Failure of Pre-ischemic Lidocaine Administration to Ameliorate Global Ischemic Brain Damage in the Rat

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The cerebral protective effects of lidocaine were evaluated using a rat model of severe (near-complete) global ischemia produced by 10 min of bilateral carotid artery occlusion combined with systemic hypotension (MAP 45–55 mmHg). Prior to the induction of ischemia, 16 rats were given incremental doses of lidocaine intravenously until EEG slowing with sharp wave activity became evident. An equal number of rats (controls) received saline prior to the ischemic insult. Normoxia, normocapnia, and normothermia were maintained at all times. Following ischemia, the animals were allowed to recover. At 1.5 h post-ischemia, eight rats from each treatment group were reanesthetized, and regional brain water content was assessed gravimetrically. Brain specific gravity was significantly reduced from normal values in both treatment groups, and was unaltered by pre-ischemic lidocaine administration. Seven days post-ischemia, the remaining animals were reanesthetized; the brains were formalin fixed and processed for identification of irreversibly injured neurons in the hippocampus, neocortex, and caudate nucleus. Saline-treated rats displayed 75 ± 4% (mean ± SD) dead cells in the hippocampus (CA1); lidocaine-treated rats had similar injury (78 ± 7%). In the neocortex and caudate nucleus, injury was graded as moderate, and no difference in severity could be distinguished between the treatment groups. The authors conclude that pre-ischemic treatment with maximal sub-epileptogenic doses of lidocaine had no effect on either early post-ischemic cerebral edema or delayed neuronal necrosis in this rat model of near-complete global ischemia. (Key words: Anesthetics, local: Lidocaine. Brain delayed neuronal necrosis; edema; forebrain ischemia.)

LIDOCAINE, AN AMIDE local anesthetic, significantly reduces cerebral metabolic rate (CMR) when administered in sub-epileptogenic doses.1,2 In addition, its anesthetic mechanism of action has been associated with blockade of energy-dependent sodium channels in excitable nervous tissue.3,4 Because adverse sequelae of cerebral ischemia are related to cellular energy failure and loss of neuronal ionic homeostasis,5-7 theoretical potential exists for lidocaine to ameliorate injury resulting from a transient ischemic event.

Under conditions of focal ischemia, lidocaine protection is consistent with neurophysiologic findings reported by two laboratories. Evans et al. found that a low dose of intravenous lidocaine (5 mg/kg) administered prior to feline vertebral artery air embolism resulted in a reduced decrement and more rapid recovery of cortical somatosensory evoked potentials (SSEP).8 If administered post-ischemically, lidocaine (5 mg/kg) was also associated with a more rapid recovery of SSEP when compared to untreated controls.9 Similarly, Gelb et al. found a transient preservation of SSEP if cats were pretreated with low-dose lidocaine (5 mg/kg) prior to middle cerebral artery occlusion, although histologic outcome remained unaltered.10

Lidocaine protection against global ischemia has only been evaluated in the context of massive doses (160 mg/kg) administered to halothane-anesthetized dogs, where some preservation of energy-dependent cortical ionic homeostasis has been identified.11 In this dose range, however, cardiopulmonary bypass support was necessary to control for the cardiac depressant properties of lidocaine and, therefore, extrapolation of these results to potential clinical use of lidocaine for cerebral protection is difficult. Our study was designed to evaluate the effects of lidocaine, given in clinically relevant doses prior to ischemia, on post-ischemic cerebral edema and delayed neuronal necrosis in a rat model of reversible near-complete forebrain ischemia.

Materials and Methods

The experimental protocol was approved by the institutional Animal Research Committee. Fasted male Sprague-Dawley rats (age range: 9–10 weeks) were prepared for forebrain ischemia as follows. Each rat was anesthetized with 3.0% halothane in 30% O₂ and balance N₂O. Following tracheal intubation, the rats were ventilated with a small animal respirator delivering 0.7% halothane and 30% O₂ in N₂O. Tidal volume and respiratory rate were adjusted to maintain normocapnia and normoxia. The tail artery was catheterized to monitor blood pressure and to sample blood. A transverse ventral neck incision was made, and the common carotid arteries were isolated under magnification with care taken to preserve the vagus nerves and cervical sympathetic plexi. Finally, the right jugular vein was cannulated with a silicone catheter.

In the pre-ischemic interval, muscle paralysis was
provided by a 1-mg intravenous bolus of succinylcholine, repeated as necessary. Heparin (50 IU) was given intravenously prior to the first blood gas measurement. Bipolar EEG recordings (Grass® Electroencephalograph, Model 8-10G; Quincy, MA) were obtained from a pair of needle electrodes inserted into the temporalis muscle on each side of the head, with a reference needle electrode placed subcutaneously in the midline scalp. Rectal temperature was maintained between 36.5–37.5°C by surface heating or cooling. Following surgical preparation, halothane was discontinued and the rats were allowed a stabilization period of 30 min. Twenty-five minutes after discontinuation of halothane, 0.4 cc of arterial blood was withdrawn for determination of \( P_{\text{aCO}_2}, P_{\text{aO}_2}, \text{pH} \), and hemoglobin concentration.

Four rats then served as subjects for a pilot study to determine a lidocaine dose-response relationship to EEG activity. In these animals, 1.0% lidocaine in 0.9% NaCl (Astra, Westborough, ME) was incrementally infused intravenously, while the EEG was continuously observed. A characteristic pre-epileptogenic pattern of background slowing with sharp wave activity was observed in each animal. This EEG pattern was accepted as the end-point for administration of lidocaine prior to induction of ischemia. Additional lidocaine administration resulted in a transient electrical seizure, followed by a more prolonged epileptiform discharge and then electrocortical silence.

Experimental rats were randomly divided into two groups, after completion of the 30-min halothane washout interval. With the EEG being continuously monitored, one-half of the animals (n = 16) received 1.0% lidocaine intravenously over 10 min administered as an initial bolus of 0.1 ml (1.0 mg), followed by 0.05 ml (0.5 mg) every 45 s (approximately 8 mg total), until EEG slowing with sharp wave activity was observed. At this point, 1 cc of venous blood was aspirated for determination of plasma glucose (Beckman Glucose Analyzer, Fullerton, CA) and lidocaine concentrations (Enlit® Lidocaine Assay, Syva Co., Palo Alto, CA). The remaining animals (n = 16) served as controls, and were given saline intravenously in volumes similar to that used for lidocaine infusion over the same 10-min interval.

All rats then immediately underwent a 10-min interval of reversible near-complete forebrain ischemia induced by a single intravenous bolus of 0.3 ml (8 mg) trimethaphan camphor sulfonate, followed immediately by temporary bilateral carotid artery occlusion and simultaneous central venous exanguination, as required, to maintain MAP at 50 ± 5 mmHg throughout the ischemic interval.1213 The EEG was continuously recorded during this period. Ischemia was reversed by simultaneous removal of the carotid artery clips and rapid restoration of intravascular volume by reinfusion of the previously shed blood, thereby completing a square wave hemodynamic profile. All animals received 0.3 ml of 0.6 M \( \text{NaHCO}_3 \) at termination of ischemia to counteract systemic acidosis.

Blood pressure was continuously monitored during the early recovery period. At 5-min intervals, the EEG was recorded. Body temperature was kept near 37°C by surface heating or cooling. The neck and tail incisions were closed with sutures. After a recovery period of 25 min, arterial \( P_{\text{O}_2}, P_{\text{CO}_2} \), and \( \text{pH} \) were measured, and the arterial catheter was removed. Thirty minutes post-ischemia, the \( \text{N}_2\text{O} \) was discontinued, the animals regained consciousness and resumed spontaneous ventilation, and the tracheas were subsequently extubated. The rats soon became ambulatory, and were housed in cages with free access to pellet food and water.

At 1.5 h post-ischemia, eight lidocaine-treated and eight saline-treated rats were reanesthetized with 3.0% halothane, 30% \( \text{O}_2 \), and balance \( \text{N}_2\text{O} \), and were immediately decapitated. The brains were rapidly removed and placed in a chamber with gloved periholes. Relative humidity within the chamber was maintained at 90% saturation by a humidifier, while the temperature was maintained at 13–15°C with ice.14 The brains were dissected into samples weighing approximately 25 mg derived from the neocortex (gray matter), caudoputamen, and dorsolateral hippocampus bilaterally. Two samples from each region were introduced into a Percol® (Pharmacia, Uppsala, Sweden) linear density gradient with a sucrose concentration of 0.125 M for determination of specific gravity, using the technique described by Tengvall et al.1516 The column was calibrated prior to the analysis of each brain using glass beads with known densities of 1.0300, 1.0350, 1.0400, 1.0450, and 1.0500 gm/cc (SGA Scientific, Bloomfield, NJ).

An additional eight rats were studied to establish normal regional specific gravity. These animals were anesthetized with 3.0% halothane in 30% \( \text{O}_2 \) and balance \( \text{N}_2\text{O} \), and immediately decapitated without other surgical intervention or ischemic insult. Brains from these animals were dissected and subjected to gravimetric analysis, as described above.

On day 7 of the experiment, the remaining lidocaine-treated (n = 8) and saline-treated (n = 8) rats were reweighed and anesthetized with 3.0% halothane in 30% \( \text{O}_2 \) and balance \( \text{N}_2\text{O} \). Following tracheal intubation, the rats were ventilated by a small animal respirator delivering 0.7% halothane in 30% \( \text{O}_2 \) and balance \( \text{N}_2\text{O} \). Via the ascending aorta, the brains were perfusion fixed with 4% formaldehyde buffered to a \( \text{pH} \) of 7.35, preceeded by a 30-s rinse period with saline. Both solutions were prewarmed to 37°C and infused at a pressure of 135 mmHg. The brains were allowed to stabilize.
in situ until removal the subsequent day for storage in cold fixative.

The brains were then cut coronally into 3.0-mm-thick slices and dehydrated in graded strengths of ethanol. Following clearing in xylol and embedding in paraffin (Reichert-Jung, West Germany) and stained with celestine blue and acid fuchsin. Sectioning intervals were adapted to obtain specific standard levels of the caudate nucleus, neocortex, and hippocampus for quantification of injury. The damage was assessed as the presence of necrotic acidophilic (pink, red) neurons in the different brain areas. The acidophilic neurons were considered to have irreversible ischemic damage, since they have been consistently found to undergo cytology and removal from brain tissue. Injury was quantified in the lateral and dorsal aspects of the caudate nucleus where the septal nuclei were widest, the neocortex at the level of the subfrontal organ, and the hippocampus at two levels throughout its septotemporal extent. Quantification of hippocampal injury was performed by the experimenters, blinded to the experimental condition, by direct visual counting of acidophilic neurons at a magnification of 320X. Damage in the caudate nucleus and neocortex was classified according to a crude damage index (CDI) as follows: 0 = no damage; 1 = rare to occasional acidophilic cells per field (approximately 10% damage); 2 = moderate number of acidophilic cells per field (approximately 10-50% damage); and 3 = frequent acidophilic cells per field (greater than 50% damage).

Five rats (two lidocaine-treated and three saline-treated) were eliminated from data analysis due to post-extubation upper airway obstruction secondary to traumatic intubation, and were replaced by the appropriate number of rats in each treatment regimen. Specimens from the caudate nucleus and neocortex were lost from one animal in each treatment group during histologic processing.

Physiologic and regional specific gravity values were compared by analysis of variance with post hoc multiple comparison testing for between and within group differences, where indicated by a significant F ratio. The Wilcoxon rank sum test was used to compare histologic injury scores in the neocortex and caudate nucleus, and to compare per cent dead cells in the hippocampal CA1 sector. Significance was assumed when $P < .05$. Data values are presented as mean ± standard deviation.

**Results**

Physiologic values are presented in table 1. Both lidocaine and saline-treated animals were normoxic, normocapnic, and normothermic prior to ischemia and during the immediate recirculation period. Blood pressure was similar between groups, as were pre-ischemic hemoglobin and plasma glucose concentrations.

Table 1. Values (Mean ± SD) for Physiologic Variables Measured at Various Intervals during Experimental Procedure

<table>
<thead>
<tr>
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<th>Control (n = 16)</th>
<th>Lidocaine (n = 16)</th>
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<tr>
<td>Pre-ischemic plasma glucose (mg/dL)</td>
<td>126 ± 11</td>
<td>119 ± 9</td>
</tr>
<tr>
<td>Body temperature at ischemia (°C)</td>
<td>37.0 ± 0.2</td>
<td>37.0 ± 0.2</td>
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<tr>
<td>MAP (mmHg)</td>
<td></td>
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<tr>
<td>1 min pre-ischemia</td>
<td>126 ± 13</td>
<td>114 ± 10</td>
</tr>
<tr>
<td>2 min post-ischemia</td>
<td>130 ± 12</td>
<td>115 ± 11</td>
</tr>
<tr>
<td>15 min post-ischemia</td>
<td>192 ± 12</td>
<td>116 ± 8</td>
</tr>
<tr>
<td>25 min post-ischemia</td>
<td>117 ± 11</td>
<td>113 ± 13</td>
</tr>
<tr>
<td>Pre-ischemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{10}$ (mmHg)</td>
<td>127 ± 17</td>
<td>118 ± 14</td>
</tr>
<tr>
<td>$P_{10}$ (mmHg)</td>
<td>38.2 ± 2.6</td>
<td>38.6 ± 2.6</td>
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<tr>
<td>pH</td>
<td>7.37 ± 0.03</td>
<td>7.37 ± 0.02</td>
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<tr>
<td>Hemoglobin (g/L)</td>
<td>17.1 ± 0.7</td>
<td>17.1 ± 0.9</td>
</tr>
<tr>
<td>25 min post-ischemia</td>
<td></td>
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<tr>
<td>$P_{10}$ (mmHg)</td>
<td>117 ± 23</td>
<td>115 ± 15</td>
</tr>
<tr>
<td>$P_{10}$ (mmHg)</td>
<td>39.5 ± 5.6</td>
<td>38.4 ± 3.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.38 ± 0.05</td>
<td>7.38 ± 0.03</td>
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**FIG. 1.** Representative EEG recordings from a lidocaine-treated rat during successive stages of the experimental procedure. A. Thirty minutes after halothane discontinued. B. EEG end-point for lidocaine administration. C. Isoelectricity during ischemia. D. Five minutes of reperfusion. E. Twenty-five minutes of reperfusion.
with frequent spike wave complexes and absence of normal background activity.

Regional brain specific gravity values are depicted in figure 2. No interhemispheric differences were noted in the regions tested; therefore, regional values were averaged for each animal in each group for statistical analysis. In normal (non-ischemic) rats, specific gravity values (gram/cc) were: hippocampus 1.0431 ± .0001; caudoputamen 1.0442 ± .0003; and neocortex 1.0446 ± .0001. In the saline-treated animals at 1.5 h post-ischemia, mean specific gravity in the hippocampus, caudoputamen, and cortex was decreased by 0.0011, 0.0013, and 0.0014 gm/cc, respectively, when compared to normal (P < .01). Lidocaine-treated animals showed a similar profile at this time. In these animals, mean values in the hippocampus, caudoputamen, and cortex were decreased by 0.0009, 0.0018, and 0.0014 gm/cc, respectively, when compared to normal (P < .01). There was no statistically significant difference between saline-treated and lidocaine-treated specific gravity values in any region. Macroscopic swelling was not observed on gross examination in either group.

Neurologically, treated and untreated animals were indistinguishable throughout the 7-day observation period. Rats in both groups were typically docile on days 1 and 2, but by day 3 showed signs of hyperexcitability to both sound and handling. No overt seizure activity was noted in any animal, although the hyperexcitability persisted through day 7. All rats had resumed ambulation within 6 h, with no gross evidence of residual paralyis or plegia throughout the remainder of the observation period. Body weights were similar between groups before ischemia (control = 294 ± 21 gm; lidocaine = 297 ± 9 gm). Over 7 days, rats in both groups increased body weight (control = 325 ± 18; lidocaine = 320 ± 12) suggesting a similar resumption of feeding behavior (P < .05).

Histologic analysis of selectively vulnerable regions 7 days post-ischemia revealed a dense hippocampal CA1 sector injury in both groups. In the saline-treated group, 75 ± 4% of the neurons were acidophilic, while 78 ± 7% of CA1 neurons were acidophilic in the lidocaine-treated group. These values were not statistically different (fig. 3). CA3 and CA4 sectors showed few acidophilic neurons without a difference being evident between groups. The dentate gyrus was spared from injury with the exception of rare dead neuron in both groups. The caudate nucleus and neocortex demonstrated moderate injury, again without differences being evident between groups. Individual crude damage index scores are reported in figure 3. Cortical injury was localized in layers III–V in both groups. Frank infarction was absent in both the caudate and cortex in both groups, with the exception of one lidocaine-treated animal which showed severe bilateral cortical spongiosis with an uncharacteristically dense phagocytic infiltration.

Discussion

Several procedural aspects of our study deserve comment. The forebrain ischemia model developed by Smith et al. appears reasonable for evaluating the potential protective benefits of lidocaine. The injury is
sufficiently mild that recovery is possible, thereby allowing full maturation of neuropathologic changes and analysis of outcome at various post-ischemic intervals. The insult, however, is severe enough that very distinct pathologic changes can be identified. Finally, outcome from such an insult can be pharmacologically modified, since histologic changes have been shown to be reduced by either pre-ischemic or post-ischemic therapy with the calcium channel blocker, flunarizine.19

The model has one disadvantage in that nitrous oxide is contained in the respiratory gas mixture and may present an adverse interaction with the reduction in cerebral metabolic rate afforded by various agents.20 This effect has questionable significance in the rat, however, as the cerebral metabolic rate of glucose utilization is relatively unaffected by N₂O.21

The dose of lidocaine used in our study was felt to be the maximum amount possible within the clinically applicable range, i.e., without producing seizure activity. Doses sufficient to generate convulsive activity have dramatic effects on cerebral blood flow and metabolic rate which may adversely influence outcome.7,22 Even larger doses, sufficient to suppress seizure activity, are associated with marked hypotension which would necessitate aggressive hemodynamic support. The smaller dose employed here which minimizes cardiovascular depression but still offers a significant reduction in cerebral metabolic rate (CMR) would seem preferable during restitution of cerebral blood flow and O₂ delivery.

In experimental models of severe global ischemia, an early post-ischemia increase in brain water content has been observed and is believed to contribute to a worsened neurologic outcome.23-27 The post-ischemic interval of 1.5 h was chosen for determination of brain water content in our study for two reasons. First, it has been demonstrated that a significant increase in brain-water content following 10 min of forebrain ischemia in the rat occurs at 1.5 h post-ischemia.23 Secondly, early post-ischemic cerebral edema is predominantly cytotoxic in nature, mechanistically derived from cellular energy depletion with subsequent failure of the Na⁺-K⁺-ATPase pump leading to influx of Na⁺ and H₂O into the intracellular space.28 Both a reduction of cellular energy requirements and blockade of Na⁺ channels might be expected to reduce cytotoxic edema.

Given these considerations, no protective benefit from lidocaine could be demonstrated. Early post-ischemic cerebral edema and delayed neuronal necrosis were unaltered by pre-ischemic administration of lidocaine. This “failure” may be interpreted in consideration of several factors. First, lidocaine does not uniformly suppress regional metabolism. When pre-convulsive spike and wave activity is present in lidocaine-treated rats, blood flow and metabolism are similarly reduced in all regions with the exception of limbic structures, including the hippocampus.1,22 Our failure to demonstrate a protective effect from lidocaine in the hippocampus may have been related to this localized relative increase in metabolic activity.

Second, the “failure” of clinically relevant doses of lidocaine to provide cerebral protection during global ischemia appears consistent with past experience with other agents that decrease CMR. Barbiturates have repeatedly been ineffective in protecting against global ischemia.28-30 Similarly, isoflurane, when administered pre-ischemically to produce EEG isoelectricity, has no protective effect against global ischemia.31 If the common mechanism of these agents in reducing CMR is via reduction of energy required for cortical electrical activity, no protection would be expected against an ischemic event severe enough to abolish electrocortical activity.32 This may, in part, explain the disparity between our results and those obtained by Evans et al. and Gelb et al., who found a protective effect from low-dose lidocaine pretreatment. Their models (arterial air embolism and MCA occlusion) reflect focal ischemia which has been shown to be beneficially influenced by barbiturate (i.e., CMR reduction) therapy.33-35

Finally, the dose of lidocaine administered (23.5 ± 3.9 mg/kg) may have been inadequate to produce the more specific ion-channel effects associated with larger doses used by Astrup et al.11,36 When 160 mg/kg of lidocaine was administered to halothane-anesthetized dogs on cardiopulmonary bypass, CMRO₂ reduction was equal to that produced by pentobarbital. If, however, massive doses of lidocaine were administered to dogs where maximal pentobarbital-induced reduction of cerebral metabolism was already present, an additional 15% decrease in CMRO₂ was produced. This was attributed to an independent membrane stabilizing effect of lidocaine, and was substantiated by a lidocaine-induced delay in cortical surface K⁺-efflux when circulatory arrest was achieved by discontinuing cardiopulmonary bypass. This membrane stabilizing effect is consistent with the Na⁺-channel blockade produced by lidocaine in the peripheral nervous system, and could theoretically block, at least in part, the ischemically induced leak of ions into and out of the cell during energy failure. The absence of infarction in our rats supports classification of the edema present at 1.5 h as being primarily cytotoxic in nature.37 Our failure to reduce post-ischemic edema suggests that, with low doses, membrane stabilization against ischemically induced ionic flux is not a property of lidocaine.

In conclusion, in this model of near-complete global ischemia, there was no difference between saline-treated rats and those receiving lidocaine administered in maximal sub-epileptogenic doses prior to onset of
ischemia. While no deleterious effect from lidocaine was seen, the results of this study indicate that, in clinically tolerated doses, pre-ischemic treatment with lidocaine will not provide cerebral protection under conditions of transient global ischemia.

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References