Fatal Strokes in Hyperglycemic Cats

Gabrielle M. de Courten-Myers, MD, Marla Kleinholz, BS, Kenneth R. Wagner, PhD, and Ronald E. Myers, MD, PhD

Hyperglycemia is associated with three- to fourfold larger infarcts than normoglycemia following permanent middle cerebral artery occlusion in cats. We investigated the effects of glycemia on brain outcome when middle cerebral artery blood flow was restored (clip release) after 4 hours of occlusion. Seven of 13 hyperglycemic (22 mM) and one of 12 normoglycemic (6 mM) anesthetized cats developed total middle cerebral artery territory infarcts and hemispheric edema and died of brainstem compression. The remaining six and 11 cats recovered fully and later showed no or only small infarcts. Compared with permanent occlusion, restoration of blood flow after 4 hours reduced infarct volume in all normoglycemic and hyperglycemic cats that survived, but caused a much higher proportion (54% vs. 17%) of hyperglycemic and, for the first time, one normoglycemic cat, to die of infarct extension, hemorrhagic infarct conversion, and total territory edema. Thus, clip release after 4 hours caused some cats to show reduced and others to show augmented tissue damage. Rendering cats hyperglycemic substantially worsened their outcome after reperfusion by increasing their death rate from total territory edema sevenfold. Our results demonstrate that risk/benefit analyses for recanalization efforts in humans should take serum glucose concentrations into account. (Stroke 1989;20:1707-1715)

Restoring blood flow to an ischemic brain territory by clot lysis in the treatment of acute stroke has the potential for harm by extending infarcts, increasing brain edema, and converting ischemic into hemorrhagic infarcts.1-7 Thus, though stroke treatment by fibrinolysis may improve outcome in some patients,8-18 it may in others violate the basic concept of nihil nocere, or “never do harm.” The present uncertainty regarding the effects of thrombolytic therapy makes it important to clarify those mechanisms that may aggravate or reduce tissue injury due to restoration of blood flow and to develop methods to identify those patients who will benefit and those who risk aggravation of their neurologic outcome or even death from such treatment.

Whereas longer durations of ischemia are recognized as enhancing the probability of reperfusion injury,6,7 the impact that metabolic factors, such as level of glycemia, may have on outcome has not been investigated. That level of glycemia may affect outcome from focal ischemia is made plausible by studies in our19-22 and other23-26 laboratories that have demonstrated that hyperglycemia markedly reduces brain tolerance to circulatory arrest and other forms of systemic anoxia/ischemia and strikingly increases brain injury. Our earlier animal studies demonstrated that hyperglycemia extends infarct size resulting from permanent middle cerebral artery (MCA) occlusion.27 These findings parallel those of the clinic, which indicate that hyperglycemic patients who suffer strokes experience worse prognoses than do their normoglycemic contemporaries.28-33

We used a 4-hour MCA occlusion model in cats to investigate the influence of normoglycemia versus hyperglycemia on the brain pathologic effects of restoring blood flow and compared these results with those from permanent MCA occlusion in cats with similar levels of glycemia.

**Materials and Methods**

Occlusion of the MCA in a number of animal species reliably produces cerebral infarcts,34-39 whereas release of the occlusion restores blood flow to the ischemic territory.40,41
We deprived 81 cats of food for 48 hours before surgery to deplete their stores of liver glycogen. Either 10% glucose solutions (in 37 cats, 22 mM glucose) or 0.9% saline (in 44 cats, 6 mM serum glucose) were infused starting 1 hour before and for 6 and 8 hours, respectively, after MCA occlusion (10 ml/kg for 1 hour and 5 ml/kg/hour for 8 hours). All cats then received saline for the last 2 hours of observation.

Our surgical techniques are the same as those described earlier.\textsuperscript{27} Anesthesia was induced with 35 mg/kg i.v. pentobarbital. Additional 2-mg/kg doses were administered hourly during surgery and for 8 hours afterward to sustain anesthesia for postsurgical monitoring. A transorbital approach was used to occlude the right MCA to minimize brain exposure and retraction. We used a surgical microscope to identify the MCA and its adjoining structures through a 4×6 mm bone opening created in the posterior roof of the orbit. A Yasargil aneurysm clip was atraumatically placed and left in place or later removed to permanently or temporarily occlude this vessel, respectively. The surgeon was blinded as to the glycemia class of each cat. Cats that survived the acute postrelease period (i.e., those that failed to die of hemispheric edema) were maintained for 2 weeks afterward to provide for precise delineation of the margins of tissue damage (infarcts).

We prepared four groups of cats: Group 1, temporary MCA occlusion in glucose-infused hyperglycemic cats with clip release at 4 hours (n=17); Group 2, temporary MCA occlusion in saline-infused normoglycemic cats with clip release at 4 hours (n=20); Group 3, permanent MCA occlusion in glucose-infused hyperglycemic cats (n=20); and Group 4, permanent MCA occlusion in saline-infused normoglycemic cats (n=24). Groups 3 and 4 were expanded from eight cats each, the results of which have been published.\textsuperscript{27}

All cats were monitored physiologically before, during, and for 8 hours after MCA occlusion regarding a number of parameters, disturbances of any one of which might affect outcome. Particular attention was paid to maintaining the blood volume unchanged (all blood losses were replaced by repeated, small transfusions; all cats with surgical blood losses of >10 ml were excluded from the study). Deviations of >2 standard deviations (SDs) in blood pressure, hematocrit, body temperature, level of anesthesia, blood gases, and acid-base values triggered exclusion of the cat from the study.

We excluded 31 cats (38% of the 81 prepared), mostly during the study’s early phase. The decision for exclusion was always made in a blinded fashion as to group identity. Reasons for exclusion included 1) surgical blood loss of >10 ml or hemorrhage into the cranial cavity in nine cats (three from Group 2, three from Group 3, and three from Group 4); 2) other technical problems such as incomplete, incorrect, or unverifiable MCA clip placement in five cats (one from Group 1, three from Group 3, and one from Group 4); 3) postoperative illness such as pneumonia, feline rhinotracheitis, etc. in five cats (one from Group 1, one from Group 2, and three from Group 4); 4) hypotension (mean arterial blood pressure of <85 mm Hg) during the first 8 hours after MCA occlusion in six cats (one from Group 1, two from Group 2, one from Group 3, and two from Group 4); and 5) metabolic disturbances such as hypothermia or hyperthermia or blood gases, pH, or hematocrit >2 SDs from the mean in six cats (one from Group 1, two from Group 2, one from Group 3, and two from Group 4). Therefore, we studied 50 cats in the four groups: 13 in Group 1, 12 in Group 2, 12 in Group 3, and 13 in Group 4.

After 2 weeks of survival, the cats were anesthetized and their vascular systems were perfused with 10% buffered formalin. The perfusion-fixed brains were immediately removed, stored for 2 weeks in formalin, and then examined grossly, microscopically, and morphometrically to quantify any resulting infarcts.

The cerebrum was cut into six standard blocks and embedded in paraffin. Whole-mount sections were stained with hematoxylin and eosin, and the areas of infarction and of preserved ipsilateral brain parenchyma were measured using computer-assisted morphometry (Bioquant, R&M Biometrics, Nashville, Tennessee). Infarct volume was expressed as a percentage of the MCA territory by multiplying by 2 since earlier carbon black injections suggested that the MCA territory in cats encompasses one half of the cerebral hemispheric volume. Sham-operated control cats exhibit no or slight damage to the cortex, restricted to the region immediately overlying the bone window (<1% of the MCA territory). Thus, we classified cats that exhibited damage of <1% as showing no injury. The brains of cats that died during the first 2 days of MCA territory (hemispheric) edema were also perfusion-fixed and similarly examined; the extent and localization of infarction and the associated tissue edema were assessed semiquantitatively. Morphometric determinations were not performed on these brains since edematous infarcts in cats dying acutely do not compare with 2-week-old organizing infarcts. All brain examinations were performed without knowledge of group identity.

Since the physiologic parameters monitored among the groups exhibited a normal distribution, the data were analyzed using one-way analysis of variance (ANOVA) with correction for unequal group sizes. Because the brain findings after temporary MCA occlusion exhibited a bimodal distribution, Fisher’s exact test in conjunction with the Bonferroni correction was used to compare the groups as to the probability that their respective incidences of long-term survivors and of cats dying acutely of MCA territory edema differed significantly. Because infarct size in survivors also failed to be normally distributed, a Wilcoxon’s scores two-sample test with a continuity correction of 0.5 was used.
and in conjunction with the Bonferroni correction was applied to this component of the data analysis. For all tests, $p < 0.05$ indicated significance.

Results

The two infusion schedules divided the cats into two distinct glycemia classes: normoglycemic and hyperglycemic. Each class, in turn, was subdivided into cats that underwent permanent or temporary MCA occlusions. The serum glucose concentrations in the resulting four groups at the time of MCA occlusion and during the 8 hours of monitoring that followed are depicted in Figure 1. Table 1 presents the physiologic parameters monitored. The absence of significant differences by ANOVA among the groups at all but two times regarding these parameters confirms group comparability.

The cats exposed to temporary MCA occlusion showed a striking dichotomy of their brain pathologic outcomes; that is, they showed either no or small infarcts or they developed a necrosis that affected the entire MCA territory accompanied by massive hemispheric edema and died (Figures 2 and 3). Figure 4 illustrates a brain cross-section from one of these cats showing upper brainstem compression and transtentorial herniation of the edematous and infarcted hemisphere.

The brain pathologic findings are summarized in Table 2. Group 1 cats died of hemispheric edema significantly more frequently than did Group 2 cats ($54\%$ vs. $8\%$). Further, death from hemispheric edema occurred four times more frequently among cats with temporary MCA occlusion than among cats with permanent MCA occlusion. On the other hand, cats that survived temporary MCA occlusion fared better than those that survived permanent MCA occlusion in that the former showed a $>30$-fold smaller mean infarct volume.

Figure 2 shows the four groups and their brain pathologic outcomes expressed as frequency of occurrence of no ($0-1\%$), small ($1-10\%$), medium ($10-40\%$), and large ($>40\%$) infarcts in survivors and of total MCA territory infarcts, marked edema, and death from brainstem compression within 5-40 hours after clip application. Group 3 cats showed a wide spectrum of outcomes, ranging from small infarcts to hemispheric edema deaths; large infarcts and deaths from hemispheric edema were twice as frequent as small and medium infarcts. In contrast, all Group 4 cats survived and showed only small or medium infarcts. The hemispheric infarcts in Group 4 cats, on average were one third the volume of those in surviving Group 3 cats ($p < 0.01$). Cats exposed to temporary MCA occlusion showed no infarcts significantly more frequently (12 of 25) than did cats exposed to permanent MCA occlusion (one of 25).

Seven of the 13 Group 1 cats died 5-36 hours after clip application; all showed total MCA territory infarcts, and the entire territory displayed marked edema. The infarcted territories in these cats also showed focal, small-vessel hemorrhages visible both grossly and microscopically and affecting mainly the gray matter (Figure 5). These same territories also showed marked, small-vessel dilatation, with their lumina packed with erythrocytes (vasoparalysis). Three of the 12 Group 3 cats also showed similar focally hemorrhagic infarcts, but only one such infarct occurred in a cat dying of edema. No normoglycemic cat developed hemorrhage into the infarct, including the one cat that died of hemispheric edema. Thus, hemorrhage into infarcted tissue was restricted to hyperglycemic cats and affected mainly those with temporary MCA occlusion.

Discussion

The clinical diagnosis of “stroke” corresponds to a variety of pathologic brain changes that include hemorrhages, ischemic infarcts, and hemorrhagic arterial and venous infarcts of variable sizes and locations. Thromboembolic events constitute up to $80\%$ of strokes.\textsuperscript{4, 43}

Individual animal stroke models can address only a subset of the complex entity known as stroke. Temporary and permanent MCA occlusion in cats is a model of large-vessel occlusive disease that leads to a wide range of infarct sizes, with purely ischemic or ischemic-hemorrhagic lesions with or without development of massive edema within the affected territory causing transtentorial herniation, brainstem compression, and death. This model appears relevant for approximately $40\%$ of strokes and, as in our study, edema of infarcted tissue with
transient herniation is considered a major cause of death in autopsy series of cerebral infarctions in humans.42–44

Restoring blood pressure and, therefore, blood flow within the blood vessels supplying brain tissue rendered focally ischemic for 4 hours in our study produced dichotomous results both in normoglycemic and hyperglycemic cats. In some cats reperfusion after 4 hours of MCA occlusion markedly extended the zone of tissue injury within the ischemic territory, and this was accompanied by massive tissue edema affecting the same territory. This mechanism killed 8% of the normoglycemic (Group 2) and 54% of the hyperglycemic (Group 1) cats with temporary MCA occlusion within 5–36 hours. However, in other cats, reperfusion under manifestly the same circumstances permitted survival with no (six of 13 hyperglycemic [Group 1] and six of 12 normoglycemic [Group 2] cats) or only small infarcts (five of 12 normoglycemic [Group 2] cats). Thus, our results confirm a bipolarity or dichotomy of outcome after reperfusion of ischemic brain, that is, ameliorating the expected outcome by prevention of that permanent brain damage shown to take place after permanent blood vessel occlusion in some animals, while exacerbating the brain damage in other animals by extension of the infarct to include the entire ischemic vascular territory and causing death from marked edema in that territory in a much higher proportion than after permanent occlusion (54% and 8% vs. 17% and 0% in Groups 1 and 2 vs. 3 and 4, respectively). Survivors of temporary MCA occlusion exhibited significantly smaller infarcts than did their counterparts with permanent MCA occlusion, (0.2% vs. 41% infarct volume in hyperglycemic cats and 1.2% vs. 14% in normoglycemic cats).
infarct volume in normoglycemic cats). This finding of more widespread tissue injury with prolonged ischemia agrees with other studies.45-49

Our results are in accord with the pioneering findings of Sundt et al,45 who described a bimodal outcome after temporary MCA occlusion in monkeys and cats. These authors found a reversibility of tissue changes after 3 and 6 hours in 60% of monkeys and 90% of cats, respectively, whereas the remainder died of brain edema. Other studies of temporary MCA occlusion in monkeys, cats, and rats have failed to define such a dichotomous outcome. Rather, the latter studies suggest that longer durations of vascular occlusion cause stepwise augmentations of infarct sizes.46-49

Elevated serum glucose concentrations accentuate brain tissue acidosis and, consequently, accentuate damage to the brain from systemic anoxic/ischemic exposures.20,21,25 Hyperglycemia also significantly increases infarct volume in rats exposed to transient (10-15-minute) focal cerebral ischemia50 and augments infarct size in cats following permanent MCA occlusion.27 Our study similarly demonstrates that moderate hyperglycemia (400 mg% or 22 mM glucose) increases sevenfold the probability of dying of ischemic tissue edema following temporary MCA occlusion. Thus, hyperglycemia markedly increases the risk of brain damage and death in both systemic anoxia and in temporary or permanent focal cerebral ischemia.

Brain biochemical data from cardiac arrest and systemic hypoxic/hypotensive studies19-22,25,51 suggest that hyperglycemia's adverse effects are mediated through the production of a more marked tissue acidosis. Recent studies in our laboratory52 similarly demonstrate a concordance between suprathreshold lactic acid accumulation in the brain (>17-
**FIGURE 4.** Coronal brain sections illustrating (top) transtentorial herniation with midbrain compression in hyperglycemic cat after 4 hours of middle cerebral artery occlusion, subsequent reperfusion, and death 32 hours later and (bottom) normal cat brain at same level for comparison.

**TABLE 2.** Brain Pathologic Outcome in Cats With MCA Occlusion

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>Results</th>
<th>( p )</th>
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</tr>
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<tbody>
<tr>
<td><strong>Acute death vs. 2-week survival</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1 vs. 2</td>
<td>7:6 vs. 1:11</td>
<td>0.03</td>
<td>F</td>
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<tr>
<td>1+2 vs. 3+4</td>
<td>8:17 vs. 2:23</td>
<td>0.04</td>
<td>F'</td>
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<td><strong>Infarct volume in survivors</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>3 vs. 4</td>
<td>41±9 vs. 14±4</td>
<td>0.01</td>
<td>W</td>
</tr>
<tr>
<td>1 vs. 2</td>
<td>0.2±0.1 vs. 1.2±0.4</td>
<td>0.11</td>
<td>W</td>
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<tr>
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<td>0.002</td>
<td>W</td>
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<tr>
<td>1+2 vs. 3+4</td>
<td>0.8±0.3 vs. 26±5</td>
<td>0.0001</td>
<td>W</td>
</tr>
</tbody>
</table>

MCA, middle cerebral artery; 1, temporary MCA occlusion in 13 hyperglycemic cats; 2, temporary MCA occlusion in 12 normoglycemic cats; 3, permanent MCA occlusion in 12 hyperglycemic cats; 4, permanent MCA occlusion in 13 normoglycemic cats. Data are number of cats or mean±SEM % of MCA territory. F, Fisher’s exact two-tailed test; F’, Fisher’s right exact test; W, Wilcoxon scores, \( t \) test with approximate significance.
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FIGURE 5. Photomicrographs of (top) hemorrhagic and (bottom) ischemic infarcts in caudate nucleus of hyperglycemic and normoglycemic cats that died 36 and 6 hours, respectively, after temporary occlusion of middle cerebral artery. Hematoxylin and eosin stain, ×5.

Hemorrhagic infarcts have been observed in situations in which blood flow is restored to a recently ischemic area either as collateral flow or by reopening an occluded vessel.4'55'56 Further, experimental studies have demonstrated that blood-brain barrier permeability is altered after ischemia and reperfusion.40,56-60 Erythrocyte diapedesis therefore appears to represent, in extreme form, a vascular permeability defect. Our study showed hemorrhagic infarcts to occur solely in hyperglycemic cats, affecting one fourth of all those with permanent MCA occlusions and encompassing all cats that died of hemispheric edema after temporary MCA occlusion. Kamijyo et al40 also have shown that hemorrhagic infarcts are more preva-
lent among cats with temporary MCA occlusion and subsequent reperfusion. The restriction of hemorrhagic infarcts to glucose-infused cats in our study underscores hyperglycemia’s aggravating, tissue-damaging effect in focal cerebral ischemia. Hyperglycemia leading to marked tissue acidosis modulates the brain tissue response to blood flow restoration in focal ischemia, markedly increasing the risk of infarct extension and edema formation, leading to death. Hyperglycemia converts what would be a simple ischemic infarct into a hemorrhagic-ischemic infarct after identical durations of temporary or permanent MCA occlusion. Thus, in benefit/risk assessments for thrombolytic or other therapeutic recanalization procedures in the treatment of stroke, duration of cerebral ischemia must not be the only factor considered. The high incidence (54%) of rapidly fatal hemispheric edema following hyperglycemic reperfusion in our cat MCA occlusion model provides opportunity for further study and understanding of this phenomenon.

In conclusion, brain infarcts from cerebrovascular occlusion are generally preventable by restoring blood flow even after a delay as long as 4 hours in anesthetized cats. Unfortunately, the restoration of blood flow that prevents or markedly restricts brain infarction in some cats causes widespread MCA territory infarction with edema and death in others. This risk of death on restoring blood flow is especially high in hyperglycemic cats. In any case, with or without release of the vascular occlusion, elevated serum glucose concentrations substantially exacerbate brain pathologic response to focal ischemia.

References


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KEY WORDS • hyperglycemia • reperfusion • brain edema • cats
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