An Evaluation of the Effect of Lidocaine in Experimental Focal Cerebral Ischemia


SUMMARY In order to determine the effect of lidocaine in focal cerebral ischemia, the left middle cerebral artery was transorbitally occluded in twenty cats. Eleven received lidocaine hydrochloride intravenously. The infusion was begun half an hour prior to clip occlusion and the rate was adjusted to maintain an isoelectric EEG. Nine cats served as controls, receiving an equivalent volume of 5% dextrose 0.2% saline. Thirteen animals (7 lidocaine-treated and 6 control) were sacrificed after six hours of left middle cerebral artery occlusion without reperfusion. In the remaining seven cats, the vessel was occluded for four hours prior to sacrifice. Ischemic neuronal alteration was assessed by both histochemical (2',3'5'-tetrazolium hydrochloride reaction) and histological examination. With both durations of ischemia, there was no significant difference in the extent and severity of neuronal alterations between the lidocaine-treated and control groups of animals.

Methods

1. Experimental Set-Up

The study was conducted on a feline model of focal cerebral ischemia in which the middle cerebral artery (MCA) was proximally occluded by means of a Heifitz clip. Twenty animals (2.5-4.0 kg body weight) were studied. In each cat, anesthesia was commenced by intraperitoneal injection of ketamine hydrochloride (10-20 mg/kg) and atropine sulphate (0.1 mg/kg) and maintained with alphachloralose (50 mg/kg). This regimen resulted in burst suppression and eventually EEG flattening. In some cats, supplementary doses of lidocaine were required to achieve this end point. The cats in the control group received an equivalent volume of 5% dextrose/0.2 NaCl.

2. Duration of Ischemia

Thirteen animals (7 lidocaine-treated, 6 control) were sacrificed after 6 hours of ischemia. The remaining 7 cats (4 lidocaine-treated and 3 control) were subjected to only 4 hours of ischemia after which they were sacrificed.

At the end of the experiment, the heart was stopped by intracardiac injection of potassium chloride.

3. Tissue Processing and Morphological Examination

The animals were promptly perfused through the ascending aorta with 300 mls of 0.9% NaCl followed...
by 50 mls of a 2% solution of 2',3',5' triphenyl-2H-
tetrazolium chloride (TTC). The brain was immediate-
ly removed from the intracranial cavity and the cere-
brum sectioned coronally into two halves at the level of
the optic chiasm. A 5 mm thick coronal slice obtained
from the posterior end of the anterior half was incubat-
ed for 30 minutes at 37°C in 50 mls of a 2% solution of
TTC. The distribution of color change (see results sec-
tion) on the posterior surface of the slice was photo-
graphically recorded. The area of pallor in the ische-
mic cerebral cortex was calculated as a percentage of
the total cross-sectional area of the hemisphere. The
total experiment was fixed in formalin for at least 72 hours
prior to paraffin processing. The histological changes
were independently evaluated, in a blinded manner, by microscopic examination of whole mounts
of 8 micron-thick coronal sections of the cerebrum,
stained with hematoxylin and eosin. These sections
were obtained at the level of the optic chiasm. Ische-
mic neuronal damage was graded according to pre-
viously established criteria. For each grade or severity
of ischemic neuronal change, the area of cerebral cor-
tex was calculated as a percentage of the total cross-
sectional area of the grey matter of the hemisphere.
The results (control and treatment) were analyzed
using the Wilcoxon's rank sum test. A p value less than
0.05 was considered statistically significant. The con-
tralateral hemisphere was also examined for neuronal
alterations.

Results

1. Physiological Parameters

Pulse, blood pressure and temperature were main-
tained within normal limits in the control group of cats.
In the lidocaine treated animals, hypotension some-
times occurred with the infusion. This was readily
corrected by temporarily decreasing the rate of infu-
sion and then administering neosynephrine intrave-

ous if necessary. Mean arterial blood pressure was
kept within 10-20 mm Hg of control value. Metabolic
acidosis was corrected with intravenous sodium bicar-
bonate. The end-tidal pCO₂ correlated positively with
the arterial pCO₂ and was a reliable guide to the ade-
quacy of ventilation. It was maintained between 30-35
mm Hg.

2. EEG Changes (fig 1)

The EEG pattern in the unoperated anesthetized cat
consisted of low voltage waves of mixed frequencies,
the majority of which were in the alpha range. There
was no significant difference between the two hemi-

spheres. In the control group, occlusion of the left
middle cerebral artery consistently resulted in a slight
reduction of voltage and mild slowing of EEG activity
over the ischemic hemisphere. This persisted through-
out the duration of the experiment. The EEG pattern
in the lidocaine-treated cats was profoundly altered with-
in minutes of the start of the infusion. The initial
change was that of burst suppression, which usually progressed to complete flattening of the EEG. This
change was present bilaterally and was maintained to
the time of sacrifice. With the dose schedule and mode
of administration of lidocaine utilized in this study,
seizure activity was not observed. The effect of the
drug on the EEG was not significantly altered by
changes in arterial blood pressure within the range
observed during the experiment. Serum lidocaine lev-

els were in the toxic range (>21.4 μmol/l) during EEG
suppression (see table 1). The toxic level is the plasma
concentration above which significant cardiovascular
depression and seizures may be seen.

3. Gross Pathological Findings

The superolateral surface of the left hemisphere was
pale, compared to the rest of the brain, which exhibited
an intense red coloration due to the presence of the
colored formazen product of the TTC reaction (see
Discussion). The area of pallor corresponded to the
"area of the left middle cerebral artery and con-
"formed occlusion of this vessel. The external appear-
ance of the fresh uncut brain was otherwise unremarkable.

The area of infarction was better demarcated by incubation of the 5 mm slice of cerebrum in TTC. Incubation improved exposure of the cerebral tissue in the ischemic area to the compound, so that the post-incubation area of pallor was more representative of the area of infarction in the slice. Although the mean area of pallor in the lidocaine treated cats slightly exceeded that of the control, the difference was not statistically significant (table 2). The results were similar for both durations of ischemia.

4. Histology

The neuronal alterations in the cerebral cortex that resulted from occlusion of the middle cerebral artery are similar to those described in previous studies. Mild or Grade 1 change consisted of slight neuronal shrinkage with loss of Nissl substance. In severely altered neurons (Grade 3), there was severe shrinkage, bright cytoplasmic eosinophilia and nuclear pyknosis. The intermediate gradation (Grade 2 moderate change) was that of moderate neuronal shrinkage and increased nuclear basophilia without pyknosis.

The mean cross-sectional areas, expressed as percentages of the total cross-sectional area of the grey matter of the hemisphere are summarized in table 3. After six hours of ischemia, the mean total area of the cerebral cortex exhibiting ischemic neuronal alterations was similar in both treated (40 ± 17%) and untreated cats (37 ± 12%). Although the extent of severe neuronal alterations (Grade 3) in the lidocaine treated cats (mean = 8 ± 9%) was less than that of the control group (mean = 15 ± 10%), this difference was not statistically significant. Similarly, comparison of the other subgroups of severity (Grades 1 and 2) revealed no significant differences. In the animals subjected to 4 hours of left MCA occlusion, the results were comparable to those just described, with no statistically significant difference between treated and untreated groups. There was minimal ischemic neuronal alterations in the contralateral hemisphere.

Discussion

It has recently been suggested that lidocaine hydrochloride may confer protection on cerebral tissue during episodes of ischemia. This conclusion was based on the results of several metabolic and biochemical studies. Potassium efflux, which has been shown to occur from cortical neurons during ischemia, was delayed by massive doses of intravenous lidocaine during global ischemia. The dose required to achieve this effect also flattened the EEG and reduced oxygen and glucose consumption in the brain. As well, during periods of glucose lack, the addition of lidocaine hydrochloride to vagus nerve preparations decreased axonal permeability to potassium and sodium ions. It appeared, therefore, that lidocaine, by stabilizing membranes, reduced energy expenditure and delayed the onset of irreversible structural damage. This expectation is not confirmed by the results of the present investigation.

In support of a protective effect of lidocaine, Evans et al observed that neurological function (as assessed by cortical somatosensory evoked response) was substantially preserved following cerebral air embolism in the animals that received a single prophylactic dose (5 mg/kg) of lidocaine. Considering that in dogs, this dose produced less than a 30% reduction in CMRO2, the reported effect on SER's appeared dramatic and in excess of what can be predicted from neurochemical studies. It is pertinent to emphasize that cerebral air embolism in cats only transiently interrupts flow through small cerebral arteries and reperfusion occurs usually within a half hour, following dissipation of the embolus. Although the blood-brain barrier remains intact, intracranial pressure is often elevated. The salutary effect of lidocaine on neurological function during cerebral air embolism may partly be attributable to reduction in intracranial pressure. The model of focal cerebral ischemia utilized in the present study

<table>
<thead>
<tr>
<th>Duration of ischemia (hours)</th>
<th>% Area of cerebral cortical infarction (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lidocaine</td>
</tr>
<tr>
<td>6</td>
<td>10 ± 8</td>
</tr>
<tr>
<td>Grade 2</td>
<td>22 ± 14</td>
</tr>
<tr>
<td>Grade 3</td>
<td>8 ± 9</td>
</tr>
<tr>
<td>All grades</td>
<td>40 ± 17</td>
</tr>
<tr>
<td>(n = 4)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>13 ± 15</td>
</tr>
<tr>
<td>Grade 2</td>
<td>9 ± 8</td>
</tr>
<tr>
<td>Grade 3</td>
<td>6 ± 4</td>
</tr>
<tr>
<td>All grades</td>
<td>28 ± 18</td>
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</tbody>
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Consisted of major vessel occlusion (middle cerebral artery) for 6 hours without reperfusion. Significant disruption of the blood-brain barrier occurred and the duration of ischemia was long enough to allow histologic changes to be apparent. The lack of benefit in the lidocaine-treated cats may well be a reflection of the severity and extent of the ischemic injury which exceeded that resulting from air embolism.

It is appropriate to further speculate on why lidocaine failed to provide benefit in the present investigation. Haschke and Fink have shown that the addition of lidocaine to porcine brain mitochondria markedly inhibited oxygen consumption. Specifically, this resulted from enzyme blockade in the electron transport system and was similar to the effect of anoxia. Previously, Geddes and Questel had observed that potassium-stimulated increases in the respiration of slices of rat brain cortex were considerably inhibited by lidocaine. Thus, in addition to membrane-stabilizing properties, high concentrations of lidocaine appear to possess the property of impairing cellular respiration and ATP production in a specific manner. The savings in oxygen and glucose consumption reported by Astrup et al. may partly be consequent on this. If these results can be extrapolated to in vivo situations, the availability of high energy substrates may be jeopardized by substantial doses of lidocaine. However, concentrations in excess of those used in this study would probably be required to produce such effects.

The extent and severity of ischemic neuronal alterations were not reduced by dosages of lidocaine high enough to abolish the EEG. A beneficial effect was similarly not observed when the duration of ischemia was shortened to four hours. Although there was a numerical trend toward a lesser percentage of grade 2 injury in the lidocaine group, this did not approach statistical significance (p = 0.25). Furthermore, the numerical trend was towards a larger percentage of grade 3 injury in the lidocaine-treated animals. The inter-animal variation was such that it seemed highly unlikely that by enlarging this group by a few more animals that we would alter our conclusions. We cannot however exclude the possibility that lidocaine may be protective when used with shorter periods of focal ischemia.

The histological findings were corroborated by the results of TTC reaction. In the presence of intact mitochondrial enzymes, 2',3',4' triphenyl-2H-tetrazolium chloride is reduced to a red colored formazan. This color change was absent in regions where irreversible tissue damage had occurred suggesting that this technique is a reliable histochemical indicator of cerebral infarction.

Lidocaine at high dose (plasma concentrations >25.5 μmol/l) exerts significant hemodynamic effects. This is associated with increased cerebral vascular resistance and reduced cerebral blood flow. Although reduction in cerebral blood flow might contribute to a reduction in intracranial pressure, it might also be harmful to the substrate supply of damaged dysautoregulated cerebral tissue. Hypoten-
sive episodes responsive to pressor agents occurred in all animals after lidocaine administration. It is conceivable that these episodes, even though brief, contributed to the severity of ischemic alterations observed in the lidocaine-treated cats. The use of a smaller dose of lidocaine may avoid this complication.

The optimal dose required to suppress the EEG without inducing seizures has yet to be determined. Some investigators have emphasized the dual effect of this drug: EEG suppression in low doses and potent convulsant effect in 10–20 mg/kg dose range. Because of cardiotoxic effects, doses beyond this have seldom been evaluated until Astrup et al. showed that a single bolus of 160 mg/kg, rapidly administered, abolished the EEG. The corresponding peak plasma lidocaine level was 108 ± 16 mg/l (≈ 432 ± 64 μmol/l). The EEG remained flat for up to 45 minutes after the bolus. The dose was much in excess of that required to produce cardiovascular toxicity in dogs, which were, however, on extracorporeal circulation during the experiments. By administering a 50 mg bolus IV followed by a continuous infusion of lidocaine at a rate slightly below 1 mg/kg/min, it was possible in our study to achieve prolonged EEG suppression.

The dose of lidocaine required to produce maximum reduction in cerebral metabolic rate is also not known. The EEG has been used in this study as an end point of treatment. However, in dogs pretreated with pentobarbital to the point of EEG suppression, lidocaine administration resulted in additional reduction of cerebral metabolic rate. This observation suggests that EEG suppression may not be the appropriate end point for lidocaine treatment, so that the amount infused during this study may have been inadequate for cerebral protection. However, as noted above, larger doses are associated with unacceptable cardiovascular depression.

Conclusion

In doses sufficient to suppress or flatten the EEG, lidocaine hydrochloride did not reduce the extent or severity of ischemic neuronal injury. Reducing the duration of ischemia from 6 to 4 hours did not result in a favorable modification of ischemic alterations in either the treated or the control group. There was good agreement between the results of histochemical (TTC) and histological methods of assessment.

References
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**Time-Related Asymmetric Changes of Brain Microvessel β-Adrenergic Receptors in the Two Hemispheres After Carotid Occlusion**

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**SUMMARY** The effect of short term and long term ischemia induced by right carotid occlusion was studied on β-adrenergic receptor function in rat cerebral microvessels. The results show a different time-dependent responsiveness of the two hemispheres to ischemia, with a pronounced and more persistent decrease in the number of capillary β-receptors in the left side of the brain. The data suggest the existence of asymmetries in the control of brain microvasculature which may mediate the different time-course of β-receptor changes in response to ischemia.

**Stroke** Vol 17, No 5, September-October 1986

NEUROTRANSMITTER CONTENT and metabolism appear to be altered in various cerebral areas after temporary or permanent ischemia.1–11 The altered patterns of neuronal activity may influence brain capillary function. In fact, increasing evidence suggests that microcirculation in the brain is under neuronal control and that β-adrenergic receptors in cerebral capillaries may play an important role in the regulation of microvessel function.12–17

Recent studies demonstrated a different responsiveness of the microvasculature of the two hemispheres to ischemia. In fact, 48 h occlusion of the right carotid artery in the rat and in the gerbil induces a reduction in the number of β-receptors in brain capillaries of both hemispheres; notably, the reduction in the contralateral hemisphere is more marked than in the ipsilateral one, suggesting a different sensitivity of the microvasculature of the two sides of the brain to ischemia.18,19 To further investigate the responsiveness of the microvasculature of the two hemispheres to ischemia and the changes of its regulatory mechanisms, the natural evolution of the phenomenon was studied by measuring the effect of right carotid ligature on β-adrenergic receptors in brain capillaries at early (2 to 38 h) and late times (96 h and 14 days) after the occlusion.
An evaluation of the effect of lidocaine in experimental focal cerebral ischemia.
M T Shokunbi, A W Gelb, S J Peerless, M Mervart and P Floyd

Stroke. 1986;17:962-966
doi: 10.1161/01.STR.17.5.962

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