Neuroprotective Effect of Low-dose Lidocaine in a Rat Model of Transient Focal Cerebral Ischemia

Baiping Lei, M.D., Ph.D.,* James E. Cottrell, M.D.,† Ira S. Kass, Ph.D.‡

Background: A low concentration of lidocaine (10 μM) has been shown to reduce anoxic damage in vitro. The current study examined the effect of low-dose lidocaine on infract size in rats when administered before transient focal cerebral ischemia.

Methods: Male Wistar rats (weight, 280–340 g) were anesthetized with isoflurane, intubated, and mechanically ventilated. After surgical preparation, animals were assigned to lidocaine 2-day (n = 10), vehicle 2-day (n = 12), lidocaine 7-day (n = 13), and vehicle 7-day (n = 14) groups. A 1.5-mg/kg bolus dose of lidocaine was injected intravenously 30 min before ischemia in the lidocaine 2-day and 7-day groups. Thereafter, an infusion was initiated at a rate of 2 mg · kg⁻¹ · h⁻¹ until 60 min of reperfusion after ischemia. Rats were subjected to 90 min of focal cerebral ischemia using the intraluminal suture method. Infarct size was determined by image analysis of 2,3,5-triphenyltetrazolium chloride–stained sections at 48 h or hematoxylin and eosin–stained sections 7 days after reperfusion. Neurologic outcome and body weight loss were also evaluated.

Results: The infarct size was significantly smaller in the lidocaine 2-day group (185.0 ± 43.7 mm³) than in the vehicle 2-day group (261.3 ± 45.8 mm³, P < 0.01). The reduction in the size of the infarct in the lidocaine 7-day group (130.4 ± 62.9 mm³) was also significant compared with the vehicle 7-day group (216.6 ± 73.6 mm³, P < 0.01). After 7 days of reperfusion, the rats in the lidocaine group demonstrated better neurologic outcomes and less weight loss.

Conclusions: The current study demonstrated that a clinical antiarrhythmic dose of lidocaine, when given before and during transient focal cerebral ischemia, significantly reduced infarct size, improved neurologic outcome, and inhibited postischemic weight loss.

CEREBRAL ischemia is a frequent consequence during cardiac and neurologic surgery. Many surgical procedures such as coronary artery bypass graft, carotid endarterectomy, aneurysmectomy, and resection of arteriovenous malformations, are associated with a substantial risk of focal cerebral ischemia.

Prophylactic pharmacologic neuroprotective interventions could be of great benefit in patients undergoing these procedures. However, there are currently no safe and efficacious agents available to protect the brain from ischemia. Although some agents, such as anesthetics, competitive and noncompetitive N-methyl-D-aspartate (NMDA) antagonists, and glycine site antagonists, have been shown to protect against ischemic brain injury in vitro, they are effective only at the high concentrations. The neurotoxic and systemic side effects of these agents at high concentrations have limited their use as intraoperative neuroprotective agents.

Previous studies in our laboratory found that a low concentration of lidocaine (10 μM) could reduce anoxic damage in vitro without affecting electrophysiological activity. This concentration of lidocaine has little systemic toxicity and is used widely as an antiarrhythmic agent. Therefore, we conducted the present study to examine the effect of low-dose lidocaine on infract size in rats when administered before transient focal cerebral ischemia.

Materials 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–Health Science Center at Brooklyn. Male Wistar rats (weight, 280–340 g) were used in the experiments. Animals were allowed free access to food and water before surgery. Rats were placed in a plexiglas box and anesthetized by inhalation of a gas mixture of isoflurane (3%), oxygen (40%), and nitrogen (remainder). After tracheal intubation was completed, the lungs were mechanically ventilated. The isoflurane concentration was reduced to between 2 and 2.2%. The end-tidal carbon dioxide concentration, the inspiratory oxygen concentration, and the inspiratory isoflurane concentration were monitored continuously using an airway gas monitor (DATEX Intrumentarium Co., Helsinki, Finland). Ventilation was adjusted to maintain normocapnia. Temperature probes were inserted into each animal’s rectum and left-side temporalis muscle. Rectal and pericranial temperatures were kept constant at 38.0°C ± 0.2°C by surface heating or cooling using a temperature-controlled heating pad (Harvard Apparatus Ltd., Edenbridge, Kent, UK) and a temperature controller (Physitemp, Clifton, NJ) throughout the surgical procedure. The tail artery was catheterized for continuous blood pressure monitoring and periodic blood sampling for arterial gas level, pH, and blood glucose level (10 min before ischemia, at 45 min of ischemia, and 60 min after reperfusion). Mean arterial blood pressure was recorded continuously with a Macintosh type of computer (Power

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Rats that exhibited convulsion, sustained consciousness disturbance, or were without neurologic deficit 30 min after recovery from anesthesia were excluded from the study.

**Measurement of Infarct Size**

In the vehicle and lidocaine 2-day groups, the animals were anesthetized and decapitated 48 h after reperfusion. The brains were removed rapidly and kept in saline solution (0–4°C) for 2 minutes. Each brain was cut into seven 2-mm-thick coronal slices using a rat brain matrix. Slices were immediately incubated at 37°C for 30 min in 2,3,5-triphenyltetrazolium chloride (TTC), 2%. The stained slices were fixed in 10% buffered formalin solution for 2 h. Video images of slices were captured with a charge-coupled device camera (Pixera Corp., Los Gatos, CA), and the areas traced using an image analysis system (NIH Image 1.60; National Institutes of Health, Bethesda, MD). Infarct volumes in cubic millimeters were calculated using the slice thickness and the measured areas of lesion. To correct for the effect of brain edema, infarct volumes were adjusted by the ratio of the volumes of both cerebral hemispheres (left over right). The extent of infarction was also expressed as the percentage of lesion to the contralateral hemisphere.

In the vehicle and lidocaine 7-day groups, the brains were removed 7 days after reperfusion. Unlike at 48 h after reperfusion, TTC staining could not adequately differentiate the infarcted area from the area that was not infarcted, 7 days after reperfusion. Therefore, we used the hematoxylin and eosin staining method to detect infarct size. Each brain was cut into seven 2-mm-thick coronal blocks. The blocks were immediately fixed in 10% buffered formalin solution and embedded in molds with embedding compound (Sakura Finetek U.S.A., Inc., Torrance, CA) and dry ice. Coronal sections (20 μm) approximately 1 mm from the anterior surface of each block were cut on a cryostat. The sections were dried and stained with hematoxylin and eosin. Video images of individual sections were obtained with a CCD camera, equipped with a macro lens. Brain areas were traced and measured with an image analysis system (NIH Image 1.60). The volume of infarction was calculated as the integrated product of the cross-sectioned area for all sections and the distance between sections. To avoid errors associated with processing of the tissue for histologic analysis, the infarct size was also expressed as the percentage of lesion to the contralateral hemisphere.

**Measurement of Lidocaine Concentration**

An additional five rats were used to measure plasma lidocaine concentrations. These rats were prepared as described earlier in this section, except that the MCA was not occluded. The dosage regimen of the lidocaine administration was the same as for the lidocaine groups. Blood was obtained during the lidocaine infusion 75 min after the initial bolus of lidocaine. This time point was
Table 1. Physiologic Variables in the Four Experimental Groups

<table>
<thead>
<tr>
<th></th>
<th>Vehicle 2-day</th>
<th>Lidocaine 2-day</th>
<th>Vehicle 7-day</th>
<th>Lidocaine 7-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>315 ± 17</td>
<td>310 ± 19</td>
<td>310 ± 18</td>
<td>312 ± 14</td>
</tr>
<tr>
<td>Before ischemia MABP (mmHg)</td>
<td>91 ± 7</td>
<td>92 ± 8</td>
<td>96 ± 6</td>
<td>93 ± 6</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.42 ± 0.01</td>
<td>7.43 ± 0.02</td>
<td>7.42 ± 0.02</td>
<td>7.43 ± 0.02</td>
</tr>
<tr>
<td>PaO2 (mmHg)</td>
<td>143 ± 28</td>
<td>134 ± 22</td>
<td>130 ± 22</td>
<td>133 ± 16</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>41 ± 3</td>
<td>40 ± 1</td>
<td>43 ± 2</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>During ischemia MABP (mmHg)</td>
<td>182 ± 36</td>
<td>179 ± 26</td>
<td>187 ± 15</td>
<td>187 ± 20</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>98 ± 9</td>
<td>99 ± 7</td>
<td>106 ± 7</td>
<td>107 ± 6</td>
</tr>
<tr>
<td>PaO2 (mmHg)</td>
<td>7.42 ± 0.02</td>
<td>7.41 ± 0.01</td>
<td>7.43 ± 0.02</td>
<td>7.42 ± 0.02</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>172 ± 27</td>
<td>173 ± 18</td>
<td>182 ± 20</td>
<td>188 ± 21</td>
</tr>
<tr>
<td>After reperfusion MABP (mmHg)</td>
<td>97 ± 7</td>
<td>94 ± 7</td>
<td>93 ± 4</td>
<td>95 ± 8</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.43 ± 0.02</td>
<td>7.43 ± 0.02</td>
<td>7.42 ± 0.02</td>
<td>7.42 ± 0.02</td>
</tr>
<tr>
<td>PaO2 (mmHg)</td>
<td>136 ± 15</td>
<td>133 ± 23</td>
<td>131 ± 23</td>
<td>138 ± 17</td>
</tr>
<tr>
<td>Pco2 (mmHg)</td>
<td>39 ± 3</td>
<td>38 ± 3</td>
<td>41 ± 2</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>177 ± 26</td>
<td>171 ± 21</td>
<td>184 ± 21</td>
<td>188 ± 18</td>
</tr>
</tbody>
</table>

Data are mean ± SD. Before ischemia, values were measured 10 min before ischemia. During ischemia, values were measured 45 min after the onset of ischemia. After reperfusion, values were measured 60 min after the onset of reperfusion.

2-day = 2 days, 7-day = 7 days of reperfusion after middle cerebral artery occlusion; MABP = mean arterial blood pressure; PaO2 = arterial oxygen tension; PaCO2 = arterial carbon dioxide tension.

Statistical Analysis

All values, except for neurologic score, are presented as mean ± SD. Infarct size, body weight, and physiologic variables were analyzed with the unpaired 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

One animal from the vehicle 2-day group, two from the lidocaine 2-day group, two from the vehicle 7-day group, and one from the lidocaine 7-day group were excluded from the study because of subarachnoid hemorrhage. One animal from the vehicle 2-day group and one from the lidocaine 7-day group were discarded from the study because of unsuccessful MCA occlusion resulting in no neurologic disturbance 30 min after recovery from anesthesia. Three additional animals in the vehicle 7-day group died on the fifth and sixth days after reperfusion and were discarded from the study, although they did not die of subarachnoid hemorrhage. This may have underestimated the efficacy of lidocaine as a neuroprotective agent.

Physiologic variables in the four experimental groups are given in table 1. Mean arterial blood pressure, arterial oxygen tension (PaO2), arterial carbon dioxide tension (PaCO2), arterial pH, and blood glucose level did not differ significantly between vehicle and lidocaine groups. 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 was no difference between vehicle and lidocaine groups. The plasma lidocaine concentration 75 min after the initial bolus administration of lidocaine with our dosage regimen was $1.2 ± 0.4 \mu g/ml (n = 5)$.

All animals in the study lost weight at 24 and 48 h of reperfusion when compared with values before MCA occlusion (fig. 1). The weight differences at 24 and 48 h between the animals in the vehicle groups and the lidocaine groups were not statistically different. However, animals from the vehicle 7-day group continued to lose weight. At 7 days after reperfusion, the lidocaine 7-day group of rats exhibited significantly greater weight than the vehicle 7-day group of rats ($P < 0.01$).

The neurologic scores 30 min after recovery from anesthesia were not significantly different between vehicle and lidocaine groups (vehicle 2-day, 2 [range, 2–2.5] vs. lidocaine 2-day, 2 [range, 2–2.5]), $P = not
significant [NS]; vehicle 7-day, 2 [range, 2–2] vs. lido-
caine 7-day, 2 [range, 2–2], $P < 0.17$; fig. 2A). At 7 days after reperfusion, the
neurologic scores were significantly better in the lido-
caine 7-day group than in the vehicle 7-day group ($P < 0.05$; fig. 2B).

The infarct size was significantly smaller in the lido-
caine 2-day group ($185.0 \pm 43.7 \text{ mm}^3$) than in the vehicle 2-day group ($261.3 \pm 45.8 \text{ mm}^3$; $P < 0.01$; fig. 3). The reduction in the size of the infarct in the lido-
caine 7-day group ($130.4 \pm 62.9 \text{ mm}^3$) was also statistically significant compared with the vehicle 7-day group ($216.6 \pm 73.6 \text{ mm}^3$; $P < 0.01$; fig. 4).

**Discussion**

Our study demonstrates that intravenous administra-
tion of low-dose lidocaine (a clinical antiarrhythmic
dose) significantly reduces infarct size in a rat model of
transient focal cerebral ischemia when administration
begins 30 min before MCA occlusion. Pretreatment with
lidocaine also improves neurologic outcome and post-
ischemic body weight loss.

Lidocaine, which is commonly used as a local anes-
thetic and antiarrhythmic agent, has also been investi-
gated as a potential neuroprotective agent.\textsuperscript{8–16} However,
the use of lidocaine for cerebral protection during is-
chemia has produced conflicting results. The inconsis-
tent results might well reflect a divergence in animal
models, lidocaine dosage regimen, or techniques for
evaluating the effect of lidocaine. When high-dose lido-
caine (resulting in burst suppression and isoelectric elec-
troencephalogram) and severe cerebral ischemia models
were used, several studies failed to show the protective
effect of lidocaine.\textsuperscript{9,12} In contrast, a lower dose of lido-
caine has been shown to have protective effect after mild cerebral ischemia.\textsuperscript{11,14} Indeed, at high concentra-
tions lidocaine is neurotoxic.\textsuperscript{17,18} Low concentrations of
lidocaine have significant effects on other excitable tis-
sue.\textsuperscript{5} Thus, the effect of lidocaine on neurons may de-
pend on its concentration; the concentration necessary
to produce neuroprotective effects may be within the
range of lower concentrations. Recently, Mitchell et al.\textsuperscript{19}
reported that prophylactic infusion of an antiarrhythmic
dose of lidocaine could improve neurologic outcome
after cardiac operations, suggesting that this dose of
lidocaine is neuroprotective.

Neuroprotective mechanisms of lidocaine have been
postulated to include one or more of the following
pharmacologic effects of this drug: (1) deceleration of
ischemic transmembrane ion shifts and inhibition of an-
oxic depolarization,\textsuperscript{5,20} (2) reduction in cerebral metab-
olic rate,\textsuperscript{21} (3) reduction in the release of excitatory
amino acids,\textsuperscript{22–24} (4) modulation of leukocyte activity,\textsuperscript{25}
(5) increase in cerebral blood flow,\textsuperscript{15,16} (6) scavenging
of oxygen free radicals,\textsuperscript{26} or (7) reduction in the intra-
cranial pressure.\textsuperscript{27} However, the exact mechanism for
the neuroprotective effect of lidocaine, especially low-

![Fig. 1. Changes in weight after middle cerebral artery occlusion and reperfusion in the 2-day (A) and the 7-day (B) groups. *$P < 0.01$ compared with the vehicle 7-day group.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931229/)
dose lidocaine, is not known. In a previous study from our laboratory, a low concentration of lidocaine (10 μM) significantly improved recovery of the evoked population spike recorded from the CA1 pyramidal cell layer after anoxia. This concentration of lidocaine had no significant effect on potassium levels or calcium influx during anoxia but reduced cellular sodium levels and preserved adenosine triphosphate (ATP) levels during anoxia. The protective action of low-dose lidocaine observed in the current study may be related to deceleration of ischemic transmembrane ion shifts and reduction in neuronal ATP consumption.

Because most episodes of cerebral ischemia during surgical procedures are focal in nature, we chose a rat model of transient focal cerebral ischemia. It has become increasingly clear that focal cerebral ischemia produces a densely ischemic core and a perifocal area with a moderate reduction of blood flow. The latter, often referred to as the ischemic penumbra, has received a great deal of attention because the neuronal injury in this area is essentially reversible and may be salvagable by pharmacologic agents. It has been reported that the volume of the ischemic penumbra is greater than the volume of the densely ischemic core soon after the onset of focal ischemia. However, the ischemic penumbra disappears because of progressive metabolic deterioration in the first hours following MCA occlusion and becomes a part of the irreversibly damaged infarct core. Perifocal depolarizations have been suggested to play an important role in the progression of the ischemic penumbra into the infarct core. Glutamate efflux or the release of K+ from the deteriorating ischemic core produce repetitive waves of anoxic depolarization in cells in the ischemic penumbra. Repetitive depolarizations are harmful to the energetically stressed cells in the penumbra. Inhibition of perifocal depolarizations has been shown to reduce the infarct size. It is possible that low-dose lidocaine may increase the tolerance of cells in the ischemic penumbra to repetitive depolarizations by attenuating ATP depletion. In addition, lidocaine may also reduce perifocal depolarizations by blocking Na influx or inhibiting the release of glutamate; this may limit the expansion of the infarction.

Shokunbi et al. found that a continuous infusion of lidocaine preserved the blood flow in the ischemic zone in a feline model of transient focal cerebral ischemia. Muir and Ellis also found that lidocaine infusion improved posttraumatic blood flow. Although the doses of lidocaine used in those studies are different from that of the present study, it is possible that the neuroprotective effect observed in the present study may be a result of an intraischemic blood flow improvement in the infarct and perifocal areas. Because the cerebral blood flow was not measured in the present study, we do not know whether the neuroprotective effect of lidocaine is at a cellular level or by preserving the cerebral blood flow. It is necessary to clarify the effect of low-dose lidocaine on cerebral blood flow in this rat model of transient focal cerebral ischemia.

Isoflurane was used in this study. Isoflurane has been shown to have neuroprotective effects in a model of...
focal cerebral ischemia in the rat similar to that used in the present study. It has been reported that isoflurane can inhibit the release of glutamate, reduce the frequency of NMDA receptor channel opening, the mean open time of the channel in response to stimulation of NMDA and NMDA toxicity. In addition, isoflurane may reduce the degree of ischemia, particularly in the ischemic penumbra, because of the effects of its metabolic suppression. Although both control and experimental groups were subjected to the same concentration of isoflurane, it is possible that lidocaine may exert synergistic effects.

The neurologic scores at 30 min after recovery from anesthesia were similar in the vehicle and the lidocaine groups. The neurologic outcome improved in both the vehicle and the lidocaine groups over time. However, there was a better neurologic outcome in the lidocaine group after 7 days of reperfusion. It has been proposed that, initially, the neurologic deficits reflect injury to the core as well as the penumbra. Thus, neurologic deficits do not necessarily reflect a structural lesion. The similar neurologic deficits at the early stage of reperfusion do not indicate that the size of the densely ischemic core was the same in vehicle and lidocaine groups. Because the size of the infarct in the densely ischemic core is not modified by pharmacologic interventions, we postulate that there may be a greater ischemic penumbra area and a smaller densely ischemic core in the lidocaine groups than in the vehicle groups or that a larger part of the ischemic penumbra in the lidocaine groups may be salvaged and not progress into infarction.

In conclusion, we have demonstrated that intravenous administration of a clinical antiarrhythmic dose of lidocaine starting 30 min before transient focal cerebral ischemia significantly reduces infarct size, improves neurologic outcome, and inhibits postischemic weight loss.

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References

12. Warner DS, Godders JC, Maj Lis S: Failure of preischemic lidocaine administration to ameliorate brain damage in the rat. ANESTHESIOLOGY 1988; 68:73–8
20. Liu K, Adachi N, Yanase H, Kataoka K, Arai T: Lidocaine suppresses the axonic depolarization and reduces the increase in the intracellular Ca2+ concentration in gerbil hippocampal neurons. ANESTHESIOLOGY 1997; 87:1470–8
33. Dijkstra RM, Beckwilder JP, Van der Worp HB, Van der Sprenkel JWB, Tulleken KAF, Nicolay K: Correlation between tissue depolarizations and damage in acute cerebral ischemia induced by air embolism. J Neurosurg 1989; 69:73–8

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