Gangliosides (G\textsubscript{M1} and AGF2) Reduce Mortality Due to Ischemia: Protection of Membrane Function

Stephen E. Karpiak, Y.S. Li, and Sahebarao P. Mahadik

As evidenced by their ability to reduce cerebral edema, exogenous ganglioside administration exerts acute effects on CNS injury processes. We report here that ganglioside (G\textsubscript{M1} or AGF2) treatment results in a 52\% decrease in mortality 48 hours after the induction of ischemia in gerbils by permanent unilateral ligation of the common carotid artery. By comparing the occluded vs. nonoccluded sides of the brain (cortex and hippocampus) we found a significant loss of membrane Na\textsuperscript{+}, K\textsuperscript{-} ATPase activity due to ischemia in control animals, but no such differences were found between the hemispheres of ganglioside-treated gerbils. We hypothesize that gangliosides may be "protecting" membrane function as indicated by these ATPase analyses, reducing local CNS damage at the time of injury (i.e., reduced cell loss, fiber degeneration, membrane failure). By acutely limiting the extent of CNS tissue damage, conditions may be optimized for any subsequent CNS regrowth and functional recovery.

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Ganglioside treatment of animals with a variety of central nervous system (CNS) injuries has consistently resulted in facilitated levels of functional recovery.\textsuperscript{1-12} It has been hypothesized that the improved functional recovery is due to increased CNS regeneration or sprouting\textsuperscript{5,6} since exogenous gangliosides promote neuritogenesis \textit{in vitro},\textsuperscript{7,8} enhance neuronal regeneration in response to injury \textit{in vivo},\textsuperscript{5,6,9} and accelerate neonatal CNS maturation.\textsuperscript{10,11}

Although some enhancement of regeneration and functional recovery after injury occurs in the long term (2-8 weeks after injury), data indicate that ganglioside treatments also have acute effects that are seen within 24-48 hours.\textsuperscript{12,13}

The first evidence of an acute effect was seen when ganglioside-treated rats, 24 hours after having sustained large entorhinal cortical ablations, showed reduced levels of mortality and reduced impairment on learned alternation behavior.\textsuperscript{1} Since such short term effects could not be attributed to enhanced regeneration, we studied the acute phase of ganglioside treatment. We hypothesized that gangliosides reduce the extent of local CNS damage at the time of injury (i.e., reduced cell loss, fiber degeneration, membrane failure), which would account for the reduced mortality and acute functional recovery. Further, limiting the extent of CNS tissue damage might improve conditions for any subsequent regrowth.

We have found that after 4 days of ganglioside treatment, rats that had sustained open head injury showed reduced cerebral edema,\textsuperscript{14} reduced loss of intracellular K\textsuperscript{+}, and reduced loss of membrane Na\textsuperscript{+}, K\textsuperscript{-} ATPase activity.\textsuperscript{15} Using the same paradigm, no changes were found in blood–brain barrier (BBB) permeability (determined with iodinated albumin) after ganglioside treatment.\textsuperscript{13}

Studies have now focused on the effects of gangliosides on membrane Na\textsuperscript{+}, K\textsuperscript{-} ATPase activity since this activity is markedly reduced in edemic tissue, indicating failure of plasma membrane ionic function and the disruption of membrane integrity. In addition to its functional significance, Na\textsuperscript{+}, K\textsuperscript{-} ATPase is highly concentrated in CNS plasma membranes (especially synaptic membranes), and is a good marker for membrane integrity.\textsuperscript{16} Recently we found an almost complete protection of this enzyme’s activity in the denervated striatum of rats treated with gangliosides.\textsuperscript{2} We also observed a critical postsurgical period (0-2 hours after injury) when initial G\textsubscript{M1} ganglioside administration results in a reduction in functional loss.\textsuperscript{3} This further supports the hypothesis that gangliosides exert acute pharmacologic effects on damaged CNS tissue.

The following study examined the acute effects of ganglioside treatment on injured CNS tissue. We report here the effects of G\textsubscript{M1}, ganglioside and AGF2 (a G\textsubscript{M1} derivative) on a model of global ischemia in the Mongolian gerbil. We have used a model of ischemia since no previous studies have examined the effects of ganglioside on closed head injury and a recent clinical report indicates that ganglioside treatment improved functional recovery after stroke.\textsuperscript{17} In addition, since some of the molecular mechanisms underlying the pathophysiology of ischemia are understood, we should be better able to study the mechanism(s) by which gangliosides act.

Materials and Methods

Global ischemia model. The Mongolian gerbil has been widely used as an experimental model in studies of cerebral ischemia since many gerbils have an incomplete circle of Willis, with little or no communicat-
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ventral surface of the neck, the salivary glands were drochloride, 7.5 mg/kg). An incision was made in the brachial hemisphere in the majority of animals. This is advantageous since the animal can be used as its own control, allowing comparisons between the occluded and nonoccluded sides of the brain.

Male gerbils (80–90 g) were anesthetized (i.m.) with a mixture (7:3 by vol.) of Vetalar (ketamine hydrochloride, 87.5 mg/kg) and Rompun (xylazine hydrochloride, 7.5 mg/kg). An incision was made in the ventral surface of the neck, the salivary glands were moved laterally, and the right carotid sheath was exposed. Both the vagus and sympathetic nerves were separated from the right common carotid artery (CCA), which was then permanently ligated using No. 0 silk thread.

Ganglioside injections. Gerbils received ganglioside injections (20 mg/kg i.m., FIDIA Research Laboratories) 0.5 and 24 hours after surgery. There were 3 groups: animals receiving saline (controls), monosialoganglioside (GM1), and a ganglioside derivative (AGF2). AGF2, an internal ester of GM1, has been reported to have a longer half-life in serum than GM1 analoganglioside (GM1), and a ganglioside derivative (AGF2). AGF2, an internal ester of GM1, has been reported to have a longer half-life in serum than GM1 analoganglioside (GM1), and a ganglioside derivative (AGF2). AGF2, an internal ester of GM1, has been reported to have a longer half-life in serum than GM1.

Mortality 48 Hours After Permanent Carotid Ligation

<table>
<thead>
<tr>
<th>Group</th>
<th>Deaths after 48 hours</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>103</td>
<td>54</td>
</tr>
<tr>
<td>GM1</td>
<td>94</td>
<td>25</td>
</tr>
<tr>
<td>AGF2</td>
<td>69</td>
<td>18</td>
</tr>
</tbody>
</table>

![Image](https://via.placeholder.com/150)

Table 1. Mortality Rate in Gerbils 48 Hours After Permanent Carotid Ligation

Table 2. Cortical and Hippocampal Membrane Na, K-ATPase Activity 48 Hours After Unilateral Carotid Occlusion in Gerbils: GM1 and AGF2 Ganglioside Effects

<table>
<thead>
<tr>
<th>Group</th>
<th>Occluded side</th>
<th>Nonoccluded side</th>
<th>Difference</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>5.41 ± 0.49</td>
<td>6.74 ± 0.10</td>
<td>-1.33 ± 0.21</td>
<td>p &lt; 0.01*</td>
</tr>
<tr>
<td>GM1</td>
<td>3.68 ± 0.25</td>
<td>5.63 ± 0.32</td>
<td>-1.95 ± 0.07</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>AGF2</td>
<td>2.95 ± 0.26</td>
<td>3.21 ± 0.24</td>
<td>-0.26 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>3.21 ± 0.37</td>
<td>3.48 ± 0.43</td>
<td>-0.27 ± 0.06</td>
<td>NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Cortex</th>
<th>Hippocampus</th>
<th>Difference</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>4.03 ± 0.36</td>
<td>4.76 ± 0.43</td>
<td>-0.73 ± 0.08</td>
<td>NS</td>
</tr>
<tr>
<td>GM1</td>
<td>4.46 ± 0.45</td>
<td>3.77 ± 0.34</td>
<td>0.69 ± 0.43</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± SEM μmol Pi/hr/mg protein based on 18 gerbils/group.

*Occluded vs. nonoccluded, Student’s t, NS, not significant.

Discussion

Ganglioside (GM1 or AGF2) treatment results in a 52% decrease in mortality 48 hours after the induction of ischemia in gerbils by permanent unilateral ligation disected out. A membrane fraction was prepared by homogenizing specimens in ice-cold buffer (40 mM Tris-HCl pH 7.5, 10 mM MgCl2, 10 mM EDTA), and centrifuging (30 minutes, 100,000 g). The resulting pellet was suspended in the homogenizing buffer. Five hundred μl of reaction mixture contained 40 mM Tris-HCl pH 7.5, 150 mM NaCl, 40 mM KCl, 10 mM MgCl2, 2 mM EDTA, 2 mM EGTA, 4 mM ATP, and 100–200 μg of membrane protein. The amount of inorganic phosphate released in 30 minutes at 37° C was determined by the method of Eibl and Lands.24 Na, K-ATPase activity was estimated from the difference between the activities in the absence and presence of 5 mM ouabain.

Results

Forty-eight hours after permanent unilateral ligation of the CCA, saline-injected gerbils had 52% mortality (Table 1). GM1- and AGF2-injected gerbils showed almost identical mortalities of 27% and 26% respectively. Ganglioside treatments reduced mortality from 52 to 26% (p < 0.001, χ²).

By comparing levels of membrane Na, K-ATPase activity in the occluded vs. nonoccluded side of the brain in saline-injected controls, we found significant (p < 0.01) decrease in enzyme activity (Table 2): a 19.8% decrease in the cortex and a 34.7% decrease in the hippocampus. No significant differences in enzyme activity between the occluded and nonoccluded sides of the brain were found in either GM1- or AGF2-treated rats.

Na, K-ATPase activity levels were lower in the ganglioside-treated gerbils compared with controls (Table 2). This was more pronounced on the nonoccluded side compared with the occluded side between groups (Student’s t: occluded side—saline vs. GM1, p < 0.001; saline vs. AGF2, p < 0.01; nonoccluded side—saline vs. GM1, p < 0.001; saline vs. AGF2, p < 0.01).

Discussion

Ganglioside (GM1 or AGF2) treatment results in a 52% decrease in mortality 48 hours after the induction of ischemia in gerbils by permanent unilateral ligation.
of the CCA. 

G₄₄ ganglioside treatment reducing mortality after brain injury has also been reported in rats, indicating that gangliosides can reduce impairment in the acute phase following a CNS insult. If functional deficits can be minimized in the short term, the possibility for facilitated recovery in the long term should be maximized.

Although these ongoing experiments hypothesize that ganglioside treatment reduces mortality due to cerebral death, the pharmacologic effect may be indirect. Specifically, the severity of seizures (convulsions) that occur in this gerbil model may be reduced, and therefore any consequent seizure-associated mortality may be reduced. Further, gangliosides could have a systemic effect (e.g., prevention of cardiac failure).

The mechanisms by which ganglioside treatments result in reduced mortality, or, as previously reported, facilitated functional recovery, are not yet identified. Analysis of membrane Na, K-ATPase activity in this experiment indicates that one possible action may be the stabilization of plasma membranes. In untreated gerbils there was a significant decrease in membrane ATPase activity in the hippocampus and cortex on the occluded side of the brain compared with the nonoccluded side. No such differences were found in the G₄₄- or AGF₂-treated animals, although ATPase levels in the ischemic side of the brain were higher in AGF₂-treated gerbils than in those treated with G₄₄.

Preliminary studies indicate that this internal ester of G₄₄, AGF₂, activates Na, K-ATPase activity more than G₄₄.

The fact that the overall levels of Na, K-ATPase activity are lower in the ganglioside-treated gerbils vs. saline-injected controls may result from two factors. First, approximately one-third of the gerbils assayed for ATPase activity in the ganglioside groups are animals that were predicted to have died from the ischemia. This group of animals would therefore be expected to have extremely low levels of ATPase activity and are probably animals in which ischemia was not exclusively localized to the occluded side of the brain. Thus, lower levels of enzyme activity on both sides of the brain might be expected. Ideally, Na, K-ATPase activities from gerbils that died would have been useful. However, assaying this enzyme at death seems inappropriate. For better comparisons, ongoing studies are now measuring the enzyme activity in groups of animals that are severely affected, i.e., animals with a high probability of dying.

Secondly, gangliosides have been shown to affect this enzyme per se. We have reported that in vitro (in animals without brain injury or any compromise to the BBB), G₄₄ ganglioside reduces the activity of this enzyme, in both the cortex and hippocampus, by 24 and 12% respectively. The reductions in the activity of this enzyme may be even greater after injury to the BBB since higher ganglioside levels probably reach injured CNS tissue. Small quantities (<0.5%) of ganglioside reach the CNS in intact brain. Also, since these studies have been done in rats, species differences in ganglioside transport across the intact BBB and in regional cerebral vascularization may be important.

As the model proscribes, all comparisons have been made within each group, examining occluded vs. nonoccluded sides of the brain. The nonoccluded side of the brain is the most suitable control for these present studies since the amounts of ganglioside reaching the CNS in all groups would be identical. By comparing levels of ATPase activity in ganglioside-treated uninjured animals (intact BBB) with ischemic animals (compromised BBB), ganglioside dose is variable, making such between-group comparisons difficult.

In view of these factors, if there were no protection of the ATPase enzyme on the occluded side, the decreases due to the two cited factors would be identical on both sides, and the difference between the occluded and nonoccluded sides due to ischemia would persist. However, since there is no difference between enzyme activity on the occluded compared with the nonoccluded side we conclude that the enzyme activity on the occluded side has been increased, namely, protected, thereby resulting in similar levels on both sides.

In addition, detailed studies in progress show that the kinetic characteristics of Na, K-ATPase from ischemic tissue in G₄₄- and AGF₂-treated rats are different from those of enzyme from control tissue, suggesting that membranes from ganglioside-treated ischemic tissue are probably different from untreated ischemic tissue. Changes in kinetic characteristics of ATPase activity have also been reported after in vitro treatment with G₄₄. We therefore conclude that the activity of this enzyme is being "protected," possibly reflecting increased membrane stability.

We had previously reported that ganglioside treatment of rats with open head CNS injury leads to a reduction of edema, and in losses of Na, K-ATPase and intracellular K⁺ at the locus of injury. Using hippocampal slices, Bianchi et al. reported similar protection of ATPase activity by either in vitro or in vivo administration of G₄₄ ganglioside. Consequently, attention has focused on a possible membrane mechanism by which gangliosides may be pharmacologically active. This mechanism is further supported since exogenous gangliosides have been shown to functionally insert into membranes and to modulate ATPase activity in vitro and in vivo.

Ganglioside protection from loss of Na, K-ATPase activity may indicate protection of membrane structure and function since there is considerable evidence that ischemia causes significant reductions in this enzyme's activity.

By stabilizing membrane function and therefore "normal" ionic gradients, membrane failure and subsequent deterioration may well be minimized. The protection of this enzyme from the loss of activity probably reflects effects on one or a series of biochemical changes (e.g., lipid hydrolysis, phospholipase activation, production and membrane action of arachidonic acid, ionic imbalance) that occur as a result of injury and lead to membrane failure. A number of in vitro and in vivo studies have shown that G₄₄ treatments protect
neuronal as well as neuromuscular resistance to hypoxia and ionic disturbances, improve conduction velocity and axonal morphology in neuropathy, prevent ultrastructural deterioration of mitochondria, and reduce retrograde axonal degeneration in the CNS after injury. These data, together with evidence for gangliosides’ ability to reduce cerebral edema and mortality rates due to significant CNS injury, strengthen the hypothesis that gangliosides may preserve membrane structure by preventing the “deterioration of the membrane microenvironment.”

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References

Key Words: ischemia • gangliosides • cerebral edema • membrane • Na,K-ATPase
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