Protective Effects of a Monosialoganglioside Derivative Following Transitory Forebrain Ischemia in Rats

M.S. Seren, PhD, R. Rubini, MS, A. Lazzaro, BS, R. Zanoni, BS, M.G. Fiori, MD, and A. Leon, PhD

We evaluated the effects of treatment with the inner ester derivative of the monosialoganglioside GM1 on cortical electroencephalographic activity and hippocampal CA1 morphology after transitory, near-complete cerebral ischemia in rats. Ischemia was induced by the four-vessel occlusion method, and we studied only the 58 rats that showed flattening of the cortical electroencephalogram for the entire 30 minutes of occlusion. The ganglioside (n = 30) or saline (n = 28) was administered intravenously immediately after release of the carotid clips and then intramuscularly for 21 days of observation. Cortical electroencephalographic activity was monitored throughout the experiment. After 21 days of recirculation we assessed hippocampal CA1 damage by light microscopy. The results indicate that treatment with the ganglioside reduces postischemic secondary damage to the cortical circuitry (as indicated by significantly higher cortical electroencephalographic activity late after reperfusion) and limits neuronal loss in the CA1 region. Our results lend support to the possible therapeutic use of the ganglioside in human pathologic conditions associated with cerebrovascular insufficiencies. (Stroke 1990;21:1607-1612)

Transient global cerebral ischemia induces a relatively selective pattern of neuronal degeneration. Recent investigations into the underlying pathogenesis suggest that excitatory amino acid neurotransmission plays an important role. Neurons selectively vulnerable to an ischemic episode receive prominent excitatory amino acid transmitter inputs, and ablation of these pathways reduces ischemia-induced neuronal loss. In addition, the extracellular concentrations of glutamate and aspartate—potential neurotoxins both in vitro and in vivo—increase during ischemia. A current hypothesis is that excessive accumulation of glutamate or related compounds, via specific postsynaptic receptors, cause neuronal overactivation, triggering a cascade of cellular events that ultimately lead to cell death.1

A corollary to the above hypothesis is that agents capable of antagonizing specific excitatory amino acid receptor–related recognition sites or postreceptor effects may be of potential therapeutic value. In particular, great attention has recently been focused on N-methyl-D-aspartate (NMDA) receptor antago-

From Fidia Research Laboratories, Abano Terme, Italy.
Address for correspondence: M.S. Seren, Department of CNS Research, Fidia Research Laboratories, Via Ponte della Fabbrica 3/A, 35031 Abano Terme (PD), Italy.
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Materials and Methods

We subjected 92 adult male Wistar rats weighing 200–250 g to four-vessel occlusion according to the methods of Pulsinelli and Brierley. Following induction of anesthesia with 50 mg/kg pentobarbital sodium, the vertebral arteries were permanently occluded and stainless steel screws were mounted over the dura to record cortical electrical activity throughout the experiment. The rats were allowed to recover from anesthesia for 24 hours with an overnight fast. The following day, under anesthesia with 1.5% halothane in 30% O₂ and 70% N₂O, the tail artery was catheterized for measurements of mean arterial blood pressure, arterial blood gases, and arterial pH. The carotid arteries were then exposed, occluded for 30 minutes with clips, and released as described by Schmidt-Kastner and Hossmann.

At all times, body temperature was kept at 37°C by using a heated operating table and physiologic parameters were within normal ranges. In 62 rats (68%) the cortical electroencephalogram (EEG) became isoelectric 1–2 minutes after carotid occlusion and the spontaneous righting reflex was absent. Since preliminary experiments conducted by implanting an electrode deep within the hippocampus (stereotactic coordinates -3.5 anterioposterior, -2.9 vertical from the bregma) revealed that isoelectricity occurred concomitantly in the cortex and the hippocampus (data not shown), we used only cortical electrodes to avoid hippocampal damage due to electrode implantation. Following release of the carotid clips, the rats were allowed to survive for 21 days.

Siagoside was prepared and purified in our laboratory according to the methods of Sonnino et al. Purity was assessed to be >99%. All solutions were made fresh immediately before administration.

We considered only the 58 rats showing cortical EEG flattening during the entire 30 minutes of occlusion. After the carotid clips were removed, 30 randomly selected rats were treated with 20 mg/kg i.v. siagoside. The remaining 28 rats were analogously treated with saline. We counted the total number of preserved neurons in the two groups using Student's two-tailed t test for unpaired groups.

Results

Four rats (4%) died of respiratory failure very soon after occlusion. An additional 13 rats (14%) died during the 21 days of observation, with a concomitant severe reduction in body weight. We observed no difference in mortality (seven [25%] in the saline-
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FIGURE 1. Bar graphs of mean±SEM effect of siagoside treatment in rats on delta (A), theta (B), alpha1 (C), and alpha2 (D) cortical electroencephalographic (EEG) activity monitored using eight active electrodes implanted over dura 24 hours after electrode implantation and vertebral artery occlusion (baseline) (IMP), during 30-minute occlusion of carotid arteries (Car Occ), and 30 minutes (m), 3 and 24 hours (h), and 3, 7, 14, and 21 days (d) after carotid clip removal. Siagoside treatment significantly increased cortical EEG activity (p < 0.05 for A and D, p < 0.01 for B and C). Filled bars, saline-treated group; shaded bars, siagoside-treated group.

treated and six [20%] in the siagoside-treated group) between groups. In addition, control experiments conducted in separate groups (five rats per group) showed no effect of siagoside on mean arterial blood pressure, arterial blood gases, arterial pH, or blood glucose levels (data not shown).

Compared with that in the four naive control rats, the baseline cortical EEG (recorded before carotid artery occlusion) of the 92 experimental rats was characterized by increased synchronous delta activity, most probably due to previous electroateterization of the vertebral arteries. Immediately after carotid occlusion the cortical EEG amplitude began to decrease, and in 62 rats (68%) isoelectricity was observed in <2 minutes. Following 8–12 minutes of recirculation, slow, arrhythmic delta activity typically appeared in all 58 rats considered. Within the first 1–3 days of recirculation frequency and amplitude of all bands and in all derivations considered recovered, while the global cortical EEG activity declined during the 21 days.

Postischemic changes in the quantitative cortical EEG of the saline-treated group are exemplified in Figure 1. Typically, within the first 24 hours of recirculation, the delta frequency and amplitude reached values comparable to those at baseline and were organized in synchronized episodes of mixed fast (3–3.25 Hz) and slow (1–1.25 Hz) delta waves. In contrast, recovery of the theta and alpha waves was incomplete; their amplitudes 24 hours after occlusion were lower than those at baseline, and desynchronized signals were evident. Subsequently, delta activity decreased markedly to approximately 35% of baseline values at day 21. Activities of the other bands, although slightly increased, still remained below baseline values between days 1 and 7 and then declined further to approximately 40% of baseline values. At day 7, bursts of hypersynchronized fast (12–14 Hz) activity lasting a few seconds were evident, generally at the end of a synchronized episode (data not shown). Alternations between sleeping and waking reappeared by day 3 and subsequently declined to a nearly continuous state of wakefulness by day 21.

Figure 1 also reports postischemic changes in the quantitative cortical EEG of the siagoside-treated group. Although the pattern of electrophysiologic changes was similar to that observed in the saline-
treated group, the subsequent decline in cortical EEG activity was either absent or markedly reduced. Accordingly, while no difference was found in beta activity (data not shown), delta and theta activities were significantly higher in almost all derivations considered. Likewise, the alpha1 and alpha2 activities were also consistently more elevated. In addition, at these times the siagoside-treated group showed longer periods of synchronized sleep.

All coronal sections examined were well perfusion-fixed. No residual intravascular blood was present. Fixation artifacts (e.g., dark cells or dropsical cells) were absent, and neuronal morphology was well preserved.

Thirty minutes of near-complete forebrain ischemia consistently resulted in morphologic manifestation of neuronal damage in all rats considered. Although we also observed damage in the cerebral cortex and dorsolateral thalamus, the most consistently affected area was the CA1 sector of the hippocampus. The CA1 pyramidal neurons were, in most cases, severely reduced in number or had virtually disappeared (Figure 2). When present, most CA1 pyramidal neurons displayed a normal morphology; a few showed features typical of neuronal damage (i.e., shrinkage and triangulation) but not microvacuolation or hyperchromasia of the cell body and nucleus.20 Thus, we graded hippocampal damage as the density of surviving CA1 pyramidal neurons.

Figure 3 reports the density of surviving CA1 pyramidal neurons in the naive control, saline-treated, and siagoside-treated rats. While most saline-treated rats displayed a loss of >50% CA1 pyramidal neurons, this amount of damage occurred less frequently among the siagoside-treated rats. Furthermore, comparing the means, the siagoside-treated group showed a significantly higher density of surviving neurons than the saline-treated group (p<0.05).

Discussion

Transitory forebrain ischemia in rats produced by the four-vessel occlusion method resulted in a relatively circumscribed loss of most pyramidal neurons in the hippocampal CA1 region. The rats also displayed neurophysiologic signs of temporally evolving cortical damage after ischemia. Although the mean quantitative cortical EEG power spectrum recovered partially during the first 1–3 days of reperfusion, within 21 days cortical EEG activity of all bands in all derivations considered declined. Such secondary deterioration of cortical EEG activity suggests a delayed impairment of previously recovered neuronal transmission in this region. This suggestion is further supported by the observation that the initial recovery of sleep–wake alternation patterns seen after 3 days of reperfusion also disappeared, leaving a nearly continuous state of wakefulness by day 21. The neurophysiologic dysfunction of the cortical circuitry observed at relatively long reperfusion times most
probably reflects a decrease in the efficiency of or a loss of synaptic contacts. Such findings are in line with and extend previous observations regarding the occurrence of selective neuronal damage in areas other than the hippocampus (such as the neocortex and striatum) following transient global ischemia.

Systemic administration of siagoside following transitory forebrain ischemia in rats reduced the cortical EEG deterioration, as well as the loss of sleep–wake alternation patterns that developed in a time–dependent manner following ischemia. Furthermore, treatment with siagoside limited neuronal loss in the CA1 region of the hippocampus. Since the severity of ischemia per se, as monitored by mean cortical EEG suppression, was identical in both groups, we attribute this reduction in the ensuing postischemic damage to the cortical circuitry and the hippocampal CA1 region to treatment with siagoside.

Our findings are consistent with previous reports demonstrating that systemic treatment with monosialoganglioside (GM1 or its inner ester derivative, siagoside) is efficacious in limiting mortality and the loss of hemispheric Na,K-ATPase activity in gerbils subjected to permanent unilateral carotid artery occlusion; brain edema, Na\(^+\) and Ca\(^+\) intracellular loading, and behavioral deficits following focal cortical ischemia in rats; elevated concentrations of cyclooxygenase and lipoxygenase metabolites after transient hypoxia-ischemia in rats; morphologic damage and neurologic deficits subsequent to transient middle cerebral artery occlusion in cats; and neurologic impairment after global cerebral ischemia in monkeys.

The molecular mechanisms underlying the capability of siagoside to limit neuronal damage after a cerebral hypoxic/ischemic insult remain to be fully defined. When added to cultured neuronal cells, monosialogangliosides are stably incorporated into the outer layer of the cell membrane in a temperature-, time-, and concentration-dependent manner. A similar phenomenon occurs in the CNS following the systemic administration of monosialogangliosides in vivo. Furthermore, gangliosides, including the monosialogangliosides, reduce glutamate-related toxicity in cultured CNS neurons. Likewise, systemically administered monosialogangliosides are efficacious in reducing excitotoxin-induced brain damage in both neonatal and adult rats. Thus, it is conceivable that the siagoside effects that we report reflect the ability of this agent to reduce excitatory amino acid–related neurotoxicity in ischemic brain.

Also noteworthy is that the antiexcitotoxic effect of gangliosides observed in vitro has been attributed solely to inhibition of the downstream consequences of excessive excitatory amino acid–receptor activation. In addition, gangliosides are capable of reducing glutamate neurotoxicity at 37°C when given as long as 30 minutes after glutamate exposure. In contrast, agents such as phencyclidine, known to act at the NMDA receptor level, are effective only when administered concurrently with or immediately after glutamate. These aspects differentiate gangliosides from NMDA receptor antagonists.

Another, not mutually exclusive, possibility concerns the relation between monosialogangliosides and neuronotrophic factors. Recent studies indicate that nerve growth factor may protect hippocampal CA1 pyramidal neurons against ischemic injury in both gerbils and rats. Because GM1 potentiates the action of neuronotrophic factors both in vitro and in vivo, we cannot exclude a potentiation of endogenous neuronotrophic factors as contributing to the action of siagoside in reducing neuronal damage after ischemia.

Recent studies have shown that early treatment with GM1 may ameliorate outcome in patients affected by acute cerebrovascular insufficiencies. This evidence, together with our results, supports the continuation of clinical trials to assess further the efficacy of treatment, not only with GM1 but also with its inner ester derivative, siagoside.

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