Nicardipine Reduces Ischemic Brain Injury
Magnetic Resonance Imaging/Spectroscopy Study in Cats

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We investigated whether the calcium channel entry blocker nicardipine would reduce ischemic brain damage in barbiturate-anesthetized cats subjected to permanent unilateral occlusion of the middle cerebral artery. The evolution of cerebral injury was assessed in vivo in 24 cats by a combination of proton magnetic resonance imaging and phosphorus-31 magnetic resonance spectroscopy for 5 hours following occlusion. Immediately thereafter, the volume of histochemically ischemic brain tissue was determined planimetrically in triphenyl tetrazolium chloride-stained serial coronal sections. Nicardipine was initially administered as an intravenous bolus injection of 10 mg/kg/hr 15 minutes before or 15 minutes after occlusion, followed by continuous infusion at 8 mg/kg/hr for the 5 hours of the experiment. Compared with untreated controls, cats that received nicardipine before or after occlusion showed a significant reduction in the extent of edema in the ipsilateral cerebral cortex, internal capsule, and basal ganglia. The results of phosphorus-31 magnetic resonance spectroscopy studies suggest that nicardipine may protect against cerebral ischemic damage by an action on cellular metabolic processes that preserve high-energy phosphates during the ischemic period. (Stroke 1989;20:268-274)

Ischemic depolarization of nerve membranes is associated with a rapid influx of calcium into the intracellular compartment and with impaired mitochondrial adenosine triphosphate (ATP) production. The resulting disruption of the ATP-dependent Na⁺-K⁺ pump in the cell membrane causes Na⁺ and water to accumulate in the cell. Subsequently, the breakdown of the blood-brain barrier promotes macromolecular leakage from the intravascular space and accumulation of extravascular water.

Calcium channel blockers appear to inhibit calcium entry into cells via the so-called slow channels in the cell membrane and prevent or reduce metabolic disturbances associated with ischemia. Our study was designed to test whether a specific calcium channel entry blocker, the 1,4-dihydropyridine nicardipine, would reduce cerebral injury induced by permanent middle cerebral artery (MCA) occlusion in cats. Nicardipine is highly lipophilic because of its tertiary amine structure in the ester sidechain and thus readily penetrates cell membranes. Also, in the low-pH conditions found in ischemia, nicardipine is almost completely protonated and hence is preferentially sequestered in acidotic tissues. It has also been proposed that nicardipine may have a mechanism of action similar to the Class III antagonists, such as flunarizine, which have been found useful in some ischemic conditions.

Since calcium antagonists may produce different neuropathologic outcomes depending on whether they are given before or after stroke, we administered nicardipine 15 minutes before or 15 minutes after MCA occlusion. We used proton magnetic resonance imaging (MRI) to detect alterations in brain tissue water content in nicardipine-treated and untreated (control) MCA-occluded cats. Phosphorus-31 magnetic resonance spectroscopy (MRS) was performed concurrently to compare the extent of cerebral metabolic injury. Histochemical mapping of the viability of the electron transport chain enzymes was carried out with 2% 2,3,5-triphenyl tetrazolium chloride (TTC) to correlate spatially the evolution of ischemic tissue injury with the MRI/MRS results 5 hours after MCA occlusion.

Materials and Methods

Twenty-four cats were tranquilized with 1–2 mg/kg i.m. acepromazine, anesthetized with 30–35 mg/kg i.v. sodium pentobarbital, and ventilated with a Drager respirator (Telford, Pennsylvania) to maintain normal PaO₂ and PaCO₂. Femoral vein and artery catheters were placed for blood pressure monitoring and drug administration. The cats' rec-
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FIGURE 1. Triphenyl tetrazolium chloride (TTC)-stained serial coronal sections of cat brain showing areas of ischemic damage in right hemisphere 5 hours after permanent unilateral occlusion of ipsilateral middle cerebral artery (MCA). In black and white photographs, gray matter appears black to dark gray, ischemic zones are light gray, and totally ischemic or infarcted tissues (which lack electron transport chain enzymes) appear white. Cortical and subcortical tissue injury extends from septal nuclei (A) through hypothalamic area (B). At level of midbrain and anterior pons (C), injury is seen only in cortical area.

tal temperature was maintained at 37±0.5° C, and isotonic saline was administered throughout the surgical procedure.

The MCA was occluded just proximal to the origin of the lateral striate arteries with bipolar electrosurgery and then completely transected. The dural incision and orbit were covered with saline-moistened gauze or absorbable gelatin sponge. The ipsilateral temporal muscles were excised to optimize the external placement of the surface coil over the parietotemporal fossa. The cats were then immediately placed in the MRI unit.

Coronal and axial brain images were obtained using a CSI-II spectrometer/imager (General Electric, Schenectady, New York) operating at 2.0 T. An 8.3-cm i.d. “bird cage” head coil tuned to the proton resonance frequency of 85.552 MHz was used to acquire two-dimensional spin–echo images. Other machine parameters included an 8×8-cm

FIGURE 2. T₂-weighted coronal magnetic resonance images 2 and 5 hours after occlusion of right middle cerebral artery in cats. Five hours after occlusion, areas of high signal intensity indicating edema in right hemisphere are more extensive at frontal plane of anterior pons (A), hypothalamus (B), and basal ganglia (C).
Figure 3. Top: T2-weighted coronal magnetic resonance image (MRI) showing areas of cortical and subcortical edema (arrows) in cat brain 5 hours after permanent ipsilateral occlusion of middle cerebral artery. Bottom: Triphenyl tetrazolium chloride–stained coronal section of same brain. Note close correspondence of unstained ischemic–necrotic tissue (arrows) with areas of cerebral edema shown on MRI.

Field of view, a 5-mm-thick slice, and an acquisition matrix of 128 phase-encoded steps by 256 complex frequency encoding points. Two spin–echo sequences were used: a multislice (four slices) T2-weighted (TR=3000 msec, TE=100 msec, two acquisitions) sequence and a single-slice T2-weighted (TR=500 msec, TE=20 msec, two acquisitions) sequence. Region-of-interest (ROI) analyses were carried out in the injured parietal and temporal cortex, internal capsule, and caudate nucleus and in the corresponding uninjured contralateral regions in nicardipine-treated and control cats.

Spectra were obtained using a circular 1.5-cm two-turn balance–matched surface coil tuned to the phosphorus24 resonance frequency (34.631 MHz) and fixed with adhesive directly to the skull surface overlying the parietal cortex in the territory of the occluded MCA. The surface coil was centered in a standard position 1.0 cm lateral and 0.8 cm posterior to the bregma. Spectral enhancement (Gaussian line broadening of 15 Hz) was used to display the spectra. Spectra were phased and analyzed with a line-fitting computer simulation program. The beta, alpha, and gamma peaks for ATP and the peaks for phosphocreatine (PCr), phosphodiesters (PD), inorganic phosphate (Pi), and phosphomonoesters (PM) were resolved. The Pi:PCr ratio was calculated to quantify the bioenergetic status of the tissues. Intracellular pH (pHi) was calculated from the chemical shift difference between the phosphorus-31 signals of Pi and PCr using the titration curve described previously.10

Twenty-one cats were randomly assigned to control (MCA occlusion but no nicardipine, n=8), preocclusion treatment (10 mg/kg i.v. nicardipine 15 minutes before MCA occlusion, n=8), or postocclusion treatment (10 mg/kg i.v. nicardipine 15 minutes after MCA occlusion, n=5) groups. After the initial bolus injection, cats in both treatment groups received nicardipine HCl (Syntex Laborato-
Control 2 hr  Post-occlusion 2 hr  Pre-occlusion 2 hr

Control 5 hr  Post-occlusion 5 hr  Pre-occlusion 5 hr

**Results**

Occlusion of the MCA had no significant influence on mean arterial blood pressure, Pao2, Paco2, or heart rate. There was no significant difference in blood glucose concentration between the control and the combined nicardipine-treated groups; values for both remained within normal limits for this species.

At the doses we used, nicardipine induced a 10–15 mm Hg drop in mean arterial blood pressure from the control level of 120±20 mm Hg; this mild hypotensive effect was stable throughout the infusion. There was a corresponding slight increase in heart rate (peak, 22% above control).

Ischemic tissue damage was observed within the territory of the occluded MCA, including the lateral orbital gyri, the inferior and middle frontal gyri, the inferior and superior parietal lobules, the superior and middle temporal gyri, parts of the lateral gyri of the occipital lobe, the internal capsule, and most of the caudate and globus pallidus (Figure 1). These areas had the morphologic characteristics of the ischemic cell process, including coagulation necrosis and glial proliferation. At the center of the ischemic cortex the cytoarchitecture and cortical layering were completely disrupted. Neurons showed clumps of chromat substance, typical of degenerating cells. Extensive vacuolation of the neuropil, characteristic of cerebral ischemia, was also seen.

In the sequential studies of control cats, the progression of the evolving ischemic lesion was well demonstrated by MRI. Mass effect was usually detected on T2-weighted images within 45 minutes after occlusion as a shift from the midline and a
The effects of nicardipine in attenuating cerebral edema induced by MCA occlusion could be seen clearly on sequential T₂-weighted multislice MRI. One to three hours after occlusion, there was minimal edema in the cortex and basal ganglia in the injured hemisphere. Five hours after MCA occlusion, there was significantly less edema in the injured parietal and temporal cortex, internal capsule, and caudate nucleus in the nicardipine-treated cats than in the controls.

Figure 5 shows phosphorus-31 brain spectra obtained from a control cat just before and 20 minutes after occlusion of the MCA. The spectrum recorded before occlusion showed the characteristic resonances of PM, Pi, PD, and PCr and the gamma, alpha, and beta resonances of ATP. The mean PCr/3-ATP ratio found in fully relaxed spectra was 1.8±0.2 and the Pi:PCr ratio was 0.32±0.11, which are in close agreement with the values for brain tissue published by other groups. The average chemical shift of the Pi resonance with respect to PCr was 4.93±0.13 ppm, corresponding to a pH of 7.11±0.11.

The time course of changes in bioenergetic status induced by MCA occlusion is summarized in Figure 6. Within 30 minutes there was a clear increase in Pi. The Pi peak from injured tissue split from the Pi peak from normal tissue and shifted toward the PD peak. pH dropped from 7.11±0.11 before ischemia to 7.04±0.18 at 1 hour and 6.85±0.12 at 5 hours after MCA occlusion. These changes are in general agreement with other studies of MCA-occluded cats in which brain pH was measured with phosphorus-31 MRI using a surface coil or topographically using neutral red as an internal pH indicator.

Qualitative differences in spectra were seen in both nicardipine-treated groups compared with controls. Cats treated with nicardipine before or after MCA occlusion appeared better able than controls to preserve their preischemic concentrations of high-energy phosphates and pH while maintaining lower levels of Pi. Because of the variable responses of individual cats in both the treated and the control groups, however, the averaged quantitative differ-
Control Nicardipine Nicardipine
pre-occlusion post-occlusion
2.0 hr 2.0 hr 2.0 hr
post MCA-O post MCA-O post MCA-O
4.0 hr 4.0 hr 4.0 hr
post MCA-O post MCA-O post MCA-O

Figure 6. Phosphorus-31 spectra 2 and 4 hours after unilateral occlusion of middle cerebral artery in untreated control and nicardipine-treated cats. Placement of coil was standardized, enabling comparisons of peak intensities in different cats. There was progressive deterioration in metabolic status of control cat compared with cats treated with nicardipine before or after occlusion. Inorganic phosphate resonance of injured tissue in control cat is also shifted to lower frequency, indicating developing intracellular acidosis.

Discussion

Our results demonstrate that nicardipine, a substitued pyridine calcium channel blocker, can ameliorate posts ischemic brain injury if given just before or even 15 minutes after occlusion of the MCA. Nicardipine administration preserved preischemic concentrations of high-energy phosphates while maintaining low levels of Pi and significantly reduced cerebral edema 5 hours after occlusion. pH, declined only slightly in nicardipine-treated cats, whereas a progressive acidosis developed in the ischemic MCA territory of untreated controls.

Nicardipine has been shown to cross the blood-brain barrier very effectively in normal rats and in rats with cerebral ischemia (first-pass extraction). There is also some evidence that under ischemic conditions, the intraneuronal access of nicardipine is enhanced, which may explain why nicardipine seemed to protect central nervous system neurons even when administered after MCA occlusion in our study. Alps and Hass previously reported a neuroprotective action of nicardipine given before or after occlusion in a 10-minute four-vessel rat model of transient forebrain ischemia. The effects were particularly evident in cerebral tissues such as the hippocampus, which are known to be "selectively vulnerable" to ischemia-induced injury. Nicardipine has also recently been shown to protect hippocampal CA1 neurons in gerbils surviving 72 hours after 5 minutes of bilateral carotid artery occlusion, whereas similar doses of nimodipine were ineffective.

It has been suggested that nimodipine may promote the recovery of function in some models of cerebral ischemic injury by improving postischemic hyperperfusion. Nicardipine, on the other hand, appears to protect ischemic brain tissue mainly by inhibiting disturbances in cerebral metabolism.

Several observations from our study support this interpretation. Following MCA occlusion, the cerebral Pi:PCr ratio and ATP levels were better preserved in nicardipine-treated cats than in control cats. These observed differences could have resulted from increased synthesis and/or decreased consumption of high-energy phosphates. The first possibility seems less likely since, in the absence of oxygen, increased ATP synthesis would also increase lactate concentrations and result in lower pH. The reductions in cerebral pH in nicardipine-treated cats were only slight.

At the same time, there are insufficient data from this study and previous work to exclude the possibility that nicardipine exerts its cerebroprotective action by enhancing regional blood flow in the
brain. Collateral blood flow is critical with the MCA-occlusion model of stroke, and agents that improve collateral flow have been shown to decrease infarct size and edema.

Barbiturate anesthesia also probably contributed to the reduction in ischemic injury. Several recent investigations used doses of pentobarbital similar to those of our study in evaluating the effects of MCA occlusion in cats. In one of these other studies, it was shown that barbiturate anesthesia delayed the disappearance of high-energy phosphates, particularly ATP, and the concomitant accumulation of Pi but had little effect on the ischaemia-induced decrease in pH. However, in our study, pH decreased only in the control cats. This finding suggests that nicardipine, rather than barbiturate, prevented the development of intracellular acidosis. Similarly, the Pi:PCr ratio was maintained near preischemia levels only in the nicardipine-treated cats. Thus, while we cannot exclude the possibility that barbiturate reduced ischemic tissue damage, our results indicate that nicardipine exerted the more important protective action.

In summary, the calcium channel entry blocker nicardipine significantly reduced ischemic brain damage in cats subjected to permanent unilateral occlusion of the MCA. The protective action of nicardipine in this model of stroke may be related to the drug's effects on cellular metabolic processes that preserve ATP during ischemia. We did not directly evaluate the possible beneficial effects of nicardipine in increasing collateral blood flow, but they should not be excluded. More generally, our data demonstrate the feasibility of using combined MRA/MRS to study pathophysiological changes induced by acute ischemic stroke and their modification by pharmacologic agents.

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