Hyperglycemia Reduces the Extent of Cerebral Infarction in Rats

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Although hyperglycemia is known to exacerbate neuronal injury in the setting of reversible brain ischemia, its effect on irreversible thrombotic infarction is less well understood. In this study, unilateral thrombotic infarction was induced photochemically in the parietal cortex of Wistar rats. Seven days later, brains were perfusion-fixed for light microscopy. Infarct areas were measured by computer-assisted planimetry on multiple coronal sections at 250-μm intervals; these data were integrated to yield infarct volumes. Fasted, normoglycemic rats were compared with hyperglycemic rats that had received 1.2–1.5 ml of 50% dextrose i.p. 15 minutes prior to the induction of infarction. Infarct volume averaged 12.5 ± 4.0 mm³ (mean ± SD) in rats (n = 14) with plasma glucose levels of 72–184 mg/dl; this differed statistically from the average volume of 9.3 ± 3.3 mm³ observed in rats (n = 13) with elevated plasma glucose (range 264–607 mg/dl). Spearman rank correlation analysis confirmed a significant correlation of larger infarct volumes with lower plasma glucose levels. In contrast, rats receiving mannitol i.p. to produce an osmotic load comparable with that of the dextrose-pretreated animals showed larger infarct volumes than saline-treated controls. The small but definite beneficial effect of hyperglycemia in this end-arteriolar thrombotic infarction model is possibly attributable to improved local energy metabolism at the periphery of the lesion during the early period of lesion expansion. (Stroke 1987;18:570–574)

It is now well appreciated that hyperglycemia may worsen the outcome of transient cerebral ischemia. In their studies in primates, Myers and Yamaguchi noted that food-deprived animals tolerated 14 minutes of cardiac arrest with only minimal neurologic changes and showed only restricted neuropathologic abnormalities, whereas animals administered glucose prior to arrest were severely abnormal and exhibited widespread gray-matter necrosis. Ginsberg, Welsh, and their colleagues documented a marked accentuation of heterogeneous postischemic hyperperfusion together with postischemic elevations of brain lactate levels and pronounced deterioration of cerebral energy metabolites in cats given large glucose loads prior to transient global cerebral ischemia. Similar results have been reported from several laboratories and ultrastructural studies have demonstrated consistent endothelial swelling with narrowing of cortical capillary lumina.

Hyperglycemia has been thought to exert its injurious effect by elevating brain stores of glucose, the anaerobic metabolism of which during ischemia gives rise to marked tissue lactic acidosis. During incomplete ischemia, the continued delivery of glucose to brain tissue from the circulating blood further fuels this process. It has been suggested that cerebral acidosis may enhance mobilization of tissue iron, which in turn may contribute to the production of injurious active oxygen radical species.

The studies cited above were carried out in models of transient reversible high-grade cerebral ischemia — a setting that (in the absence of hyperglycemia) gives rise to ischemic neuronal alterations without frank parenchymal necrosis. In the present study, we were concerned, rather, with the influence of hyperglycemia in the condition of completed thrombotic infarction — a less well studied situation but one of obvious relevance to the clinical problem of completed stroke in humans.

Materials and Methods

Photochemical Induction of Focal Thrombotic Infarction

Food-deprived male Wistar rats weighing 275–350 g were used in these studies. Anesthesia was induced with 3% halothane and maintained with 1.5–2.0% halothane, 70% nitrous oxide, and the balance of oxygen delivered via a closely fitting face mask. The scalp overlying the parietal convexity of the left hemisphere was exposed, and a focal thrombotic infarction was then induced noninvasively in these rats by the photochemical method previously established in our laboratory. The photosensitizing dye rose bengal (1 mg in
0.133 ml of saline per 100 g body wt) was first administered i.v. With the rat restrained in a stereotactic frame, green light from a xenon arc lamp, centered at 560 nm, was directed onto the skull. Owing to the translucency of the skull, the light penetrated into the underlying brain. The interaction of intravascular rose bengal with light results in the production of singlet oxygen, a highly reactive excited state of oxygen, within the exposed blood vessels; this leads to endothelial injury and subsequent platelet aggregation within the irradiated microvasculature. For this study, the light intensity was 0.58 W/cm² and the duration of irradiation 2 minutes. Following this procedure, the scalp was sutured, and the rats were revived and returned to their cages. The antibiotic cefazolin, 40 mg/kg/day i.p., was administered postoperatively during the 7-day survival period.

Histologic Evaluation and Planimetric Analysis

Seven days after photochemical induction of thrombotic stroke, brains were prepared for histopathology. Rats were anesthetized with halothane and perfused transcardially with a mixture of formaldehyde, glacial acetic acid, and methanol (FAM, 1:1:8 by vol) delivered at a pressure of 100–110 mm Hg for 20 minutes and preceded by a 1-minute saline flush. The heads were immersed in FAM at 4°C overnight, and brains were then carefully removed. Coronal brain blocks were embedded in paraffin and dehydrated, and 10-µm sections were prepared at 250-µm intervals. These were stained with hematoxylin and eosin.

Infarct areas were quantified by one of us without knowledge of the rats' experimental status. Computer-assisted planimetry was used to measure the area of infarction on multiple coronal sections separated by 250 µm and spanning the anteroposterior extent of the infarction. Each such section was first viewed using a light microscope with a 10× objective, and the image of the infarcted zone was projected via a drawing tube onto paper. The perimeter of the infarct, encompassing the zone of macrophage infiltration, was traced onto the paper. Each such tracing was subsequently redrawn onto a digitizing tablet (Summagraphics) interfaced to a PDP 11/44 minicomputer, which computed the areas. Total infarct volume was then derived by means of numerical integration of sequential infarct areas using the trapezoidal rule. In addition, whole brain cross-sectional area at the level of the hippocampus was estimated planimetrically for each animal in a fashion similar to the above.

Production of Hyperglycemia

Rats to be made hyperglycemic received an i.p. injection of 1.2–1.5 ml of 50% dextrose 15 minutes prior to the induction of infarction. Normoglycemic controls received a comparable volume of saline. To address the concern of a possible osmotic influence of dextrose, a third group of rats was studied, which received 2.8 ml of 18% mannitol i.p. prior to the induction of infarction, to produce a comparable intraperitoneal osmotic load.

Results

Physiologic Variables

These are summarized in Table 1. Mild hypercarbia was present in all groups of rats, probably the result of the type of face mask used for anesthesia administration. The slight acidosis was compatible with the extent of hypercarbia. There were no significant differences among the various groups.

Pathologic Observations

Our previous studies have established that the photochemical method gives rise to subtle endothelial damage within the irradiated zone, leading to extensive platelet aggregation and occlusive thrombosis. The topography of the infarct is illustrated diagrammatically in Figure 1. The infarct typically involved the full thickness of the parietal cortex and touched the underlying white matter but spared underlying gray structures. Survival in all rats was excellent, without evidence of clinical abnormalities. Microscopically, there was a well-demarcated focus of ischemic necrosis with surrounding macrophage infiltration (Figure 2). Reactive astrocytes and polymorphonuclear leukocytes were occasionally seen within the pale-staining and spongiform appearing neuropil. Parenchymal vessels within the infarcted zone frequently contained thrombotic material and exhibited red cell stasis.

Relation of Plasma Glucose Level to Infarct Volume

Plasma glucose levels in the series as a whole at the induction of photochemical thrombosis ranged from 72 to 607 mg/dl. The average infarct volume in the series was 10.9 ± 3.9 mm³ (mean ± SD). It was possible to divide the rats into 2 groups according to their blood glucose levels at the time of induction of the photochemical infarction. The 14 rats with plasma glucose levels in the range 72–184 mg/dl exhibited a mean infarct volume of 12.5 ± 4.0 mm³ (mean ± SD). In contrast, the 13 rats with elevated plasma glucose, ranging from 264 to 607 mg/dl, showed an infarct volume of 9.3 ± 3.3 mm³ (mean ± SD). The difference in infarct size between these groups was significant by the two-tailed Student’s t test (p < 0.05).

In Figure 3, the individual infarct volumes in each of the dextrose- and saline-treated rats are plotted as a function of plasma glucose level. Infarct volume was not linearly related to the plasma glucose level (correlation coefficient, r = −0.27). However, of the 11 rats showing infarct volumes > 11.0 mm³, 8 had plasma glucose levels of 184 mg/dl or below. To confirm the
impression that larger infarct volumes were associated with lower levels of plasma glucose, a Spearman rank correlation test was carried out to establish the extent of correlation between ascending infarct volume and descending plasma glucose. The Spearman correlation coefficient was 0.7100, denoting significance at p<0.01. Thus, these data revealed the unexpected finding that hyperglycemia actually led to a slight diminution of infarct volume.

To ascertain that glucose pretreatment did not affect the size of the brain as a whole, a planimetric analysis of total cross-sectional area of each brain was carried out at the level of the dorsal hippocampus. There was no significant correlation between cross-sectional area of the entire brain and plasma glucose level (r = 0.21).

**Brain Glucose Levels**

In a separate series of rats, both plasma and brain glucose levels were assayed 35 minutes after administration of either dextrose or saline i.p. as described in "Materials and Methods." Brains were frozen transversely by the application of liquid nitrogen, and brain glucose was assayed in the frontoparietal cortex by an enzymatic, fluorometric technique. In normoglycemic rats, having plasma glucose levels of 102–134 mg/dl, brain glucose levels ranged from 1.45 to 2.24 mmol/kg. In contrast, hyperglycemic rats, with plasma glucose values of 330–374 mg/dl, had brain glu-
cose levels of 3.76–4.44 mmol/kg. Thus, we could be confident that dextrose administration had induced significant elevations of brain glucose.

**Effect of Mannitol Pretreatment**

Comparison of infarct volume was undertaken in mannitol-pretreated vs. saline-treated controls. Mannitol-pretreated animals (n = 7), having plasma glucose levels of 164 ± 84 mg/dl (mean ± SD), exhibited a mean infarct volume of 18.3 ± 2.5 mm³. In contrast, saline-treated controls (n = 5), having plasma glucose of 140 ± 45 mg/dl, had a mean infarct volume of 10.2 ± 2.2 mm³. The difference in plasma glucose levels between the 2 groups was not significant, but the infarct volume in the mannitol-pretreated group was significantly greater than that of the saline-treated controls (two-tailed Student’s *t* test, *p* < 0.01). This result is opposite to that observed in the dextrose-pretreated rats. (There was no difference in whole-brain cross-sectional area between the 2 groups.)

Serum osmolality was assessed by freezing-point measurements in selected animals of the saline-, dextrose-, and mannitol-pretreated groups. These data are shown in Figure 4. Only in the mannitol series was there a consistent elevation of osmolality after treatment, and this was small in its average extent.

**Discussion**

The salient finding of this study, that hyperglycemia leads to a slight reduction of infarct volume in an animal model of thrombotic stroke, is unexpected in view of the deleterious effect established for hyperglycemia in the setting of transient reversible ischemia. Our data suggest that this effect is not osmotically mediated since a comparable intraperitoneal osmotic load of mannitol actually increased infarct volume over that observed in saline-treated controls. Two published abstracts have considered the effects of hyperglycemia in animal models of unilateral ischemia. An ameliorative effect of hyperglycemia on morbidity and mortality was reported by Jemigan et al in rats subjected to unilateral common carotid artery ligation and hypoxia. In contrast, Brint et al reported that i.p. dextrose administered prior to unilateral permanent occlusion of the common carotid and middle cerebral arteries of rats led to a significant increase in infarct volume compared with saline-treated controls, although the magnitude of the effect was not specified.

It is possible that characteristics of the thrombotic lesion itself may help to explain the failure of hyperglycemia to worsen the neuropathologic outcome in this study. In our model of photochemically induced brain infarction, the infarct is sharply demarcated from the surrounding tissue, and autoradiographic studies of local cerebral blood flow have shown that there is a steep blood flow transition from the near-zero levels of the infarct itself to almost normal levels in the adjacent tissue. Because the dye–light interaction gives rise to widespread in situ thrombosis of arterioles and venules within the field of irradiation, the possibility of hemodynamic “rescue” from adjacent collateral circulation is minimal. In a restricted sense, therefore, an analogy might be drawn between this stroke model and the condition of end-arterial or lacunar infarction in humans.

As noted above, hyperglycemia results in corresponding elevations of brain glucose concentration, so that anaerobic glycolysis during ischemia is able to generate correspondingly greater amounts of lactic acid within the brain parenchyma. If the ischemia is incomplete, massive increases in tissue lactate may result from the continued intracerehmic transport of glucose to the brain. The resulting cerebral acidosis is thought to mediate ischemic brain damage. When the extent of the ischemic lesion, however, is determined primarily by the topography of the occluded microvasculature and the degree of flow reduction is permanent and virtually complete, as in the present study, this pathogenetic sequence of events may be incapable of further enhancing the degree of brain injury.

The modest reduction of infarct volume observed with hyperglycemia in this study nonetheless requires explanation. It is known from our prior study that there is a spontaneous increase in lesion volume, demonstrated by [14C]iodoantipyrine autoradiography, during the first 4 hours following photochemical initiation. Studies using 111In labelled platelets have excluded continued platelet deposition as a cause of this phenomenon. Rather, the increase in lesion volume appears to be the result of early blood–brain barrier breakdown and tissue edema, with compression of subjacent tissue zones. It is possible that, during this early period of evolving infarction, elevated brain glucose levels may serve a critical role in the zone of lesion expansion by maintaining local tissue high-energy stores at levels sufficient to escape eventual infarction.

In summary: Although one must be cautious in extrapolating our results to the situation of human stroke, the data of this study indicate that, in the condition of...
end-arteriolar thrombotic occlusion resulting in cortical infarction, hyperglycemia fails to worsen neuropathologic outcome but rather is associated with a modest reduction of infarct size.

Acknowledgments
The authors are grateful to Mayra Gonzalez-Carvajal and Ofelia F. Alonso for their expert technical assistance.

References

Key Words • photochemical thrombosis • plasma glucose • cerebral infarction
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*Stroke*. 1987;18:570-574
doi: 10.1161/01.STR.18.3.570

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/18/3/570

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