, the brain was removed and separated grossly into frontal and parietal portions and each portion was weighed immediately. The frontal portion was used for determination of water content. The brain was calculated from the wet and dry weights of the brain.

The parietal portion of the brain was used for determination of ATP, lactate and pyruvate. ATP was analyzed with the luciferin-luciferase reaction and bioluminescence was monitored on the ATP photometer (SAI Technology, Model 2000). Lactate and pyruvate were determined with an enzymatic method.

Regional cerebral blood flow
Regional cerebral blood flow (r-CBF) was measured using a hydrogen clearance method. Teflon coated platinum electrode was placed in thalamus through small burr holes which were made in the skull 2 mm lateral and 2.5 mm posterior to the bregma on the each side. The reference electrode was inserted under the skin. Rats were anesthetized with 1% halothane, and tracheotomy was performed. The femoral artery and vein were cannulated for monitoring arterial blood pressure, for periodic arterial blood gas analysis, and for the administration of osmotic agent. After the above procedures, halothane was discontinued. Thereafter, artificial ventilation was initiated with a gas mixture of 70% N\textsubscript{2}O and 30% O\textsubscript{2} following the administration of 1 mg/kg suxamethonium chloride (I.V.), and PaCO\textsubscript{2} and PaO\textsubscript{2} were maintained between 30–35 mmHg and 100–120 mmHg, respectively, by adjusting the respiratory rate. About 10% of hydrogen gas was given directly into the endotracheal tube for 5 min. r-CBF was measured periodically before and after BLCL until 4h of ischemia. r-CBF values were calculated as described previously.

Through the above procedures, the rectal temperature was kept at 37.5±0.5 °C using a heating blanket.

We calculated mean and standard deviation (S.D.) for all data. For between-group comparison, we used Scheffe’s multiple comparison procedure. For within-group comparisons of r-CBF values, we used repeated-measure analysis of variance (ANOVA) and Dunnett’s test. We considered p<0.05 to be significant.
<table>
<thead>
<tr>
<th>Variables</th>
<th>NIK-242 inj.</th>
<th>Mannitol</th>
<th>Glycerol</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MABP (mmHg)</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Before</td>
<td>178 ± 11.7</td>
<td>177 ± 11.6</td>
<td>180 ± 13.6</td>
<td>184 ± 14.8</td>
</tr>
<tr>
<td>After</td>
<td>165 ± 7.7</td>
<td>155 ± 17.3</td>
<td>167 ± 15.0</td>
<td>171 ± 10.6</td>
</tr>
<tr>
<td><strong>PH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>7.33 ± 0.11</td>
<td>7.37 ± 0.12</td>
<td>7.31 ± 0.11</td>
<td>7.39 ± 0.19</td>
</tr>
<tr>
<td>After</td>
<td>7.41 ± 0.06</td>
<td>7.42 ± 0.06</td>
<td>7.42 ± 0.07</td>
<td>7.44 ± 0.20</td>
</tr>
<tr>
<td><strong>PaCO2 (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>35.7 ± 6.4</td>
<td>37.2 ± 5.0</td>
<td>35.6 ± 3.0</td>
<td>33.3 ± 3.9</td>
</tr>
<tr>
<td>After</td>
<td>22.7 ± 5.4</td>
<td>23.5 ± 0.56</td>
<td>24.9 ± 5.8</td>
<td>25.0 ± 6.4</td>
</tr>
<tr>
<td><strong>PaO2 (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>105.6 ± 9.5</td>
<td>104.5 ± 10.3</td>
<td>101.1 ± 6.8</td>
<td>102.9 ± 8.6</td>
</tr>
<tr>
<td>After</td>
<td>114.4 ± 8.4</td>
<td>127.5 ± 6.1</td>
<td>124.0 ± 8.0</td>
<td>122.6 ± 7.6</td>
</tr>
</tbody>
</table>

*Data are mean ± S.D. MABP: mean arterial blood pressure*

Physiological data in each group are presented in Table I. After occlusion, the rats in all groups developed mild respiratory alkalosis and blood pressure depression. However, there were no significant differences in the levels of blood pressure and the values of blood gas analysis (PaCO2, PaO2, PH) among groups.

2. Changes in brain water content

As shown in Fig. 2, the brain water content 3h after BLCL was 77.12 ± 0.54% (mean ± S.D.) in the NIK-242 inj., 77.69 ± 0.51% in the mannitol-treated group, 77.51 ± 0.25% in the glycerol-treated group, and 78.46 ± 0.86% in the saline group. The brain water contents of NIK-242 inj., mannitol and glycerol administration group were statistically lower than that of saline group. The decrease in brain water content in the NIK-242 inj. -treated group did not attain a significant difference from either the mannitol or glycerol administration groups.

3. Changes in brain metabolites

Brain levels of energy metabolites are summarized in Table II. ATP was 1.47 ± 0.31 μmols/g (mean ± S.D.) in NIK-242 inj. The concentration of ATP in NIK-242 inj. group was maintained at higher levels compared to the saline group. However, there was no significant difference in ATP concentration between the NIK-242 inj., mannitol and glycerol administration groups. Lactate levels were 7.56 ± 3.75 μmols/g.

**RESULTS**

1. Physiological parameters

![Graph showing brain water content (%)](image)

*Fig. 2. Effect of NIK-242 inj., mannitol, glycerol and saline on brain water content 3h after ischemia. Decrease of increased brain water content was demonstrated in the NIK-242 inj. administered group as well as these administered mannitol and glycerol. Bar graphs of mean ± S.D. *p < 0.05 by Scheffe's multiple-comparison procedure.

*Japanese Circulation Journal Vol. 55, December 1991*
TABLE II  BRAIN ENERGY METABOLITES AT 3 HOURS AFTER ISCHEMIA

<table>
<thead>
<tr>
<th></th>
<th>ATP</th>
<th>lactate</th>
<th>pyruvate</th>
<th>L/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated control</td>
<td>2.64±0.11</td>
<td>1.38±0.28</td>
<td>0.36±0.05</td>
<td>3.84±0.43</td>
</tr>
<tr>
<td>Saline</td>
<td>0.87±0.45</td>
<td>15.29±13.47</td>
<td>0.24±0.05</td>
<td>62.46±53.65</td>
</tr>
<tr>
<td>NIK-242inj.</td>
<td>1.47±0.31*</td>
<td>7.56±3.75*</td>
<td>0.41±0.14*</td>
<td>17.39±9.12*</td>
</tr>
<tr>
<td>Mannitol</td>
<td>1.11±0.28*</td>
<td>16.42±7.19**</td>
<td>0.43±0.10**</td>
<td>40.8±24.02**</td>
</tr>
<tr>
<td>Glycerol</td>
<td>1.24±0.29*</td>
<td>6.19±2.92*</td>
<td>0.30±0.12*</td>
<td>21.64±9.48*</td>
</tr>
</tbody>
</table>

Values of ATP, lactate and pyruvate are indicated as µmol/s/g brain. Data are mean±S.D
*p<0.05 different from saline group.
**p<0.05 different from NIK-242inj. group by Scheffe's multiple-comparison procedure.

Fig.3. Change of r-CBF after BLCL. Note that the reduction of r-CBF at 3 and 4h was suppressed significantly in both NIK-242inj. and glycerol administration group. The values shows mean±S.D. in each group.
*p<0.05 different from saline group by Dunnett's multiple comparison test followed by repeated-measures analysis of variance (ANOVA).

4. Regional cerebral blood flow (r-CBF)
Blood pressure, PaO2, PaCO2 and arterial PH were kept within normal physiologic limits in all rats before and after ischemia.

r-CBF in the thalamus showed an initial large reduction following BLCL. The values of r-CBF immediately after BLCL were 28.46±6.32 ml/100g/min (mean±S.D.) in the saline group. There were no statistical differences in r-CBF between the groups before treatment. The values at 2h of ischemia were about 30% of the resting state in all groups (Fig. 3). At 3h after BLCL, the values for r-CBF were 20.43±7.66 ml/100g/min (mean±S.D.) in the NIK-242inj., 13.70±9.01 ml/100g/min in the mannitol, 20.86±4.83 ml/100g/min in the glycerol and 11.76±4.71 ml/100g/min in the saline treated group. NIK-242inj. and the glycerol-treated animals maintained the r-CBF at significantly higher levels compared to saline group. At 4h after ischemia, the reduction of r-CBF was significantly smaller in the groups of NIK-242inj. and glycerol. On the other hand, mannitol administration had no effect on the decrease of r-CBF during the period of the experiment.
DISCUSSION

Brain edema is defined as a remarkable increase of water content in brain tissue. The progression of brain edema should cause impediment of cerebral circulation and energy failure, followed by increased intracranial pressure. Furthermore, edema causes increased ischemia which in turn aggravates the edema. Since Weed et al. reported that hyperosmolar solutions have an effect on brain edema, these solutions have been widely used. In the past, hyperosmotic glucose and urea solutions have been used as a hypertonic solution; however, they caused deleterious side effects such as hyperglycemia or rebound phenomenon. In recent years, therefore, instead of those agents, mannitol and glycerol, which are water soluble alcohols have been used.

NIK-242inj. is a water soluble tetravalent alcohol normally present in foods such as mushroom. NIK-242inj. does not influence glucose or lipid metabolism because it is excreted to urine while glycerol can enter into the glycolytic pathway. The dose of NIK-242inj. used for this experiment was chosen according to the volume of glycerol and mannitol used in patients with clinical brain edema.

In this study, SHR were employed for the induction of cerebral ischemia. Three hrs after occlusion of the bilateral common carotid arteries, brain edema, metabolic alteration and reduction of r-CBF have been described in SHR.

In the present experiments, administration of NIK-242inj., mannitol, glycerol or saline had no effect on the physiological parameters, such as blood pressure, pH, PaCO₂ and PaO₂.

First in this study, the effects on ischemic brain edema of 3 different kinds of hyperosmotic agents were tested. NIK-242inj. had almost same effect on ischemic brain edema as did glycerol and mannitol. The dehydrating effect of hyperosmotic agents is generally attributed to the establishment of an osmotic gradient between the blood and cerebrospinal fluid or the brain tissue, with a resultant net removal of water from the brain tissue.

It has been reported that free radicals play a role in the development of ischemic brain edema. It is known that mannitol and glycerol have free radical scavenging actions. It is believed that the amelioration of brain edema is partly attributed to this activity in addition to the dehydrating effect. It is suggested that NIK-242inj. may also have free radical scavenging actions, thereby reducing brain edema. Moreover, mannitol and glycerol are known to inhibit the production of cerebrospinal fluid. NIK-242inj. may have also this action as it belongs to the same group of water-soluble alcohols.

Concerning brain energy metabolites levels of ATP were higher, and these of lactate and the L/P ratio were lower in the NIK-242inj. group than in the saline group. Thus, NIK-242inj. ameliorated the disruption of brain energy metabolism in ischemia. Glycerol also brought about a similar amelioration of the ischemic change of ATP, lactate and L/P ratio. NIK-242inj. may ameliorate the disruption of brain energy metabolism after ischemia due to improvement of cerebral microcirculation and suppression of anaerobic glucose metabolism, this reducing brain edema.

The levels of r-CBF at 3 and 4h after ischemia were higher in the NIK-242inj. and glycerol groups than in the saline group. However, in this experiment, we could not detect a beneficial effect of mannitol on r-CBF in ischemia, as has been previously reported.

Numerous previous experimental studies have reported that hypertonic solutions improve the cerebral circulation in ischemia but the mechanism is not well understood. It has been suggested that hypertonic solutions achieve beneficial effects through not only direct lowering of intracranial pressure due to reduction of brain edema, but also by hemorrheological actions such as enhancement of PG₁₂, increased red blood cell deformability and lowering of red blood cell agglutination. Those hemorrheological actions in NIK-242inj. have not yet been described, but it is possible that NIK-242inj. ameliorates disruption of cerebral blood flow and metabolism following ischemia through such actions.

In conclusion, NIK-242inj. may have beneficial effects on brain edema and disruption of cerebral blood flow and metabolism in ischemia. NIK-242inj. administration had
almost same effect on ischemic alteration as glycerol in the dose used for these experiments.

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Japanese Circulation Journal Vol.55, December 1991