Failure of GM1 Ganglioside to Influence Outcome in Experimental Focal Ischemia

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Background and Purpose: Reports of improved short-term (<72 hours) outcome in experimental models of mechanical and ischemic central nervous system injury suggest that exogenous ganglioside administration may confer a protective effect on neural tissue. We studied the effect of the monosialoganglioside GM1 on cerebral infarction and edema in spontaneously hypertensive rats subjected to permanent focal cerebral ischemia.

Methods: GM1 or normal saline was injected intramuscularly once a day for 3 days before and 30 and 120 minutes after occlusion of the right middle and common carotid arteries. Following a 24-hour survival period, the volume of infarction was measured by computer-assisted image analysis, and the extent of edema was assessed by measurements of tissue water content and hemispheric volume.

Results: Infarct volume was similar among the GM1-treated (n=10) and saline-treated (n=10) rats (212±10 versus 220±13 μl, respectively). In a second series of experiments, the brain water content and edema volume of the ischemic right hemisphere in GM1-treated rats (n=10) did not differ from saline-treated controls (n=10).

Conclusions: GM1 ganglioside does not effectively reduce cerebral infarction caused by permanent focal ischemia. (Stroke 1992;23:242-246)

Gangliosides are naturally occurring acidic glycosphingolipids that play a well-established role in the promotion of developmental and regenerative processes in the central nervous system.1-5 Exogenously administered gangliosides penetrate the blood-brain barrier6 and have been demonstrated to actively insert into neuronal membranes.7 Recent reports based on a variety of animal models of central nervous system injury suggest that the monosialoganglioside GM1 may in addition exert acute protective effects on neural tissue, presumably by preserving the functional integrity of cell membranes.8 Early findings that exogenous GM1 administration can limit the extent of functional deficits9-12 and cerebral edema13 caused by acute mechanical trauma have been followed by reports of improved outcome in GM1-treated animals subjected to both global14-16 and focal17-19 ischemic insults. The protective effects of gangliosides in ischemia have also been demonstrated in vitro: GM1 can attenuate glutamate-induced neurotoxicity20,21 and preserves mitochondrial structure22 and membrane excitability23 in hypoxic neural tissue. To further test the use of ganglioside therapy during the acute phase of stroke, we studied the effect of GM1 treatment on the volume of cortical infarction and extent of cerebral edema in spontaneously hypertensive rats (SHR) subjected to permanent focal ischemia.

Methods and Materials

Focal necortical ischemia was produced according to the method described by Brint et al.24 Fasted male SHR (Taconic Farms Inc., Germantown, N.Y.) weighing 245–315 g were anesthetized with a 70% N2:28% O2:2% halothane mixture. The tail artery was cannulated to monitor blood pressure and to obtain blood samples for physiological variables. Arterial pH, PO2, and PCO2 and glucose and hematocrit values were obtained immediately after right middle cerebral and common carotid artery (MCA/CCA) occlusion and again 2 hours later when the animals had recovered from anesthesia. Mean arterial blood pressure was measured throughout the surgical procedure and was recorded at the time of MCA/CCA occlusion and at 2 hours after occlusion. Body temperature was maintained at 37.5±0.5°C via a rectal thermistor connected to a heating lamp. After MCA/CCA occlusion the rats were allowed to survive for 24 hours, at which time they were reanesthetized with...
ether and decapitated. Immediately thereafter the brains were dissected from the cranium for postmortem tissue analysis.

Treated rats received hind limb intramuscular injections of GM1 (30 mg/kg) dissolved in a phosphate buffer (0.05–0.1 ml) once a day for 3 days before surgery and at 30 and 120 minutes after occlusion. Control rats received equivalent volumes of normal saline (NS) injections at similar intervals. Pharmacological studies have documented that intramuscular injections in rats result in detectable brain levels of tritium-labeled ganglioside.7 Our dosing regimen was based on evidence that some of the in vitro effects of GM1 on membrane function require 3 days of pretreatment2021 and on reports that exogenous gangliosides need to be present during the early phase of injury (0–2 hours after a mechanical insult)12 to achieve optimal benefit.

The investigation was conducted in three stages, during which rats from common shipments were randomly assigned to different treatment groups. The number of rats in each treatment group and the type of postmortem tissue analysis conducted are presented in Table 1. In the first experiment (series A), the brains of GM1- and NS-treated rats were studied histologically in order to determine the volume of cortical infarction. In the second experiment (series B), the extent of cerebral edema was assessed in GM1-treated, NS-treated, and untreated, unoperated rats by determining the volume of the right and left hemispheres, followed by measurements of brain regional water content. We chose to measure changes in hemispheric volume as well as tissue water content to determine whether any detectable reduction of cerebral edema was associated with a clinically relevant amelioration of brain swelling. To verify that the method for hemispheric volume measurements (see below) did not result in significant alterations of regional water content, the brains of an extra group of operated, untreated controls (series C) were analyzed for regional water content only.

Infarct volume was calculated using computer-assisted image analysis as described previously.24 For the determination of hemispheric volume, the olfactory bulbs, cerebellum, and brain stem were removed, and the freshly dissected brain was sagitally sectioned in the midline. The right and left hemispheres were suspended by a hanging wire hook through the occipital lobe in a preweighed (T) beaker of distilled water placed on a digital automatic balance. The weight of the beaker, water, and suspended hemisphere was recorded (S). Each hemisphere was subsequently blotted dry, frozen in Freon over dry ice, and stored in an airtight plastic bag at –70°C. The volume of each hemisphere was calculated by subtracting T from S, the difference representing the weight of the water displaced by the suspended brain. Since the specific gravity of distilled water is 1.0, this measurement is in turn equivalent to the volume of water displaced25 and therefore the volume of the suspended hemisphere.

The water content of punch samples (Figure 1) from the basal ganglia (area 1), paramedian neocortex (area 2), and infarcted frontoparietal neocortex (area 3) was measured as percentage of wet tissue weight by subtracting the dry (100°C for 24 hours) weight from the wet weight and dividing by the wet weight.

Student's t test (two-tailed) was used to evaluate differences between GM1- and NS-treated groups with regard to physiological variables, infarct volume, hemispheric volumes, and brain regional water content. Right and left regional water contents were compared using the paired t test. Group means that differed by values of p≤0.05 were considered significant. Power analysis was applied to the results to estimate the percent reduction of infarct volume, hemispheric volume, and regional water content that could be accurately detected given the numbers of animals used, a power of 80%, and a significance level of 5%.26

Results

Two rats were excluded from the study: one from group 1 because of intraoperative hypotension, and one from group 2 because of abnormal cerebral vascular anatomy.

Physiological data are not presented but are available upon request. Anesthetized rats in all groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Surgery</th>
<th>Treatment</th>
<th>End point analysis</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>MCA/CCAo</td>
<td>NS</td>
<td>Infarct volume</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>MCA/CCAo</td>
<td>GM1</td>
<td>Infarct volume</td>
<td>10</td>
</tr>
<tr>
<td>Series B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>None</td>
<td>Hemispheric volume only</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>None</td>
<td>Hemispheric volume, RWC</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>MCA/CCAo</td>
<td>NS</td>
<td>Hemispheric volume, RWC</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>MCA/CCAo</td>
<td>GM1</td>
<td>Hemispheric volume, RWC</td>
<td>10</td>
</tr>
<tr>
<td>Series C</td>
<td></td>
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</tr>
<tr>
<td>7</td>
<td>MCA/CCAo</td>
<td>None</td>
<td>RWC only</td>
<td>6</td>
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</tbody>
</table>

MCA/CCAo, tandem occlusion of the middle cerebral and common carotid arteries; NS, normal saline; RWC, regional water content analysis.

Table 1. Composition of Experimental Groups of Rats

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developed mild hypercapnia, respiratory acidosis, and hypotension, which normalized within 2 hours after the surgery. Hematocrit, arterial PO₂, blood glucose, and body temperature remained in the normal range throughout. There were no significant differences between the GM1- and NS-treated groups with regard to any of the recorded variables.

Gross examination of the brain revealed large, well-demarcated neocortical infarctions in all rats subjected to focal ischemia. The mean (±SEM) neocortical infarct volume of the GM1-treated rats (211.5±9.7 μl) was not significantly different from that of the NS-treated animals (220.3±12.9 μl).

Hemispheric volume measurements revealed no effect of GM1 on the extent of brain swelling in rats subjected to focal ischemia (Table 2). Among both GM1- and NS-treated rats, there was a mean increase of 96 μl in the volume of the ischemic right hemisphere when compared with the left, representing a change of approximately 16%. Compared with untreated, unoperated rats, the right hemisphere volumes of both groups were significantly increased, whereas the left hemisphere volumes were not.

Normal unoperated rats had reproducible tissue water concentrations (mean, 79.0%) that did not vary significantly between the brain regions sampled (Table 3). The administration of GM1 had no effect on regional water content in either the ischemic or nonischemic hemispheres of operated rats. GM1- and NS-treated rats demonstrated similar elevations of water content in the infarcted right frontoparietal neocortex, which in both groups was significantly increased when compared with the opposite side or with untreated, unoperated rats. Water concentrations in the ipsilateral peri-infarct regions (basal ganglia and paramedian neocortex) were also elevated in both groups when compared with the nonischemic side, but these differences were not statistically significant, nor were any of these values significantly higher than corresponding areas in unoperated animals. The data obtained in series C indicate that brief immersion in distilled water did not lead to significant alterations of tissue water content.

Discussion

The purpose of this study was to evaluate the potential benefit of GM1 therapy during the acute phase of stroke by assessing its ability to prevent tissue infarction caused by ischemia. Because of reports that GM1 can limit cerebral edema caused by ischemia as well as trauma, we also measured tissue water content and edema volume to ensure that apparent reductions of infarct volume were not due to an anti-edema effect.

Previous estimates have calculated that brain tissue swelling accounts for approximately 20% of infarct volume in the SHR MCA/CCA occlusion model. The present data indicate that this figure is closer to 30%. Our measurements of regional water content indicate that the majority of edema within the ischemic hemisphere is localized to the area of infarction. Based on the conservative assumption that 80% of the increase in volume of an ischemic hemisphere is localized to the infarct, at least 58–77 μl of a 220-μl infarct (approximately 26–35%) can be accounted for by tissue swelling from edema.

Our data indicate that GM1 ganglioside administered before and during the acute phase of irreversible focal ischemia does not significantly influence the subsequent development of infarction and cerebral edema. In light of the negative results of our study, the ability to detect a treatment effect is an

Table 2. Hemispheric Volume Measurements in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
<th>n</th>
<th>Right</th>
<th>Left</th>
<th>Total</th>
<th>Right−Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3 and 4</td>
<td>9</td>
<td>617.9±7.7</td>
<td>606.4±14.5</td>
<td>1224.3</td>
<td>+11.5</td>
</tr>
<tr>
<td>Normal saline</td>
<td>5</td>
<td>10</td>
<td>690.5±10.0*</td>
<td>594.2±6.7</td>
<td>1284.7</td>
<td>+96.3</td>
</tr>
<tr>
<td>GM1</td>
<td>6</td>
<td>10</td>
<td>698.8±8.6*</td>
<td>602.7±5.9</td>
<td>1301.5</td>
<td>+96.1</td>
</tr>
</tbody>
</table>

Values expressed as mean±SEM in microliters.

*p<0.001 compared with contralateral hemisphere or ipsilateral hemisphere of unoperated controls.
important consideration. Applying power analysis to the number of animals in each group and the inter-animal variability observed, this study had the power to detect a ≥22% reduction of infarct volume, a ≥22% reduction of edema in the infarcted right frontoparietal cortex, and a ≥6% reduction of volume in the ischemic right hemisphere.

Our failure to observe a treatment effect contrasts with previous reports of improved short-term outcome associated with GM1 therapy in both global14-16 and focal17-19 models of ischemia. However, none of these studies have demonstrated an unequivocal protective effect in permanent ischemia based on histological analysis.

Using a model of reversible focal ischemia (2 hours of MCA occlusion in cats), both Tanaka et al17 and Komatsumo et al18 have observed a tendency toward improved histological outcome in GM1-treated animals. Tanaka et al17 found significantly increased regional cerebral blood flow in the ischemic regions of treated animals during both occlusion and reperfusion. The association of morphological improvement with areas of decreased glucose utilization in the peripheral MCA territory of some treated animals prompted speculation that GM1 may exert a protective effect either by maintaining a more normal flow–metabolism couple17 or by limiting anaerobic glycolysis and lactic acid formation.27 Komatsumo et al18 similarly found that extended posttreatment with GM1 was associated with improved histological scores and recovery from neurological deficits. However, in neither study did GM1 appear to influence the overall severity of ischemia as assessed by depression of cortical electrical activity. We feel that the relatively modest treatment effects observed and the high degree of variability inherent in focal brain ischemia in cats28 make these studies inconclusive regarding the ability of GM1 to prevent cell death caused by ischemia. Given our failure to demonstrate a treatment effect in permanent focal ischemia, it remains possible that GM1 may limit tissue damage caused by temporary ischemia either by delaying cell death or by modifying pathological mechanisms specific to reperfusion injury. Some evidence for the latter comes from Cahn et al,16 who found that treatment with a monosialoganglioside similar to GM1 (AGF2) immediately after 30 minutes of global ischemia in monkeys led to less severe reductions in cerebral blood flow and accelerated rates of neurological recovery.

Exogenous GM1 has also been shown to improve outcome when tested in models of permanent ischemia. In Mongolian gerbils subjected to unilateral CCA ligation, Karpik et al and Mahadik et al14,15 found that GM1 treatment protects plasma membrane function (as evidenced by preserved levels of hippocampal and neocortical Na,K-ATPase activity, mitochondrial Mg-ATPase activity, and membrane fatty acid levels) and reduces mortality by 52%. Reductions in ischemic damage were not histologically verified, however, leading the experimenters to conclude that they could not rule out a beneficial effect of GM1 on systemic or seizure-related causes of death.

A preliminary report by Karpik et al19 has also found GM1 to be of benefit in Sprague-Dawley rats subjected to permanent focal ischemia (MCA/CCA occlusion). Treatment with GM1 at 0, 24, and 48 hours after occlusion resulted in less edema, less Ca2+ and Na+ accumulation, and preserved levels of K+ and Na,K-ATPase activity. Behavioral deficits were similarly reduced, but again, histological data were not presented. Our findings are difficult to reconcile with this report. Although it seems unlikely that the different dosing regimens were an important factor, it should be noted that Karpik et al19 used a lower dose of GM1 and gave more extended posttreatment. The beneficial effects observed by Karpik et al19 might also be explained by interspecies differences.

It can be argued that MCA/CCA occlusion in the SHR does not lend itself to pharmacological protection because it leads to more uniform and severe reductions of blood flow than in normotensive strains.24,29 It may be that the benefit of GM1 in permanent focal ischemia is more evident in normotensive rats, perhaps because of a stronger "membrane stabilizing effect"78 in brain regions with less severe reductions of cerebral blood flow. However, experiments in several laboratories have shown that the SHR is fully amenable to neuroprotection with nimodipine, MK-801,32 and idazoxan,33 compounds that also attenuate
damage caused by focal ischemia in nonhypertensive species. Our inability to show a similar treatment effect with GM1 suggests that more robust therapies are necessary to significantly lessen brain damage caused by permanent focal ischemia.

Acknowledgments

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References


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