

# Effects of Delayed Administration of Low-dose Lidocaine on Transient Focal Cerebral Ischemia in Rats

Baiping Lei, M.D., Ph.D.,\* Susanna Popp, B.S.,† Christine Capuano-Waters, B.S.,‡ James E. Cottrell, M.D.,§ Ira S. Kass, Ph.D.||

**Background:** The authors' previous study demonstrated that a clinical antiarrhythmic dose of lidocaine, when given before ischemia, is neuroprotective in a rat model of transient focal cerebral ischemia. In this study, the authors investigated whether the administration of this dose of lidocaine, when delayed until 45 min after the onset of ischemia, also reduces ischemic brain injury.

**Methods:** Lidocaine was administered as an intravenous bolus (1.5 mg/kg) followed by an intravenous infusion ( $2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ) for 165 min, beginning 45 min after the onset of a 90-min period of transient focal cerebral ischemia. Control animals were given the same volume of saline. Focal cerebral ischemia was induced by occluding the right middle cerebral artery using an intraluminal suture. Neurologic outcome and body weight loss were quantified 7 days later. The brain was fixed 7 days after ischemia and brain sections were stained with hematoxylin and eosin for assessment of infarct size and the number of intact neurons. In separate experiments, local cerebral blood flow and the electroencephalogram were measured during ischemia and 180 min into the reperfusion period. Infarct size was assessed after 24 h.

**Results:** Infarct size, at either 24 h or 7 days after ischemia, was not significantly reduced in the lidocaine group. However, the number of intact neurons was significantly increased in both the ischemic penumbra and core of the lidocaine group 7 days after ischemia, compared with the vehicle group. Rats treated with lidocaine demonstrated better neurologic outcome and less weight loss ( $P < 0.05$ ). Lidocaine treatment had no significant influence on local cerebral blood flow and electroencephalogram during ischemia and reperfusion.

**Conclusions:** Administration of a clinical antiarrhythmic dose of lidocaine, beginning 45 min after the onset of ischemia, reduces ischemic brain injury after transient focal cerebral ischemia in the rat. This indicates that delayed administration of neuroprotective agents may reduce brain damage resulting from ischemia.

THERE are a number of *in vitro* and *in vivo* investigations suggesting that lidocaine can reduce ischemic cerebral injury.<sup>1-13</sup> Our recent study demonstrated that a clinical antiarrhythmic dose of lidocaine is neuroprotective in a rat model of transient focal cerebral ischemia.<sup>14</sup> However, all of these studies involved administration of lidocaine before the onset of ischemia. In the clinical

setting, therapeutic procedures are frequently started after the onset of cerebral ischemia.

Experimental and clinical studies have shown that focal cerebral ischemia produces a densely ischemic core and an ischemic penumbra with a moderate reduction of blood flow.<sup>15,16</sup> Recent evidence suggests that ischemic brain injury after focal ischemia progresses slowly within the ischemic region, especially the ischemic penumbra.<sup>17-20</sup> The infarct develops over a period of many hours. Although there would seem to be little hope of rescuing cells in the ischemic core, delayed therapeutic interventions may still protect neurons in the penumbra. A few studies have shown that several agents are neuroprotective in the rat model of transient focal cerebral ischemia even if administration is delayed.<sup>21-23</sup> Therefore, in the current experiment, we investigated whether administration of a clinical antiarrhythmic dose of lidocaine 45 min after the onset of ischemia reduces brain injury following transient focal cerebral ischemia in rats. In addition, we examined the effect of this dose of lidocaine on local cerebral blood flow (LCBF) using a laser Doppler flowmeter.

## Material and Methods

### Animals and Surgical Preparation

This study was approved by the Institutional Animal Care and Use Committee of the State University of New York Downstate Medical Center. Male Wistar rats (weight, 280-340 g) were allowed access to food and water before surgery. Anesthesia was induced with a gas mixture of isoflurane (3%), oxygen (40%), and nitrogen (remainder); the animals were then orally intubated and mechanically ventilated with 2-2.2% isoflurane in the same gas mixture. The end-tidal carbon dioxide, inspiratory oxygen, and inspiratory isoflurane concentrations were monitored continuously using an airway gas monitor (DATEX Intrumentarium Co., Helsinki, Finland), and ventilation was adjusted to maintain normocapnia. Temperature probes were inserted into the rectum and the left-side temporalis muscle of each animal. Rectal and pericranial temperatures were kept constant at  $38.0 \pm 0.2^\circ\text{C}$  by surface heating or cooling using a temperature-controlled heating pad (Harvard Apparatus Ltd., Edenbridge, Kent, United Kingdom) and a temperature controller (Physitemp, Clifton, NJ) throughout the surgical procedure. The tail artery and right femoral vein were cannulated for monitoring of arterial blood pressure, periodic blood sampling for arterial gases, pH, blood glucose concentration, and hematocrit (10 min

\* Research Assistant Professor, ‡ Research Associate, § Professor and Chairman, Department of Anesthesiology, † Ph.D. Student, Program in Neural and Behavioral Sciences, || Professor, Departments of Anesthesiology and Physiology and Pharmacology.

Received from the Departments of Anesthesiology and Physiology and Pharmacology, State University of New York Downstate Medical Center, Brooklyn, New York. Submitted for publication April 15, 2002. Accepted for publication July 30, 2002. Supported by the National Institute of General Medical Sciences, Bethesda, Maryland, and Brooklyn Anesthesia Research, P.C., Brooklyn, New York. Presented in part at the annual meeting of the American Society of Anesthesiologists, New Orleans, Louisiana, October 13-17, 2001.

Address reprint requests to Dr. Kass: Department of Anesthesiology, Box 6, State University of New York Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York 11203. Address electronic mail to: ikass@downstate.edu.

before ischemia, at 45 min of ischemia, and 60 min after the end of ischemia) and for drug administration. Animals were prepared for middle cerebral artery (MCA) occlusion according to the method of Koizumi *et al.*<sup>24</sup> An incision was made in the midline of the neck, the right-side carotid bifurcation was exposed, and the external carotid artery and the internal carotid artery were dissected. After completion of the surgical preparation, the isoflurane concentration was reduced to 1.7%.

#### *Transient Focal Ischemia and Drug Administration*

After a 30-min period of physiologic stabilization, the right-side common carotid artery and the external carotid artery were ligated, and the blood flow of the internal carotid artery was transiently interrupted. An arterial incision was made on the carotid bifurcation near the internal carotid artery, and a 19-mm length of silicone-coated 4-0 nylon suture was inserted into the lumen of the internal carotid artery until it blocked the origin of the MCA. At 45 min after the onset of ischemia, animals were randomly allocated to either the vehicle group ( $n = 15$ ) or the lidocaine group ( $n = 14$ ). The number of successful MCA occlusions was 11 for each group. Two animals from the vehicle group and one from the lidocaine group had a subarachnoid hemorrhage due to vessel perforation. Two animals from each group had no neurologic disturbance 30 min after recovery from anesthesia, presumably due to unsuccessful MCA occlusion. All seven of these animals were excluded from the study. In the lidocaine group, a 1.5-mg/kg (1 mg/ml in saline) bolus dose of lidocaine was administered intravenously over a period of 2 min beginning 45 min after the onset of ischemia. Thereafter, a continuous infusion of lidocaine was initiated at a rate of  $2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  and continued until 120 min after the end of ischemia. A previous study demonstrated that the plasma lidocaine concentration with the same dosage regimen was  $1.2 \pm 0.4 \text{ } \mu\text{g/ml}$  at 75 min after the beginning of lidocaine administration.<sup>14</sup> In the vehicle group, the animals were given the same volume of saline. After 90 min of ischemia, reperfusion was accomplished by withdrawal of the intraluminal suture, and the neck incision was closed. At 180 min of reperfusion, the temperature probes and the catheters were removed, the trachea was extubated, and the animal was allowed to survive for 7 days. The animal's body weight was measured before the experiment and 7 days after ischemia.

#### *Quantification of Ischemic Cerebral Damage*

A neurologic examination was done at 30 min and 7 days after recovery from anesthesia. This was performed by an observer who was blinded to the experimental groups. A standard scoring scale was used: 0, normal; 1, fails to extend the left forepaw; 2, circles to the left; 3, falls to the left; and 4, does not walk spontaneously or exhibits a consciousness disturbance.<sup>25</sup> Rats

that exhibited convulsions, that sustained consciousness disturbances, or that were without neurologic deficits 30 min after recovery from anesthesia were excluded from the study.

Seven days after ischemia, the animals were anesthetized with 3% isoflurane and perfused transcardially with saline, followed by a 10% buffered formalin solution for the fixation of tissues. The brain was removed and post-fixed in a 10% formalin solution for 4 to 5 days at 4°C. Each brain was cut into seven 2-mm-thick coronal blocks using a rat brain matrix. The blocks were cryoprotected with a series of 20, 25, and 30% sucrose solutions in phosphate buffer solution at 4°C, embedded in molds with embedding compound (Sakura Finetek U.S.A., Inc., Torrance, CA) and dry ice, and stored at -70°C until sectioned. A thin coronal section (16  $\mu\text{m}$ ), 1 mm from the anterior surface of each coronal block, was cut on a cryostat. The sections were dried and stained with hematoxylin and eosin. Video images of individual sections were obtained with a charge-coupled device camera (Pixera Corp., Los Gatos, CA) equipped with a macro lens. Brain areas were traced and measured with an image analysis system (NIH Image 1.60; National Institutes of Health, Bethesda, MD). The volume of infarction was calculated as the integrated product of the cross-sectioned area for the sections and the distance between sections.

For quantitation of neuronal damage, intact neurons were counted in an area of  $0.22 \text{ mm}^2$  on hematoxylin-and-eosin-stained sections (from the third coronal block) at  $\times 250$  magnification using a light microscope. The ischemic penumbra and the ischemic core were chosen for cell counting. In accordance with previous studies on the measurement of local cerebral blood flow in this focal ischemia model,<sup>15,26</sup> the primary somatosensory cortex was defined as the ischemic penumbra, and the secondary somatosensory cortex was defined as the ischemic core. Both regions were delineated on each slice by referring to a rat brain atlas.<sup>27</sup> The counting was performed by an observer who was blinded to the experimental groups.

#### *Local Cerebral Blood Flow Measurement and Electroencephalography*

In a separate experiment, 14 animals were used to determine the effect of lidocaine on cerebral blood flow and the electroencephalogram. These rats were prepared as described earlier, except that a laser Doppler probe (Probe 407-1; Perimed, Jarfalla, Sweden) and a monopolar electroencephalographic electrode (a stainless steel screw) were placed on the right side of the skull. The skull was exposed, and a small area of the skull overlying the right parietal cortex was thinned. The laser Doppler probe was firmly fixed on the skull (2 mm posterior and 5 mm lateral to the bregma). LCBF was continuously recorded throughout the experiments. The electro-

**Table 1. Physiological Variables in the Experimental Groups**

	24-h Experiment		7-day Experiment	
	Vehicle	Lidocaine	Vehicle	Lidocaine
Number	7	7	11	11
10 min before the onset of ischemia				
MABP (mmHg)	95 ± 6	93 ± 11	87 ± 5	85 ± 6
Arterial pH	7.44 ± 0.02	7.45 ± 0.02	7.43 ± 0.02	7.42 ± 0.04
Pao <sub>2</sub> (mmHg)	137 ± 17	146 ± 20	148 ± 19	134 ± 25
Paco <sub>2</sub> (mmHg)	43 ± 3	41 ± 4	42 ± 2	43 ± 2
Glucose (mg/dl)	187 ± 21	198 ± 30	182 ± 23	169 ± 23
45 min after the onset of ischemia				
MABP (mmHg)	103 ± 8 *	101 ± 11 <sup>†</sup>	105 ± 7*	108 ± 7*
Arterial pH	7.44 ± 0.03	7.45 ± 0.04	7.43 ± 0.04	7.43 ± 0.02
Pao <sub>2</sub> (mmHg)	142 ± 23	158 ± 8	158 ± 18	150 ± 18
Paco <sub>2</sub> (mmHg)	44 ± 4	42 ± 4	41 ± 3	42 ± 2
Glucose (mg/dl)	185 ± 16	199 ± 26	186 ± 22	175 ± 19
60 min after the end of ischemia				
MABP (mmHg)	90 ± 3	96 ± 7	92 ± 8	94 ± 4
Arterial pH	7.44 ± 0.01	7.44 ± 0.01	7.42 ± 0.02	7.42 ± 0.01
Pao <sub>2</sub> (mmHg)	131 ± 23	154 ± 16	158 ± 9	152 ± 15
Paco <sub>2</sub> (mmHg)	43 ± 3	42 ± 3	42 ± 2	43 ± 2
Glucose (mg/dl)	189 ± 17	189 ± 25	188 ± 14	175 ± 22

Data are mean ± SD. †  $P < 0.05$ , \*  $P < 0.01$  (paired Student *t* test) versus 10 min before the onset of ischemia.

MABP = mean arterial blood pressure; Pao<sub>2</sub> = arterial oxygen tension; Paco<sub>2</sub> = arterial carbon dioxide tension.

encephalographic electrode was attached to the right skull (4 mm lateral and 0 mm posterior to the bregma), and the reference electrode was placed under the skin of the neck. Electroencephalographic activity was continuously recorded with a Macintosh type of computer using a Maclab Bio Amp and a Maclab/4e analog-to-digital converter (AD Instruments Pty Ltd., Castle Hill, Australia). The bandpass was set at 0.5–30 Hz, and changes in electroencephalographic amplitude were quantified using spectral analysis. Both LCBF and electroencephalographic data were reported as percent of the baseline before ischemia. The animals were randomly assigned to the vehicle or lidocaine group ( $n = 7$  for each), and the dosage regimen for lidocaine administration was the same as described before. The animal was allowed to recover from anesthesia 180 min after the end of ischemia; 24 h later, the brain was fixed and removed. The third of seven coronal blocks was processed for measuring infarct size as described above. This block was chosen since it has a maximal lesion area that is well correlated with total infarct volume.<sup>21,28</sup> To correct for the effect of brain edema, infarct areas were adjusted by the ratio of the areas of both cerebral hemispheres (left over right). Cell counting was not performed on these sections because 24 h after the onset of an ischemic event is too short a time to evaluate cellular ischemic neuronal injury accurately.<sup>17</sup>

#### Statistical Analysis

All values, except for neurologic scores, are presented as mean ± SD. Infarct size, body weight, number of intact neurons, and physiologic variables were analyzed

with the unpaired Student *t* test. LCBF and electroencephalographic data were analyzed with two-way analysis of variance followed by Bonferroni posttests. LCBF and electroencephalographic data for each time point were further analyzed with the more sensitive unpaired Student *t* test. Neurologic scores are reported as the median (quartile deviation). Neurologic scores were compared by the use of the two-tailed Mann-Whitney U test. A value of  $P < 0.05$  was considered significant.

#### Results

Mean arterial blood pressure, arterial oxygen tension, arterial carbon dioxide tension, arterial pH, blood glucose concentration, and hematocrit did not differ significantly between the vehicle and lidocaine groups for the 7-day and the 24-h experiments. During the period of ischemia, there was a slight increase in mean arterial blood pressure in all groups. Moderate hyperglycemia was also noted in all groups, but there were no differences between the vehicle and lidocaine groups (table 1).

Middle cerebral artery occlusion induced an immediate reduction of LCBF to approximately 20% of baseline (fig. 1). Throughout the 90-min ischemic period, LCBF remained at 20–35% of baseline. Following withdrawal of the intraluminal suture, LCBF gradually recovered, but it did not return completely to baseline. Delayed hypoperfusion persisted until the end of the 180-min post-ischemic observation period. There were no significant differences between the vehicle and lidocaine groups at any time point during ischemia and reperfusion, even when the more sensitive Student *t* test was used.

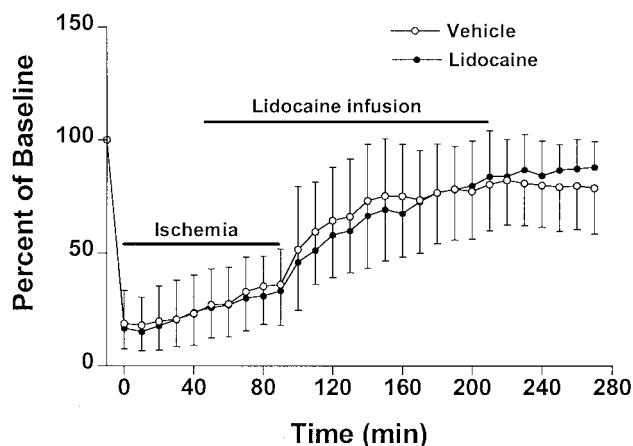


Fig. 1. Changes in local cerebral blood flow (LCBF) measured by a laser Doppler flowmeter during ischemia and reperfusion in the vehicle and lidocaine groups. Flow values shown are averages of measurements made during 30-s periods every 10 min and expressed as percent of baseline before ischemia (mean ± SD, n = 7 for each group). The first ischemic point is the value at 2 min after the onset of ischemia. The lidocaine group was not significantly different from the vehicle group at any time point.

The electroencephalographic amplitude decreased to approximately 30% of baseline immediately after MCA occlusion (fig. 2). Upon reperfusion, the amplitude did not recover significantly in either group. There was a trend toward higher amplitudes in the lidocaine-treated group after 120 min of reperfusion, but there were no significant differences between the vehicle and lidocaine groups at any time point.

Neurologic scores 30 min after recovery from anesthesia were not significantly different between the vehicle and lidocaine groups (vehicle, 2 [range, 2-2] vs. lidocaine, 2 [range, 2-2]). Seven days after ischemia, neurologic scores were significantly better in the lidocaine

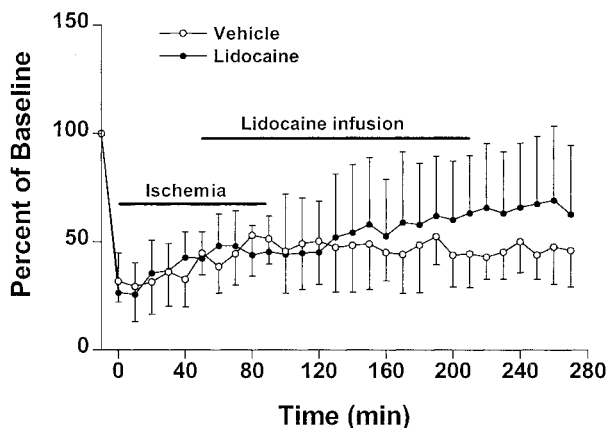


Fig. 2. Changes in the electroencephalographic amplitude during ischemia and reperfusion in the vehicle and lidocaine groups. Values shown are averages of measurements made during 30-s periods every 10 min and expressed as percent of baseline before ischemia (mean ± SD, n = 7 for each group). The first ischemic point is the value at 2 min after the onset of ischemia. The lidocaine group was not significantly different from the vehicle group at any time point.

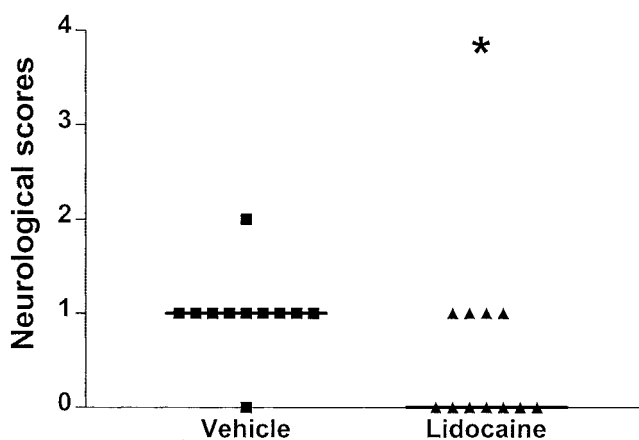


Fig. 3. Neurologic scores 7 days after ischemia. The following standard scoring scale was used: 0, normal; 1, fails to extend the left forepaw; 2, circles to the left; 3, falls to the left; and 4, does not walk spontaneously or exhibits a consciousness disturbance. Each point depicts the value for a single rat determined by an observer blinded to the treatment. Horizontal bars indicate median values for each group. \*P < 0.05 versus the vehicle group.

group than in the vehicle group (P < 0.05; fig. 3). The lidocaine-treated rats were also significantly heavier than the vehicle-treated rats 7 days after ischemia (P < 0.05; fig. 4).

Table 2 enumerates the values of total infarct area, subcortical infarct area, and cortical infarct area in the vehicle and lidocaine groups at 24 h of reperfusion. Differences between the vehicle and lidocaine groups for total, cortical, and subcortical infarct areas were not significant. Total infarct volume, cortical infarct volume, and subcortical infarct volume were also not significantly different between the lidocaine group and the vehicle group 7 days after ischemia (fig. 5).

Figure 6 shows the number of intact neurons in the ischemic penumbra and the ischemic core of the vehicle and lidocaine groups 7 days after ischemia. In the vehicle group, only a very small number of intact neurons were observed in the ischemic penumbra and the ischemic core. The number of intact neurons was significantly

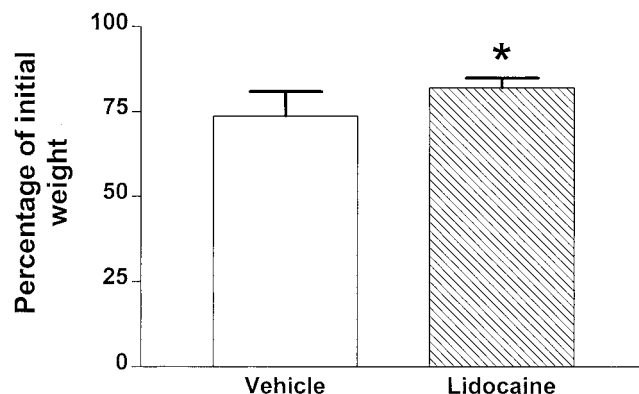


Fig. 4. Body weight 7 days after ischemia. Data are expressed as percent of initial weight and presented as mean ± SD. \*P < 0.05 versus the vehicle group.

**Table 2. Infarct Areas at 24 h after Ischemia (mm<sup>2</sup>)**

	Total Infarct Area	Cortical Infarct Area	Subcortical Infarct Area
Vehicle (n = 7)	22.5 ± 6.7	12.3 ± 5.3	9.2 ± 4.2
Lidocaine (n = 7)	17.6 ± 8.6	9.1 ± 7.0	8.6 ± 2.1

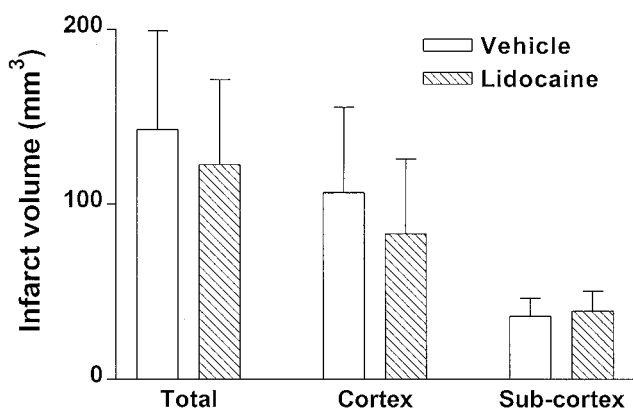
Values are mean ± SD. Infarct area was measured using hematoxylin and eosin stained sections from the third coronal block of brain tissue.

increased in both the ischemic penumbra and the ischemic core of the lidocaine group compared with the vehicle group.

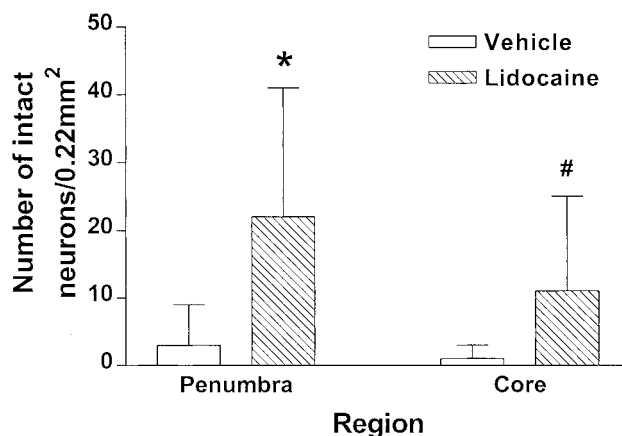
## Discussion

This study demonstrates that a clinical antiarrhythmic dose of lidocaine, when administered 45 min after the onset of a 90-min period of transient focal cerebral ischemia, is effective in reducing ischemic cerebral injury. Although the size of the infarct was not significantly reduced in the lidocaine-treated rats, there was an increase in the number of surviving neurons in both the ischemic penumbra and the ischemic core, an improvement of neurologic outcome, and an increase of post-ischemic body weight. This dose of lidocaine did not significantly alter cerebral blood flow during ischemia and reperfusion.

The measurement of infarct size is a standard method for evaluating the extent of cerebral damage after focal cerebral ischemia. However, there may not be a precise 1:1 correspondence between infarct size and the amount of cell death.<sup>17</sup> Infarct measurement is based on differences in staining between the infarct and the non-infarct areas. Identification of the infarct requires a certain percentage of the cells in that region to die. In the periinfarct area, there is some cell death but no infarct; even in the infarct area there are surviving neurons, especially at early stages of ischemia.<sup>20</sup> Thus, we mea-



**Fig. 5.** Total, cortical, and subcortical volumes of infarct 7 days after ischemia. Infarct volume (mm<sup>3</sup>) was measured in brain sections stained with hematoxylin and eosin. Data are presented as mean ± SD.



**Fig. 6.** The number of intact neurons in the ischemic penumbra and the ischemic core 7 days after ischemia. Intact neurons were counted in an area of 0.22 mm<sup>2</sup> on hematoxylin and eosin stained sections at ×250 magnification using a light microscope. The histologist was blinded to the treatment. The primary somatosensory cortex was defined as the ischemic penumbra and the secondary somatosensory cortex as the ischemic core. Values are mean ± SD. \**P* < 0.01, #*P* < 0.05 versus the vehicle group.

sured both the infarct size and the number of intact neurons as an index of ischemic cerebral damage. We found no significant differences in infarct size at 24 h and 7 days after ischemia between the lidocaine and vehicle groups; however, the number of intact neurons at 7 days after ischemia was significantly increased in both the ischemic penumbra and the ischemic core of the lidocaine group compared with the vehicle group.

The precise mechanism by which lidocaine reduced the cerebral damage after transient focal cerebral ischemia is unknown. Recent experimental and clinical studies indicate that focal cerebral ischemia produces a densely ischemic core and an ischemic penumbra.<sup>15,16</sup> Neuronal necrosis after MCA occlusion progresses from the core of densest ischemia toward the penumbral region of less dense ischemia, the latter is thought to contain potentially salvageable neurons.<sup>17,18</sup> The onset of irreversible damage in the penumbra may occur after several hours and perhaps even days after ischemia. Most therapeutic attempts have been directed toward increasing the survival of salvageable neurons in the penumbra. Although the pathophysiology of the penumbral ischemic damage is not completely understood, repetitive depolarizations due to glutamate efflux and/or the release of potassium from the deteriorating ischemic core have been suggested to play an important role.<sup>17,18,29-32</sup> In addition, oxidative metabolism of glucose and ATP generation may be impaired as a result of a reduction of blood flow in the penumbra.<sup>33-35</sup> Repetitive depolarizations are especially harmful to the energetically stressed cells in the penumbra. Lidocaine may reduce ischemic cell damage by attenuating ATP depletion, reducing repetitive depolarizations, blocking Na influx, and/or inhibiting the release of glutamate.<sup>1,2,4,12,13,36</sup>

Li *et al.*<sup>37</sup> reported that there were some intact neurons in the ischemic core 46 h after 2 h of MCA occlusion. This suggests that delayed therapeutic interventions might be effective in rescuing neurons in this region. We found significantly more intact neurons in the ischemic core of the lidocaine group compared with the vehicle group. This indicates that delayed administration of lidocaine can rescue neurons in the ischemic core. Although there would be little lidocaine available to the ischemic core during the period of MCA occlusion, lidocaine can enter this region after the onset of reperfusion. Thus, in this region, lidocaine administration may protect those neurons that are damaged but not dead from secondary damage.

The current study found that lidocaine-treated rats had better neurologic outcome and less body weight loss 7 days after ischemia, despite the absence of significant reduction of infarct size. Histo-morphometry of the ischemic cerebral damage is the conventional end point for evaluating the efficacy of neuroprotective therapies. However, the most important clinical end point is a functional one, because the goal of clinical therapies is not only to reduce infarct size, but also to improve function.

The neurologic scoring system used in the current study is mainly designed to test sensorimotor function.<sup>38,39</sup> Sensorimotor dysfunction is commonly noted in the rat subjected to transient MCA occlusion. The mechanism of motor dysfunction after transient MCA occlusion is unclear. Bolay *et al.*<sup>40</sup> has demonstrated that motor dysfunction after transient MCA occlusion is due to a persistent synaptic transmission failure within the motor cortex. Thus, impairment of sensorimotor function may reflect changes in synaptic electrophysiologic function and may not necessarily reflect changes in infarct size. It is well known that extensive connections exist between motor cortex and sensory cortex and between the cortex and the subcortex.<sup>41-43</sup> These structures are involved in the processing and integration of sensorimotor information.<sup>44,45</sup> The preservation of more neurons in the primary somatosensory cortex with lidocaine may be one of the factors in the improvement of neurologic function. It has been reported that abnormal glucose metabolism after MCA occlusion occurred not only in the MCA territory area but also in the "unaffected area," such as the substantia nigra, pars reticularis, and superior colliculus.<sup>46</sup> Lidocaine has multiple pharmacologic effects that are potentially neuroprotective, such as decelerating ischemic transmembrane ion shifts,<sup>1</sup> inhibiting anoxic depolarization,<sup>13</sup> reducing the release of excitatory amino acids,<sup>2,12,36</sup> scavenging oxygen free radicals,<sup>47</sup> and reducing intracranial pressure.<sup>48</sup> It may also reduce swelling by blocking Na influx.<sup>1</sup> Thus, lidocaine may mitigate impairment of those brain regions distant from the area of infarction and allow the animal to compensate for lost function using other brain areas.

This may have partially contributed to improvement of neurologic outcome observed in the lidocaine-treated rats.

In the current study, we measured LCBF using a laser Doppler flowmeter and did not find a significant effect of lidocaine on LCBF. Thus, the neuroprotective effect of lidocaine observed in this study appears to be not a result of an in-traschemic blood flow improvement. Using a rather high concentration of lidocaine ( $\approx 10$  times higher than the concentration used in the current study), Shokunbi *et al.*<sup>8</sup> found that lidocaine can increase the blood flow in the ischemic areas. It is possible that the effect of lidocaine on cerebral blood flow is dose dependent, and a clinical antiarrhythmic dose of lidocaine may not have a significant effect on cerebral blood flow. The laser Doppler probe in this study was placed near the secondary somatosensory region, which is the core of the infarct. Our measurements only reflect LCBF changes in the core of the infarct. We cannot rule out improvements in cerebral blood flow that might have occurred in the ischemic penumbra.

The data in the current study suggest a promising therapeutic use for lidocaine in cerebral ischemia. A recent clinical study has shown that lidocaine is neuroprotective in patients undergoing cardiac operations.<sup>49</sup> A major practical advantage is that this dose of lidocaine has little systemic toxicity and can be administered very shortly after the onset of ischemia. Our previous study demonstrated that this dose of lidocaine reduces infarct size when given before ischemia.<sup>14</sup> Although infarct size was not significantly reduced by delayed administration of lidocaine in the current study, the number of intact neurons in both the penumbra and core was increased and neurologic outcome improved. The difference in the efficacy of neuroprotection between preadministration and delayed administration of lidocaine may reflect the fact that some neurons in both the penumbral and core areas became irreversibly damaged before lidocaine administration was started.

It should be noted that in the current study, the animals were killed at 7 days after ischemia. Several studies have shown that ischemic brain injury after focal ischemia progresses slowly and the maturation of the lesion may take longer than 1 week.<sup>50-52</sup> We cannot exclude the possibility that administration of lidocaine may have simply delayed the progression of ischemic brain injury and the observed difference in the number of intact neurons and the neurologic outcome might not differ between treated and untreated groups after a longer survival period. However, delaying the progression of ischemic brain injury is important because this may provide a therapeutic window for other interventions.

In conclusion, we have demonstrated that administration of a clinical antiarrhythmic dose of lidocaine, beginning 45 min after the onset of transient focal cerebral ischemia, reduces ischemic cerebral damage and improves neurologic and physiologic outcomes. The neu-

roprotective effect of lidocaine observed in this experiment appears not to be due to an improvement of the intraschemic blood flow.

## References

- Fried E, Amorim P, Chambers G, Cottrell JE, Kass IS: The importance of sodium for anoxic transmission damage in rat hippocampal slices: Mechanisms of protection by lidocaine. *J Physiol (Lond)* 1995; 489:557-65
- Taylor CP, Burke SP, Weber ML: Hippocampal slices: Glutamate overflow and cellular damage from ischemia are reduced by sodium-channel blockade. *J Neurosci Methods* 1995; 59:121-8
- Weber ML, Taylor CP: Damage from oxygen and glucose deprivation in hippocampal slices is prevented by tetrodotoxin, lidocaine and phenytoin without blockade of action potentials. *Brain Res* 1994; 664:167-77
- Raley-Susman KM, Kass IS, Cottrell JE, Newman RB, Chambers G, Wang J: Sodium influx blockade and hypoxic damage to CA1 pyramidal neurons in rat hippocampal slices. *J Neurophysiol* 2001; 86:2715-26
- Gelb AW, Steinberg GK, Lam AM, Manninen PH, Peerless SJ, Rassi-Neto A: The effects of a prophylactic bolus of lidocaine in focal cerebral ischemia. *Can J Anesth* 1988; 35:489-93
- Nagao S, Murota T, Momma F, Kuyama H, Nishimoto A: The effect of intravenous lidocaine on experimental brain edema and neural activities. *J Trauma* 1988; 28:1650-5
- Evans DE, Catron PW, McDermott JJ, Thomas LB, Kobrine AI, Flynn ET: Effect of lidocaine after experimental cerebral ischemia induced by air embolism. *J Neurosurg* 1989; 70:97-102
- Shokunbi MT, Gelb AW, Wu XM, Miller DJ: Continuous lidocaine infusion and focal feline cerebral ischemia. *Stroke* 1990; 21:107-11
- Rasool N, Farouqi M, Rubinstein EH: Lidocaine accelerates neuroelectrical recovery after incomplete global ischemia in rabbits. *Stroke* 1990; 21:929-35
- Sutherland G, Ong BY, Louw D, Sima AAF: Effect of lidocaine on forebrain ischemia in rats. *Stroke* 1989; 20:119-22
- Evans DE, Kobrine AI, LeGrys DC, Bradley ME: Protective effect of lidocaine in acute cerebral ischemia induced by air embolism. *J Neurosurg* 1984; 60:257-63
- Fujitani T, Adachi N, Miyazaki H, Liu K, Nakamura Y, Kataoka K, Arai T: Lidocaine protects hippocampal neurons against ischemic damage by preventing increase of extracellular excitatory amino acids: A microdialysis study in Mongolian gerbils. *Neurosci Lett* 1994; 179:91-4
- Liu K, Adachi N, Yanase H, Kataoka K, Arai T: Lidocaine suppresses the anoxic depolarization and reduces the increase in the intracellular  $Ca^{2+}$  concentration in gerbil hippocampal neurons. *ANESTHESIOLOGY* 1997; 87:1470-8
- Lei B, Cottrell JE, Kass IS: Neuroprotective effect of low-dose lidocaine in a rat model of transient focal cerebral ischemia. *ANESTHESIOLOGY* 2001; 95:445-51
- Memezawa H, Minamisawa H, Smith M-L, Siesjö BK: Ischemic penumbra in a model of reversible middle cerebral artery occlusion in the rat. *Exp Brain Res* 1992; 89:67-78
- Kaufmann AM, Firlirk AD, Fukui MB, Wechsler LR, Jungries CA, Yonas H: Ischemic core and penumbra in human stroke. *Stroke* 1999; 30:93-9
- Lipton P: Ischemic cell death in brain neurons. *Physiol Rev* 1999; 79:1431-568
- Dirnagl U, Iadecola C, Moskowitz MA: Pathobiology of ischaemic stroke: An integrated view. *Trends Neurosci* 1999; 22:391-7
- Dyker AG, Lees KR: Duration of neuroprotective treatment for ischemic stroke. *Stroke* 1998; 29:535-42
- Garcia JH, Liu KF, Ho KL: Neuronal necrosis after middle cerebral artery occlusion in Wistar rats progresses at different time intervals in the caudoputamen and the cortex. *Stroke* 1995; 26:636-42
- Reglodi D, Somogyvari-Vigh A, Vigh S, Kozicz T, Arimura A: Delayed systemic administration of PACAP38 is neuroprotective in transient middle cerebral artery occlusion in the rat. *Stroke* 2000; 31:1411-7
- Markgraf CG, Velayo NL, Johnson MP, McCarty DR, Medhi S, Koehl JR, Chmielewski PA, Linnik MD: Six-hour window of opportunity for calpain inhibition in focal cerebral ischemia in rats. *Stroke* 1998; 29:152-8
- Nagayama M, Zhang F, Iadecola C: Delayed treatment with aminoguanidine decreases focal cerebral ischemic damage and enhances neurological recovery in rats. *J Cereb Blood Flow Metab* 1998; 18:1107-13
- Koizumi J, Yoshida Y, Nakazawa T, Ooneda G: Experimental studies of ischemic brain edema: I. A new experimental model of cerebral embolism in rats in which recirculation can be introduced in the ischemic area. *Jpn J Stroke* 1986; 8:1-8
- Longa EZ, Weinstein PR, Carlson S, Cummins R: Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 1989; 20:84-91
- Nagasawa H, Kogure K: Correlation between cerebral blood flow and histologic changes in a new rat model of middle cerebral artery occlusion. *Stroke* 1989; 20:1037-43
- Paxinos G, Watson C: *The Rat Brain Atlas in Stereotaxic Coordinates*. Sydney, Academic Press, 1982
- Clark WM, Lessov NS, Dixon MP, Eckenstein F: Monofilament intraluminal middle cerebral artery occlusion in the mouse. *Neurol Res* 1997; 19:641-8
- Dijkhuizen RM, Beekwilder JP, Van der Worp HB, Berkelbach Van der Sprenkel JW, Tulleken KA, Nicolay K: Correlation between tissue depolarizations and damage in focal ischemic rat brain. *Brain Res* 1999; 840:194-205
- Patel PM, Drummond JC, Cole DJ, Kelly PJ, Watson M: Isoflurane and pentobarbital reduce the frequency of transient ischemic depolarizations during focal ischemia in rats. *Anesth Analg* 1998; 86:773-80
- Chen Q, Chopp M, Bodzin G, Chen H: Temperature modulation of cerebral depolarization during focal cerebral ischemia in rats: Correlation with ischemic injury. *J Cereb Blood Flow Metab* 1993; 13:389-94
- Mies G, Kohno K, Hossmann K-A: Prevention of periinfarct direct current shifts with glutamate antagonist NBQX following occlusion of the middle cerebral artery in the rat. *J Cereb Blood Flow Metab* 1994; 14:802-7
- Yao H, Ginsberg MD, Eveleth DD, LaManna JC, Watson BD, Alons OF, Lloor JY, Foreman JH, Busto R: Local cerebral glucose utilization and cytoskeletal proteolysis as indices of evolving focal ischemic injury in core and penumbra. *J Cereb Blood Flow Metab* 1995; 15:398-408
- Folbergrova J, Memezawa H, Smith ML, Siesjö BK: Focal and perifocal changes in tissue energy state during middle cerebral artery occlusion in normo- and hyperglycemic rats. *J Cereb Blood Flow Metab* 1992; 12:25-33
- Back T, Zhao W, Ginsberg MD: Three-dimensional image analysis of brain glucose metabolism/blood flow uncoupling and its electrophysiological correlates in the acute ischemic penumbra following middle cerebral artery occlusion. *J Cereb Blood Flow Metab* 1995; 15:566-77
- Diaz L, Gomez A, Bustos G: Lidocaine reduces the hypoxia-induced release of an excitatory amino acid analog from rat striatal slices in superfusion. *Prog Neuropharmacol Biol Psychiat* 1995; 19:943-53
- Li Y, Powers C, Jiang N, Chopp M: Intact, injured, necrotic and apoptotic cells after focal cerebral ischemia in the rat. *J Neurol Sci* 1998; 156:119-32
- Zausinger S, Hungerhuber E, Baethmann A, Reulen HJ, Schmid-Elsaesser R: Neurological impairment in rats after transient middle cerebral artery occlusion: A comparative study under various treatment paradigms. *Brain Res* 2000; 863:94-105
- Corbett D, Nurse S: The problem of assessing effective neuroprotection in experimental cerebral ischemia. *Prog Neurobiol* 1998; 54:531-48
- Bolay H, Dalkara T: Mechanisms of motor dysfunction after transient MCA occlusion: persistent transmission failure in cortical synapses is a major determinant. *Stroke* 1998; 29:1988-94
- Hall RD, Lindholm EP: Organization of motor and somatosensory neocortex in the albino rat. *Brain Res* 1974; 66:23-38
- Sanderson KJ, Welker W, Shambes GM: Reevaluation of motor cortex and of sensorimotor overlap in cerebral cortex of albino rats. *Brain Res* 1984; 292:251-60
- Heimer L, Zahm DS, Alheid GF: *Basal ganglia, The Rat Nervous System*, 2nd edition. Edited by Paxinos G. San Diego, Academic Press, 1995, pp 579-628
- Whishaw IQ, O'Connor WT, Dunnet SB: The contributions of motor cortex, nigrostriatal dopamine and caudate-putamen to skilled forelimb use in the rat. *Brain* 1986; 109:805-43
- Andersen CS, Andersen AB, Finger S: Neurological correlates of unilateral and bilateral "strokes" of the middle cerebral artery in the rat. *Physiol Behav* 1991; 50:263-9
- Jaspers RMA, Berkelbach Van Der Sprenkel JW, Tulleken CAF, Cools AR: Local as well as remote functional and metabolic changes after focal ischemia in cats. *Brain Res Bull* 1990; 24:23-32
- Das KC, Misra HP: Lidocaine: A hydroxyl radical scavenger and singlet oxygen quencher. *Mol Cell Biochem* 1992; 115:179-85
- Evans DE, Kobrine AI: Reduction of experimental intracranial hypertension by lidocaine. *Neurosurgery* 1987; 20:542-7
- Mitchell SJ, Pellett O, Gorman DF: Cerebral protection by lidocaine during cardiac operations. *Ann Thorac Surg* 1999; 67:1117-24
- Nakano S, Kogure K, Fujikura H: Ischemia-induced slowly progressive neuronal damage in the rat brain. *Neuroscience* 1990; 38:115-24
- Du C, Hu R, Csernansky CA, Hsu CY, Choi DW: Very delayed infarction after mild focal cerebral ischemia: A role for apoptosis? *J Cereb Blood Flow Metab* 1996; 16:195-201
- Kawaguchi M, Kimbro JR, Drummond JC, Cole DJ, Kelly PJ, Patel PM: Isoflurane delays but not prevent cerebral infarction in rats subjected to focal ischemia. *ANESTHESIOLOGY* 2000; 92:1335-42