Methionine Sulfoximine Reduces Cortical Infarct Size in Rats After Middle Cerebral Artery Occlusion

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The methionine analogue methionine sulfoximine was administered to 10 rats 24 hours before occlusion of the proximal left middle cerebral artery. Three days later the rats were decapitated and the brain infarct volumes were compared with those in 10 control rats that received saline before middle cerebral artery occlusion. The mean volume of the infarct in the cerebral cortex was reduced by 33% in the group treated with methionine sulfoximine (p<0.01). This protective effect may be mediated by a presynaptic mechanism; methionine sulfoximine profoundly inhibits brain glutamine synthetase, thereby interrupting the astrocyte-neuron glutamate shuttle and impairing neuronal glutamate release. Methionine sulfoximine also increases brain glycogen stores, and this increased energy reserve may benefit penumbral tissue during the peri-infarct period. Further study of the mechanisms by which methionine sulfoximine decreases infarct volume could lead to new therapeutic approaches for stroke. (Stroke 1990;21:322-327)

The methionine analogue L-methionine-\textsuperscript{d/-sulfoximine (MSO) first attracted notice in 1946, when it was identified as the convulsant agent formed in wheat flour bleached by the nitrogen trichloride process.\textsuperscript{1,2} The epileptogenic potential of MSO has since prompted numerous and diverse investigations, resulting in a broad characterization of its effects on brain metabolism.\textsuperscript{3-6} These metabolic effects may be of use in understanding the pathogenesis of ischemic brain injury and in developing novel agents for stroke treatment.

One effect of MSO is a profound and irreversible inhibition of brain glutamine synthetase.\textsuperscript{7,8} This enzyme is localized exclusively to glia\textsuperscript{9,10} and is essential to the astrocyte-neuronal shuttle by which glutamine is provided to neurons.\textsuperscript{11,12} Glutamine serves as the major precursor for the neurotransmitter pools of the excitatory amino acids glutamate and aspartate.\textsuperscript{13-15} The brain extracellular concentrations of these compounds have been found to increase markedly during ischemia,\textsuperscript{16} and a growing body of evidence supports a role for excitatory amino acids in the production of ischemic brain injury.\textsuperscript{17} In vitro studies have shown that elimination of glutamine from the media can prevent the hypoxic neuronal death that is mediated by excitatory amino acids.\textsuperscript{18} Additionally, MSO has been shown to reduce the release of glutamate from rat striatal tissue.\textsuperscript{19,20} Inhibition of glutamine synthetase by MSO during ischemia could reduce the available pool of glutamine and thereby reduce excitatory amino acid release. MSO could act via this presynaptic mechanism to prevent excitatory amino acid-mediated ischemic brain injury.

A second effect of MSO is a marked increase in brain glycogen content. The mechanism of this effect is as yet uncertain,\textsuperscript{20-23} but glycogen is known to accumulate in all regions of the brain\textsuperscript{24,25} and to be localized predominantly to astrocytes.\textsuperscript{26} Under ischemic conditions this increased glycogen reserve represents a greater than twofold increase in potentially available adenosine triphosphate (ATP).\textsuperscript{21,27} This energy reserve could be used in a number of processes vital for the maintenance of brain homeostasis during ischemia, such as uptake of extracellular excitatory amino acids, K\textsuperscript{+}, and H\textsuperscript{+}.\textsuperscript{28-30}

Since these effects of MSO might protect the brain during ischemia, our study was designed to assess the effects of MSO in an animal model of stroke. Rats were given either MSO or saline 24 hours before
middle cerebral artery (MCA) occlusion and decapitated 3 days later. Brain infarct volume was taken as the histologic end point.

Materials and Methods

Male Sprague-Dawley rats weighing 300–400 g were housed on a 12-hour light:dark cycle with free access to food and water. The rats were fasted beginning 12 hours before the administration of MSO or saline because animals not fasted before MSO administration were found to have variable increases in brain glycogen content, presumably because plasma methionine competes with MSO for transport into the brain. A fresh solution of MSO (Sigma Chemical Co., St. Louis, Missouri) was prepared by dissolving 50 mg/ml in normal saline and sterilized by passage through a 0.2-μm filter. This MSO solution was injected intraperitoneally at a dose of 100 mg/kg. Control rats received 1 ml sterile saline. All rats were fasted until after the surgical procedure.

Twenty-four hours after the injections 20 rats (10 control, 10 MSO-treated) were subjected to proximal MCA occlusion by the method of Shiraishi and Simon as outlined below. After the administration of 400 mg/kg i.p. chloral hydrate, a femoral artery was cannulated to monitor blood pressure, glucose concentration, and blood gases. The foramen ovale was enlarged with a dental drill under continuous saline irrigation to gain access to the proximal left MCA. When sufficiently exposed, the overlying dura was cut and the vessel was lifted from the cortical surface. Bipolar diathermy was used to coagulate a segment of the MCA between its origin and the olfactory tract (approximately 2.5 mm). All visible lenticulostriate branches originating from this segment were also coagulated. Blood gases and glucose concentrations were measured just before the initial scalp incision and again 40 minutes after the MCA occlusion, and blood pressure was monitored continuously. All rats survived the procedure and were able to eat and drink within 18 hours.

Three days after the procedure, the rats were decapitated. The brains were immediately removed, frozen in 2-methyl butane at −30°C, and stored at −70°C until cryostat sectioning. Twenty-micron frozen coronal sections were taken at 100-μm intervals, beginning at the anterior edge of the corpus callosum and ending at the caudal aspect of the posterior commissure, yielding approximately 60 sections per brain. These landmarks were chosen so as to span the entire extent of the infarcts, except for a small portion extending into the frontal poles. The sections were stained alternately for succinate dehydrogenase and for Nissl substance using cresyl echt violet. These stains were chosen because they provide an excellent contrast in staining intensity between normal and infarcted gray matter at 72 hours after stroke. The two stains were used to obtain independent determinations of the infarct volumes for each brain. These determinations were in every case within 10% of one another.

The infarct volumes were measured using an image analysis system as described by Swanson et al. Normal gray matter in lesioned structures is recognized by optical densities equal to or greater than the lowest optical density in gray matter of the corresponding structure in the unlesioned hemisphere. An MCID system (Imaging Research, St. Catharines, Canada) was used to measure four areas in each section: the areas of normal cortical gray matter and normal basal ganglia gray matter in the lesioned hemisphere and the areas of normal cortical gray matter and normal basal ganglia gray matter in the unlesioned hemisphere. The respective volumes were obtained by integrating the area measurements over the span of the sections. The infarct volumes were obtained by subtracting the surviving gray matter volumes in the lesioned hemispheres from the gray matter volumes in the unlesioned hemispheres and are expressed as percentages of the gray matter volumes in the unlesioned hemispheres. This approach (i.e., measurement of surviving [normal] gray matter areas rather than direct measurement of infarcted areas) provides reproducible volume determinations (standard deviations [SDs] of <5%) and minimizes the error introduced by edematous expansion of the infarcted tissue and surrounding white matter.

For the determination of brain glycogen content, 24 hours after the injections 10 rats (five control, five MSO-treated) were killed using a Gherling Moore Model 4104 Microwave Fixation System (Cober Electronics, Stamford, Connecticut) by focused microwave irradiation of the head, using a 1.85-second exposure to a 3.5 kW, 2450-MHZ signal. Samples of the parietal cortex were removed, sonicated in 10 volumes of 0.03N HCl, and stored at −20°C. Glucose and glycogen concentrations were assayed using the amyloglucosidase method of Passonneau and Lowry, and the values were normalized to protein as determined by the method of Lowry et al.

For the determination of whether the increased glycogen stores induced by MSO could be used under ischemic conditions, 24 hours after the injections six rats (three control, three MSO-treated) were decapitated. After 20 minutes the brains were removed, and samples of the parietal cortex were taken and assayed for glucose, glycogen, and protein content as above.

The results are presented as mean±SEM. Student's two-tailed t test was used to analyze differences between control and MSO-treated groups.

Results

All 28 MSO-treated rats exhibited characteristic behavioral changes beginning 4–5 hours after the intraperitoneal injections, consisting of decreased exploratory behavior, ataxic locomotion, and brief episodes of repetitive limb movements. These effects decreased over the subsequent 8–10 hours, and by 15
hours after the injections the MSO-treated rats were indistinguishable from the 26 control rats.

Of the 20 rats that underwent MCA occlusion, one control animal was found to have a hemorrhage at the surgical site. Data from the remaining 19 rats are presented in Table 1. The infarcts produced by the MCA occlusion were reproducible, exhibiting SDs of <20% of the means in both the control and MSO-treated groups.

A mean of 52% of the cortical gray matter ipsilateral to the MCA occlusion was infarcted in the control group compared with 35% in the MSO-treated group (Table 1); this represents a 33% reduction in infarct volume (p<0.01). The spared tissue generally comprised the lateral parietal cortex, a region that was usually infarcted in the control rats (Figure 1). In contrast to the difference in cortical infarct volumes, the basal ganglia infarct volumes were nearly identical in the two groups (Table 1).

There were no significant differences in body weight, glucose concentration, blood pressure, arterial pH, or PCO2 between the two groups. PO2 was higher in the control group (91.6 vs. 83.2 torr, p<0.05) before but not 40 minutes after MCA occlusion.

The brain glycogen content of MSO-treated and control rats is shown in Figure 2. An 81% increase in glycogen content was evident 24 hours after the MSO injections, with no significant change in brain glucose content. In both control and MSO-treated rats the brain glycogen content fell to negligible levels after 20 minutes of ischemia (Figure 2).

**Discussion**

We found that systemic administration of MSO 24 hours before MCA occlusion significantly decreased the infarct volume in the rat cerebral cortex. In contrast, MSO had no effect on infarct volume in the basal ganglia. Partial protection of the neocortex but not the basal ganglia may be related to patterns of collateral blood flow. Much of the striatum in rats and humans is...
Glucose concentrations fell to negligible levels in both saline- and glucose (open bars) levels in normal and ischemic rat brain. Methionine sulfoximine (MSO) increased glycogen bars) and glucose (open bars) levels in normal and ischemic flow during the acute postocclusion period is ganglia may indicate that a minimal level of blood volume caused solely by pharmacologic reduction in such that acute MCA occlusion results in a zone of reduced but not absent perfusion in the lateral cortex. The failure of MSO to protect the basal cortex. The failure of MSO to protect the basal supple- ed by the MCA receive collateral blood flow such that acute MCA occlusion results in a zone of supply ed by "end arteries" such that occlusion of these penetrating arteries as occurs with the technique that we used completely and irreversibly interrupts the blood supply. In contrast, cortical regions supplied by the MCA receive collateral blood flow such that acute MCA occlusion results in a zone of reduced but not absent perfusion in the lateral cortex. The failure of MSO to protect the basal ganglia may indicate that a minimal level of blood flow during the acute postocclusion period is required for efficacy. At 72 hours after stroke, infarcted tissue and the peri-infarct white matter are expanded by edema. In assessing the effect of pharmacologic intervention on infarct size, it may be important to distinguish a true reduction from an apparent reduction in infarct volume caused solely by pharmacologic reduction in peri-infarct edema. The method that we used to measure infarct volume is essentially independent of edema effects as only surviving (normal) gray matter areas are measured directly and normal gray matter is only minimally affected by peri-infarct edema. In addition to frank infarction, focal ischemia causes selective neuronal death, particularly in regions near the infarct. It is possible that substantial neuronal loss occurs in the cortex that appears "spared" by MSO treatment in this model. On the other hand, there may be less neuronal injury in the regions surrounding this spared tissue than in the corresponding regions of control rats. The question of whether MSO also lessens selective neuronal injury after focal ischemia is not addressed by our data and warrants further study. Although the mechanism of the observed protective effect of MSO is uncertain, the known effects of this compound on brain metabolism suggest two new approaches that could potentially be exploited in therapy. First, MSO causes an 80–90% reduction in brain glutamine synthetase activity for several days after a single administration. Glutamine is a major precursor for the neurotransmitter pools of glutamate and aspartate such that the inhibition of brain glutamine synthetase by MSO may mitigate increases in the concentrations of these excitatory amino acids in the extracellular space during ischemia. Postsynaptic blockade of excitatory amino acid action by both competitive and noncompetitive receptor antagonists have been reported to decrease infarct size. The inhibition of glutamine synthetase by MSO or other agents may represent an alternative, presynaptic approach to the prevention of excitatory amino acid neurotoxicity. A second effect of systemic MSO administration is a marked increase in brain glycogen content. Glycogen accumulates in astrocytes but not in neurons or the liver. The increased glycogen concentration represents an increase in the available energy stores in penumbral tissue that has both a compromised blood supply and a transiently increased metabolic demand. A transient increase in glucose utilization in tissue surrounding the ischemic core has been demonstrated by 2-deoxyglucose autoradiography and has been attributed to efflux of K+ and excitatory amino acids. Increased glial energy reserves in the form of glycogen may be used to fuel active uptake of K+, H+, and excitatory amino acids, all of which have been implicated in ischemic neuronal death. Glial glycogen might also be mobilized to furnish neurons with a metabolic substrate during ischemia. In our study, MSO increased the glycogen content of the cerebral cortex by 81% (Figure 2). Essentially complete utilization of brain glycogen was seen following decapitation (Figure 2), confirming that the additional glycogen can be metabolized under ischemic conditions. The potential benefit of increased brain energy stores in the form of glycogen is tempered by the potentially detrimental effects of the lactic acid generated by glycogenolysis. Within limits, however, increases in brain glycogen content would not be expected to contribute significantly to ischemic lactate accumulation. The lactic acid generated after even a tripling of normal brain glycogen stores would not approach the levels considered toxic. This contrasts with the effect of hyperglycemia during ischemia, when the continued delivery of plasma glucose to damaged tissue results in very high lactate accumulations. Moreover, glycogenolysis yields 1.5 ATP molecules per lactate molecule generated, as opposed to 1 ATP per lactate generated from free glucose. Whether the observed protective effect of MSO is due to inhibition of glutamine synthetase, increased glycogen stores, or some other action is uncertain. The mechanism of this effect may be clarified by studies using agents with more selective metabolic effects. For example, alkyl analogues of MSO have been shown to inhibit glutamine synthetase but may not alter brain glycogen levels. Other agents may increase glycogen content without affecting gluta-
mine synthetase. Another potential approach to blocking the astrocyte-neuronal glutamine shuttle may be via inhibition of neuronal glutaminase.

These approaches represent relatively unexplored avenues for stroke therapy. Increasing brain glycogen content or inhibiting neuronal glutamate release may be particularly useful as prophylactic treatments in patients at high risk for stroke, such as those undergoing cardiac, carotid, or intracranial surgery and those with cardiac wall or valvular thrombi.

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References


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