Effect of Lidocaine on Forebrain Ischemia in Rats

Garnette Sutherland, MD, Bill Yuk Ong, MD, Deon Louw, MBChB, and Anders A.F. Sima, MD, PhD

We examined the effect of lidocaine on ischemic neuronal injury in the rat forebrain ischemia model. Cerebral ischemia was achieved with bilateral carotid artery occlusion and controlled hypotension to a mean of 50 torr for 10 minutes. Perfusion-fixation was performed 7 days after ischemia, subsequent to which the brains were sectioned coronally and stained with hematoxylin and eosin. Ischemic neuronal injury was quantitatively expressed (after direct counting) as a percentage of total neurons, that is, ischemic neurons/(ischemic neurons + normal neurons). Predictably, the selectively vulnerable hippocampal areas exhibited the most marked neuronal injury. In the CA1/CA2 sectors, lidocaine-treated rats demonstrated less injury (34±14%) than untreated (64±9%) or saline-treated (70±10%) rats. However, these superficially pronounced numerical differences were not of statistical significance (p>0.05). In the CA3 sector, neuronal injury in lidocaine-treated rats (31±14%) was significantly different at p<0.05 from the untreated (80±5%) but not the saline-treated (59±13%) group. We conclude that lidocaine may have an only marginal beneficial effect on forebrain ischemia in rats. (Stroke 1989;20:119-122)

Conflicting results have been obtained on the effect of lidocaine in ameliorating cerebral ischemia.1-9 The contrasting conclusions might well reflect a divergence in methodology and materials. Significant interstudy discrepancies include variations in lidocaine dosage, species of laboratory animal, and methods of producing the ischemic insult. Additionally, the effect of lidocaine was evaluated with varied techniques that included somatosensory evoked potentials and histopathologic changes of injured neurons. In all of the foregoing studies, evaluation was restricted to the acute phase of ischemic injury. In view of the conflicting reports and their limitation to the early phase following ischemic injury, we have undertaken an experiment that evaluates the effect of lidocaine on mature ischemic lesions with histologic analysis undertaken on Day 7 after ischemia.

Materials and Methods

We used 18 male Sprague-Dawley rats weighing 400-500 g. The rats were divided into three treatment groups, two control groups (an untreated control group that received maintenance fluids while a saline-treated control group received maintenance fluids plus a 1.5-ml normal saline bolus) and a third group that received 5 mg/kg lidocaine diluted in normal saline to 1.5 ml.

Forebrain ischemia was induced by a method similar to that used by Smith et al,10 differing only in the type of anesthesia. Smith et al used a combination of inhalation agents (nitrous oxide and halothane) and produced muscle paralysis with suxamethonium chloride. As previously reported, our rats were pretreated with 0.5 mg/kg atropine and anesthetized with 15 mg/kg pentobarbital sodium and 150 mg/kg chloral hydrate.11 Following tracheal intubation, mechanical ventilatory support was maintained, and expired CO₂ was continuously monitored using an infrared gas analyzer. Adjustments were made in ventilatory rate and/or tidal volume to maintain normal arterial blood gases and pH. A thermostatically controlled heating unit maintained normothermia (37°C).

A 25-gauge Teflon catheter was inserted through the tail artery into the descending aorta for blood pressure monitoring. Both carotid arteries were exposed, and a 22-gauge Teflon catheter was inserted into the external jugular vein for infusion of maintenance fluids (1.5 ml/kg/hr Ringer's lactate) and
agents (normal saline and lidocaine). After 20 minutes of stabilization, the agents were infused over 5 minutes. Ten minutes after this infusion, forebrain ischemia was induced through bilateral carotid artery occlusion using temporary vascular clips coincident with a reduction in systemic blood pressure to a mean of 50 torr through aspiration of heparinized blood from the aortic cannula. Following a 10-minute ischemic interval, the vascular clips were removed and the aspirated blood was rapidly reinfused. Both before and after the ischemic insult, blood gas analyses were obtained.

Hematocrit was determined before (baseline) and after all infusions before ischemia, as well as during and after ischemia. Postischemia ventilatory support was continued until the rat was breathing well and moving its extremities, at which time the oropharynx was suctioned and the endotracheal tube was removed. The rat was returned to its cage and allowed tap water and food pellets ad libitum.

On Day 7 after ischemia, the rats were anesthetized again with 35 mg/kg pentobarbital sodium and the left ventricle of the heart was cannulated using a 20-gauge Teflon cannula. Normal saline (50 ml at 37°C) was used to flush the intravascular compartment. Each rat was perfusion-fixed with 1 l of 2.5% glutaraldehyde in cacodylate buffer (pH 7.25). After fixation, the brain was removed and placed in the same fixative for 2 weeks before sectioning. The brains were cut coronally into 2.8-mm-thick slices, dehydrated in graded concentrations of ethanol, and embedded in paraffin. Eight-micrometer-thick serial sections were cut and stained with hematoxylin and eosin.

We examined all sections to determine the qualitative extent of ischemic brain damage. For quantification of ischemic neuronal injury, a standard section of cerebral cortex and hippocampus was used (Figure 1). Predetermined regions of this section (subiculum [S], frontal cortex [FC], inferior frontal cortex [IFC], hippocampus [sectors CA1–CA4], and dentate gyrus [inner and outer blade]) were photographed, and ischemic neuronal injury was determined by counting all the neurons. Ischemic neuronal injury was calculated by dividing the number of acidophilic and/or pyknotic neurons (ischemic neurons) by the total number of neurons (ischemic neurons+normal neurons). During this analysis, the examiner was unaware of the treatment group of the rats from which the sections were obtained.

All results are presented as mean±SEM. Intergroup comparisons were made using Student’s two-tailed t test. We compared the three treatment groups using analysis of variance followed by Duncan’s intergroup comparison test.

Results

Physiologic Observations

Table 1 presents mean blood pressure and hematocrit before and after administration of the agents and ischemic manipulation. Infusion of both normal saline and lidocaine resulted in similar transient decreases in hematocrit, which returned to baseline values 20 minutes after forebrain ischemia. The infusion of 5 mg/kg lidocaine resulted in a transient decrease in mean arterial blood pressure from 109.2±6.1 to 77.9±6.8 torr (p<0.01). Blood pressure returned to baseline values over 5–7 minutes. Following the ischemic insult, mean blood pressure increased in all groups with no significant intergroup differences (Table 1). Preischemic and postischemic acid–base indexes did not differ significantly (Table 2).

Histopathology

Qualitative findings. The distribution of ischemic changes was similar in all groups, varying only in severity. Neocortical damage, restricted to cortical layers III–IV, was most severe in the zone between the territory supplied by the anterior and middle cerebral arteries, tapering posteriorly as the posterior cerebral artery zone was approached. Infarction with cavitation was not observed. Minimal striatal damage was found in three of six rats in both the untreated and the saline-treated groups and in one of the six lidocaine-treated rats. In the hindbrain, ischemic change was restricted to the superior vermis and was observed in only those rats that also exhibited striatal damage. In all brain regions, ischemic change was most severe in the hippocampus, with ischemic neurons observed in all 18 rats. Within this area, the CA1/CA2 and CA3 pyramidal neurons were equally involved, followed by the CA4 region and the inner dentate blade. The degree of ischemic change was less pronounced in both the subiculum and the outer dentate blade. In all 18
TABLE 1. Mean Arterial Blood Pressure and Hematocrit Before and 20 Minutes After Administration of Agent and Ischemia Manipulation in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Before agent</th>
<th>After agent</th>
<th>After ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BP (mm Hg)</td>
<td>Hct (%)</td>
<td>BP (mm Hg)</td>
</tr>
<tr>
<td>Untreated</td>
<td>6</td>
<td>112.0±5.6</td>
<td>45.0±1.1</td>
<td></td>
</tr>
<tr>
<td>1.5 ml normal saline</td>
<td>6</td>
<td>108.8±5.0</td>
<td>45.7±1.1</td>
<td>117.7±5.0</td>
</tr>
<tr>
<td>5 mg/kg lidocaine</td>
<td>6</td>
<td>109.2±6.1</td>
<td>43.6±1.5</td>
<td>77.9±6.8*</td>
</tr>
</tbody>
</table>

Data are mean±SEM. BP, mean arterial blood pressure; Hct, hematocrit.

*p<0.01, two-tailed Student's t test.

rats, hippocampal injury was bilateral and of equal severity.

Quantitative findings. Total cell counts for the regions examined did not differ between groups. Ischemic neuronal injury in all regions examined is presented in Table 3. Within the hippocampal CA1/CA2 sector, the lidocaine-treated rats showed less ischemic neuronal injury (0.34±0.14) than the other two groups (untreated, 0.64±0.09; saline-treated, 0.70±0.10) although this difference failed to reach statistical significance. The difference between the lidocaine-treated (0.31±0.14) and the untreated (0.80±0.05) groups was significant at p<0.05 in the CA3 region. However, in this region ischemic neuronal injury in the saline-treated group (0.59±0.13) was not significantly different from either group. In no other region examined were there significant differences between groups.

Discussion

Our experimental model produces transient, incomplete forebrain ischemia that is uniformly reproducible and restricted in part to watershed territories. Smith et al demonstrated a "rank order of vulnerability" in which CA4 was the region most susceptible to short periods of ischemia. This selectivity was, however, time-dependent, and after a 10-minute ischemic insult neuronal injury was most evident in the CA1 sector. In our study, ischemic neuronal injury was equally apparent in both the CA1/CA2 and CA3 sectors. Although this distribution is different than that in other reported studies, all investigations have revealed extensive damage in afflicted areas after 10 minutes of ischemia. Our definition of CA1/CA2 and other regions is based on a standard reference and is unlikely to explain the differences in the results. A rational explanation for the difference in the region of greatest injury is not immediately apparent, but differences in rat age, mass, and strain and anesthetic techniques could be contributory.

Our data indicate that lidocaine exerts a marginally beneficial effect on neurons subjected to partial, transient ischemia. This benefit was observed in only the selectively vulnerable hippocampal (CA3) region. It would be appropriate to speculate on factors responsible for differentiation of our results from those that fail to exhibit amelioration of injury. Shokunbi et al based their histologic findings on neurons that had been injured for 4–6 hours, whereas we made our observations 1 week after injury. Ito et al explained that there is progression of ischemic injury following recirculation, what they call the "maturation phenomenon," with the rate of matu-

TABLE 2. Blood Gas Analysis Before and After Ischemia in Rats

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Untreated (n=6)</th>
<th>1.5 ml normal saline (n=6)</th>
<th>5 mg/kg lidocaine (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>92.6±8.9</td>
<td>95.9±5.8</td>
<td>90.7±4.47</td>
</tr>
<tr>
<td>PacO2 (mm Hg)</td>
<td>37.3±2.3</td>
<td>36.2±1.6</td>
<td>35.4±1.22</td>
</tr>
<tr>
<td>HCO3⁻ (mm Hg)</td>
<td>24.7±0.6</td>
<td>23.8±1.0</td>
<td>23.4±1.1</td>
</tr>
<tr>
<td>Base excess (meq/l)</td>
<td>1.1±0.3</td>
<td>0.45±1.2</td>
<td>0.2±1.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.43±0.02</td>
<td>7.43±0.02</td>
<td>7.4±0.01</td>
</tr>
<tr>
<td>After</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>86.5±8.6</td>
<td>97.7±9.0</td>
<td>91.8±3.51</td>
</tr>
<tr>
<td>PacO2 (mm Hg)</td>
<td>41.0±1.1</td>
<td>37.5±1.2</td>
<td>39.8±1.6</td>
</tr>
<tr>
<td>HCO3⁻ (mm Hg)</td>
<td>21.9±0.6</td>
<td>21.0±0.4</td>
<td>22.8±0.6</td>
</tr>
<tr>
<td>Base excess (meq/l)</td>
<td>-3.0±0.7</td>
<td>-2.6±0.5</td>
<td>-1.4±0.8</td>
</tr>
<tr>
<td>pH</td>
<td>7.34±0.02</td>
<td>7.37±0.01</td>
<td>7.37±0.01</td>
</tr>
</tbody>
</table>

Data are mean±SEM.
That the protection afforded has been demonstrated to be only partial can be rationalized in two ways. First, lidocaine may possess only limited protective pharmacologic properties. Second, the drug is, in all likelihood, cleared from the circulation within several hours and would therefore exert an only temporary effect. In conclusion, lidocaine may have only a marginal beneficial effect on forebrain ischemia in rats.

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**References**


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