Influence of Monosialoganglioside Inner Ester on Neurologic Recovery After Global Cerebral Ischemia in Monkeys

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We assessed the consequences of transitory global cerebral ischemia and the influence of monosialoganglioside inner ester (AGF 2) treatment on neurologic outcome, cerebral blood flow, and cerebral metabolic rate in monkeys over 48 hours. Global cerebral ischemia was produced by a cervical tourniquet and a lowering of blood pressure to 6.65 kPa; recirculation followed after 30 minutes. AGF 2 (30 mg/kg) was administered intravenously immediately after initiation of recirculation and intramuscularly twice a day for 48 hours. Our results show that treatment with AGF 2 significantly accelerated the rate of neurologic recovery. Improvement was evident 5 hours after ischemia; full neurologic recovery was observed in half of the monkeys 48 hours after ischemia. This recovery was associated with a less severe reduction in cerebral blood flow without a concomitant increase in the cerebral metabolic rate. (Stroke 1989; 20:652-656)

Gangliosides are sialic acid–containing glycosphingolipids with particular abundance in the outer membrane surface of neuronal cells. Although their biologic function is still largely obscure, considerable evidence now indicates that systemically administered gangliosides are effective in improving the outcome following neuronal injury to adult rodents. In the initial work of Ceccarelli et al., two mixtures of bovine brain gangliosides enhanced recovery of the denervated cat nictitating membrane, an effect most probably associated with an enhanced rate of axonal regrowth. Subsequently, the monosialoganglioside GM 1 (nomenclature according to Svennerholm) alone was found to affect recovery following injury to the central nervous system (CNS). For example, following partial unilateral hemitransection of the nigrostriatal pathway, chronically administered GM 1 facilitated the recovery of dopaminergic synaptic function in the lesioned striatum. This effect was associated with both enhanced survival of nigral dopaminergic cell bodies and with the associated reduction of the imbalance in energy metabolism and blood flow between the striata of the lesioned and unlesioned sides.

GM 1 effects have also been detected during the acute phase following brain insults, including cerebral ischemia. GM 1 treatment has been shown to have beneficial effects following both transitory middle cerebral artery occlusion in cats and permanent unilateral ligation of the common carotid artery in gerbils.

We describe the effects of AGF 2, the inner ester derivative of GM 1, following transitory global cerebral ischemia in monkeys. We used AGF 2 because of its reportedly greater ability to penetrate the blood–brain barrier.

Materials and Methods

We used 27 cynomolgus monkeys (Macaca fascicularis) of either sex weighing 3–6 kg. Anesthesia was induced with 3 mg/kg i.m. ketamine hydrochloride followed by an intravenous perfusion of 25 mg/kg chloralose. The monkeys were intubated and then ventilated (Delhomme, Bird Mark 8, Paris, France) so as to maintain Paco₂ at 5.19–5.45 kPa. A collateral vessel of the left femoral artery was cannulated and connected to a pressure transducer (Statham P23DC, Cleveland, Ohio) to monitor mean arterial blood pressure (MABP). Deep body temperature was kept at 37.5°C by means of a heating lamp. PaO₂, PaCO₂, arterial pH, hemoglobin, and hematocrit were evaluated at selected intervals.

Transitory global forebrain ischemia was induced by lowering MABP to 6.65 kPa with intravenous infusion of trimethaphan camsylate and inflation of
The left jugular vein as well as the femoral artery recording. The monkeys were immobilized with were catheterized for blood sampling and MABP after ischemia by scoring the level of consciousness, basal functions, and reflexes according to a rating scale (Table 1). Monkeys that died were assigned an arbitrary score of 40, which was added to their scores for respiration and reflexes.

A cervical tourniquet up to 120 kPa. Successful induction of ischemia was judged by the occurrence of a flat electroencephalographic tracing, which was continuously monitored during this time. The ischemia that occurred within seconds after inflating the tourniquet was maintained 30 minutes, followed by tourniquet deflation to allow recirculation. One minute before deflation, MABP was normalized to 10.64 kPa using 2 µg/ml i.v. norepinephrine.

After recirculation was begun, anesthesia was discontinued and the monkeys were allowed to breathe spontaneously. Their neurologic status was evaluated by a blinded observer 5, 24, and 48 hours after ischemia by scoring the level of consciousness, basal functions, and reflexes according to a rating scale (Table 1). Monkeys that died were given the maximal score of 60. Comatose monkeys were assigned an arbitrary score of 40, which was added to their scores for respiration and reflexes. The neurologic function score was then expressed as percent deficit or recovery. A score of 0 was normal with 100% recovery and 0% deficit, while a score of 60 indicated brain death with 0% recovery and 100% deficit. The percentage of neurologic deficit was calculated from the mean score value.

After the last neurologic evaluation at 48 hours, the monkeys were anesthetized again using the procedure described above and intubated. The right lingual artery was cannulated and ligated, with the end of the catheter proximal to the bifurcation. Both external carotid arteries were ligated to minimize extracranial contamination during measurement of cerebral blood flow (CBF) with xenon-133. The left jugular vein as well as the femoral artery were catheterized for blood sampling and MABP recording. The monkeys were immobilized with gallamine and ventilated as described above. Deep body temperature was maintained at 37.5°C.

Regional CBF was measured by continuous external scintillation detection of brain xenon-133 activity (Tracor Northern, Inc., IV TN 1710, Middleton, Wisconsin) for 10 minutes by four focused and collimated NaI 2.5-cm-diameter scintillation probes anchored over the left and right frontal and occipital cortices so that the frontoparietal and the parieto-occipital regions were covered. CBF was calculated using the initial slope index (ISI).11-12 Xenon partition coefficients of 1.5 and 0.87 were used for white and gray matter, respectively.

The cerebral venous PO2 (CVPO2), which represents a mean value for tissue oxygen content, was measured by blood sampling from the jugular vein. Arterial and cerebral venous glucose concentrations were determined by photocolorimetry.13 Cerebral venous lactate concentration (CVLact) was measured enzymatically.14

The above parameters were also used to calculate the cerebral vascular resistance (CVR) as well as the oxygen extraction ratio (OER) and glucose extraction ratio (GluER). The cerebral metabolic rate of oxygen (CMRO2) was calculated as CBF×(arterial O2-internal jugular venous O2). The same method was used to determine the cerebral metabolic rate of glucose (CMRGluc).

In our randomized study, the 27 monkeys were divided randomly into control (n=13) and treated (n=14) groups. At the end of the ischemic period and after MABP normalization, treated monkeys received 30 mg/kg i.v. AGF 2 in a volume of 10 ml; the control monkeys received an equal volume of solvent (phosphate buffer). The injections were repeated intramuscularly 5, 21, 29, and 48 hours after ischemia in volumes of 5 ml. Animal care complied with the "Principles of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals."

**Results**

Five hours after ischemia, all 13 control monkeys were deeply comatose, whereas only six treated monkeys exhibited coma ($\chi^2=8.575, df=1; p=0.003$). Coma persisted at 24 hours in two control monkeys and one treated monkey. Full neurologic recovery occurred in three control and seven treated monkeys ($\chi^2=2.095, df=1; p=0.14$), with a shorter latency in the treated group. In fact, this recovery was observed within 24 hours in these seven treated monkeys, whereas complete recovery in the three control monkeys did not occur until 48 hours (Figure 1). At 48 hours, mortality was similar in both groups: five control and five treated monkeys had died.

In the treated monkeys, less neurologic impairment was evident by 5 hours after ischemia (41.8% vs. 86% in controls; $p<0.05$), based on the neurologic score. This improvement was still apparent in the treated group at 24 hours (16.5% vs. 44.8% in effect).
controls; p<0.05). At 48 hours the groups were similar; the cumulative neurologic score over the 48 hours indicates improved outcome in the treated group (p<0.05) (Table 2). Based on the number of abnormal neurologic signs, a significant difference ($\chi^2$ test) was observed between groups for all items of the neurologic scale during the entire 48-hour period except for reflexes at 48 hours.

MABP, heart rate, hematocrit, hemoglobin, blood gases, pH, and temperature at 48 hours were all within the normal range in both groups. In contrast, CBF in the control group was severely and significantly reduced with respect to values for normal monkeys previously obtained by ourselves and others15 (Table 3). However, in the treated group, CBF was significantly elevated with respect to the control group (p<0.05). This increase in CBF is associated with a moderate but significant (p<0.05) decrease in CVR compared with the control group.

When considering parameters associated with cerebral metabolism, both treated and control monkeys were in a hypometabolic state compared with anesthetized normal monkeys (Table 3). The groups exhibited similar reductions in both CMRO$_2$ and CMRGlu. CVPO$_2$ was significantly higher in the treated than in the normal group but remained within the normal range, which suggests that the oxygen supply in both groups adapted to meet metabolic demands. This absence of hypoxia is further confirmed by CVLact, which in both groups was not different from normal. Furthermore, the control group showed GluER values that were significantly higher than those in the treated group. GluER of the treated monkeys was not different from normal, which indicates that the control monkeys, in contrast to the treated ones, were not fully adapted to the metabolic demand.

**Discussion**

Our study demonstrates that monkeys subjected to transitory global cerebral ischemia can spontaneously recover from their neurologic deficits within 48 hours. The possibility of recovery is, however, presumably related to the severity of the neurologic deficit. The maximum neurologic deficit (death) in the most severely affected monkeys was more frequently observed relatively long (24 and 48 hours) after ischemia; this probably reflects a slow maturation of the phenomena underlying ischemia-induced tissue injury. In contrast, monkeys displaying mild to moderate neurologic deficit early (5 hours) after ischemia rather rapidly recovered toward

**FIGURE 1.** Evolution of neurologic recovery at 5, 24, and 48 hours after ischemia in control (■) and AGF 2-treated monkeys (●). A score of 0 was normal with 100% recovery, while a score of 60 indicated brain death with 0% recovery.

**TABLE 2.** Effect of Ganglioside AGF 2 on Neurologic Score in Monkeys Over 48 Hours Following 30 Minutes of Cerebral Ischemia

<table>
<thead>
<tr>
<th>Hours after ischemia</th>
<th>Control group</th>
<th>Treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neurologic score</td>
<td>Neurologic score</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Percent deficit</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>86.0</td>
</tr>
<tr>
<td>24</td>
<td>9</td>
<td>44.8</td>
</tr>
<tr>
<td>48</td>
<td>8</td>
<td>20.2</td>
</tr>
<tr>
<td>Cumulative score</td>
<td>8</td>
<td>49.7</td>
</tr>
</tbody>
</table>

Data are scores on neurologic rating scale (Table 1).

*p<0.05 different from control group by Mann-Whitney method.
normal neurologic parameters; nonetheless, at 48 hours these monkeys still displayed physiopathologic signs of ongoing cerebral infarction, as characterized by low CBF and hypometabolism. The presence of increased GluER with a normal OER may be due to the brain's compensating for a decreased glucose supply, thus allowing CMRGlut to remain constant before decreasing due to tissue injury. When considering individual values obtained in the monkeys, the degree of low CBF and hypometabolism correlated well with relative neurologic recovery (present observations).

Treatment with AGF 2 significantly ameliorates the neurologic outcome, but not the mortality rate, following transitory global cerebral ischemia in monkeys. This effect of the ganglioside is 1) already present early (5 hours) after ischemia, 2) associated with an enhanced rate of neurologic recovery, and 3) evident when considering the "basal functions" and/or reflexes. The improved neurologic recovery at 48 hours is accompanied by a slighter reduction in CBF, although the treated monkeys were in a hypometabolic state. Since this hypometabolism was not accompanied by abnormal OER or GluER, metabolism in these monkeys seems well-adapted to the demand.

The process underlying the short-term ganglioside effects following cerebral ischemia are still unknown. Following mechanical brain insults, the GM 1–induced improvement in outcome is, in many cases, associated with enhanced neuronal viability.16–18 Optimal GM 1 effects occur when ganglioside treatment is initiated very early, that is, within 2 hours, after the brain insult.19 Interestingly, ganglioside treatment of rats with open head CNS injury has been shown to reduce edema as well as losses of Na⁺, K⁺-ATPase activity and intracellular K⁺ levels at the site of the injury.20,21 GM 1 is also reported to decrease mortality and to protect against ischemia-induced reduction of hippocampal Na⁺, K⁺-ATPase activity in gerbils.9 Similar effects may underlie the decreased hypoperfusion and limitation of the CVR increase we observed in AGF 2–treated monkeys. In cats with transitory focal ischemia, a single dose of GM 1 increases CBF in the ischemic area, and GM 1 depresses local CMRGlut in the peripheral middle cerebral artery territory, in which the morphologic damage is less severe in treated cats.8 Thus, we hypothesize that the improved neurologic recovery we observed may result from the ganglioside's ability to maintain structural integrity and/or blood flow–metabolism coupling adapted to the needs of the brain. Which of these two possibilities plays the predominant role is currently under investigation.

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