Glucose Given After Hypoxic Ischemia Does Not Affect Brain Injury in Piglets

Michael H. LeBlanc, MD; Min Huang, MD, PhD; Daksha Patel, MD; Edward E. Smith, MD; Meenakshi Devidas, PhD

Background and Purpose  Giving glucose before hypoxic ischemia worsens brain injury in piglets. Does giving glucose after hypoxic ischemia affect severity of injury?

Methods  Forty-three 0- to 3-day-old pigs were used. All piglets received 2 U/kg insulin before injury to prevent stress-induced hyperglycemia. Hypoxic ischemic brain damage was induced by clamping both carotid arteries and reducing arterial blood pressure to two thirds of normal by hemorrhage at time 0. At 15 minutes the fraction of inspired oxygen (FiO2) was reduced to 6%. At 30 minutes FiO2 was increased to 100%, the carotids were released, and the withdrawn blood was reinfused. The piglets were then randomized to receive either 2 mL/kg of 50% dextrose followed by 2 mL/kg per hour for 2 hours or an equal volume of saline.

Results  Neurological examination scores (20 is normal, 5 is brain dead, by blinded observer) at 1 day postinjury were similar in the two groups: glucose, median 15.5 (25th percentile, 12.2; 75th percentile, 18); controls, 15.6 (9.3, 18). Piglets were killed at 3 days with brain preservation at death. Pathological examion scores (sum of scores from cortex, hippocampus, and basal ganglia: 30 is normal, 3 is total necrosis) by blinded observer were similar in the two groups: glucose, 26 (18, 28); controls, 25 (16.5, 28); NS.

Conclusions  Although elevated glucose levels during hypoxic ischemic brain injury worsens injury in the piglet, elevated glucose levels after injury do not affect the severity of the injury. (Stroke. 1994;25:1443-1448.)

Key Words  • glucose • acidosis, lactic • hypoxia • ischemia • reperfusion injury • pigs

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Materials and Methods

Using our previously described protocol,6-9 0- to 3-day-old piglets were removed from their mother the day of the experiment and randomly assigned to either a glucose or saline group (Fig 1). Sample size was chosen to provide a 90% chance of detecting a difference in pathological score one half as large as the difference seen in our study of hyperglycemia beginning before injury.4 Piglets were anesthetized with 1.5% isoflurane and 50% nitrous oxide, intubated, and ventilated with a Harvard Rodent Ventilator adjusted to obtain an initial Pco2 of approximately 5.3 kPa (40 mm Hg). Catheters were placed in the superficial artery of the right rear leg and in the right external jugular vein using a sterile technique. A snare was placed around both carotid arteries. Electroencephalograph (EEG) needle electrodes were placed beneath the perioseum in the right and left parietal regions with a ground lead placed at the base of the snout. EEG was recorded at 20 μV/cm. After surgery was completed, the isoflurane was reduced to 0.5%, and the animal was paralyzed with an infusion of 0.5 mg/kg per minute of succinylcholine. Both isoflurane and nitrous oxide have effects on cerebral blood flow and metabolism and the response of the brain to ischemia,10,11 as do most anesthetics.
PROTOCOL

The animals' rectal temperatures were maintained at 38.0°C using a servo-controlled infrared lamp.

The piglets had their serum glucose levels checked by Glucostix (Miles, Inc) immediately after the initial surgery. Those piglets having an initial glucose of greater than 3.9 mmol/L received 2 U/kg of regular porcine insulin IV push. They were then observed for 15 minutes, and their serum glucose levels were rechecked. If the glucose level remained over 5.6 mmol/L, an additional dose of 2 U/kg of insulin was given, and the animals were monitored an additional 15 minutes to make sure their serum glucose levels went below 5.6 mmol/L. At time —5 minutes, baseline measurements were taken, and 700 U/kg heparin was injected. Baseline measurements included arterial blood gases, arterial blood pressure, rectal temperature, oral temperature, and whole blood lactate and serum glucose levels. This set of measurements was repeated at 0, 15, 30, 35, 45, 60, and 90 minutes. At time 0, the carotid arteries were ligated by pulling the snares snugly around them. Blood was withdrawn from the arterial catheter into syringes to reduce the arterial pressure to approximately two thirds of normal, and maintain it at that level. Isoflurane was discontinued at 10 minutes, by which time the piglets had been rendered unconscious by the ischemia (pilot studies). Fifteen minutes after the carotid ligation and the reduction of blood pressure to two thirds of normal, the animals were switched from ventilation with 50% nitrous oxide and 50% oxygen to a gas mixture containing 70% nitrous oxide, 22% nitrogen, 2% carbon dioxide, and 6% oxygen. This reduced arterial Po2 to approximately 3.3 kPa (25 mm Hg) within 1 to 2 minutes. Approximately 2 minutes after hypoxia, the animals' EEGs became totally flat. Succinylcholine was discontinued at 20 minutes; it is required until this time to prevent gasping. At an experimental time of 30 minutes, after 15 minutes of hypoxia, the animals were reoxygenated by switching the inspired gas from 6% oxygen to 100% oxygen,22 releasing the carotid ligatures, and reinfusing the blood that had been previously withdrawn. Piglets assigned to the glucose group then received 2 mL/kg 50% dextrose followed by 2 mL/kg per hour for 120 minutes. The piglets assigned to the saline group received an equal volume of normal saline. We confirmed at autopsy that patency of the carotidis was reestablished.

Piglets were nursed in cages. Warmth was provided by heat lamp. From 2 to 17 hours postreoxygenation, they received 5% dextrose intravenously at a rate of 8 mL/h. They were then fed 60 mL/kg artificial piglet formula by gavage every 6 hours. Neurological examination was performed by the experiment staff at 2 hours after reoxygenation. Neurological examinations were also performed by a blinded observer at 1, 2, and 3 days after reoxygenation. The results were recorded and scored from 5 to 20, with 20 being normal and 5 being brain dead according to a standard scoring system. The neurological examination grades mental status, cranial nerves, reflexes, and the ability to stand and walk.3

Three days after the experiment, with piglets under isoflurane and nitrous oxide anesthesia, the chest was opened, the carotid artery cannulated, and the brain perfused with 10% formalin after flushing with 30 mL of saline. Formalin was continued until the effluent from the right atrium was clear, thus preserving the brain and killing the animal. The brain was then removed and preserved in formalin for later pathological examination. If the piglet died before completion of the 3 days, its carcass was stored in the refrigerator until morning, after which a gross autopsy was performed and its brain was preserved in formalin.

After fixation in formalin, the brains of all piglets were cut. A coronal section was taken at the level of the optic chiasm, and another was taken approximately 3 mm back to demonstrate the cerebral cortex, the hippocampus, and the basal ganglia. Paraffin-embedded sections were stained with hematoxylin and eosin and examined by light microscopy. Each section was graded on a scale of 1 to 10, with 10 being considered normal by a pathologist blinded to the experimental group of the piglets.4 Cellular changes were classified as hypoxic (considered by the pathologist to be potentially reversible, scores 5 to 9 depending on size of involved area) or necrotic (thought to be clearly irreversible, scores 1 to 4 depending on size of involved area). The final score was the sum of the scores of each of the three tissues. Hypoxic changes were largely shrunken hyperchromatic neurons but also included enlarged perivascular spaces and eosinophilic staining neurons with pyknotic nuclei, whereas necrotic changes included loss of neurons with glial and vascular proliferation and macrophage activity in the tissue. Pictures of histological sections have been previously published.5

Arterial whole blood lactate level was measured enzymatically.14 Arterial serum glucose level was measured by the glucose oxidase technique.15 Blood gases and pressures were measured by standard techniques.6 Comparisons were made between the glucose and saline groups using repeated measures ANOVA for continuous variables and the Mann-Whitney U test for ordinal variables.16 For the ANOVA calculations, the data were split into a preintervention period (before reoxygenation and glucose administration) and a postintervention period (after reoxygenation and administration of glucose to the glucose group and saline to the control group). The significance of differences between the two groups and differences occurring over time were then assessed. All results are presented as mean±SE for continuous variables and median [25th percentile, 75th percentile] for ordinal variables with a value of P>0.05 as nonsignificant (NS).

Results

Piglets in the glucose group (n=21) weighed 1506±77 g and were 1.9±0.2 days old. Those in the
Fra 2. Graphs showing the arterial pH, arterial PCO2, rectal temperature, and oral temperature versus experimental time. The vertical line shows reoxygenation. The error bars are standard error of the mean. The circles and solid lines are the glucose group, and the triangles and dashed lines are the control group. There were no statistically significant differences between the glucose and control groups.

Fra 3. Graphs showing the arterial blood pressure, arterial PO2, blood lactate, and serum glucose levels versus experimental time. The vertical line shows reoxygenation. The error bars are standard error of the mean. The circles and solid lines are the glucose group, and the triangles and dashed lines are the control group. The postreoxygenation glucose values are higher in the glucose group, P<.0001. No other differences between the glucose and control groups are statistically significant.

Neurological examination scores are shown in Table 1. There were no significant differences between the two groups. Neurological examination scores on sham-operated piglets (n=10) not subjected to hypoxic ischemic injury were 20 [20, 20] as previously reported.8 Six (27%) of the piglets in the control group died before euthanasia, as did four (19%) of the glucose group (P=NS). Pathological examination scores are shown in Table 2. Values for all pigs are shown in the top half of the table, and in the lower half, values are shown for only those pigs surviving the full 72 hours and receiving brain preservation at death. Values for the cortex, hippocampus, and basal ganglia are given, as well as the value for the sum of the scores for the three tissues. There were no significant differences between the groups. Pathological examination results on sham-operated piglets not subjected to hypoxic ischemic injury were: cortex (n=11), 9 [9, 9]; basal ganglion
There were no significant differences between the two groups.

\( n \) indicates number of piglets; Sum, sum of the three tissues for each piglet.

However, the relevance of the newborn rat as a model of human neonatal brain glucose metabolism is questionable, since unlike humans, preweanling rats have very limited capacity for brain glucose metabolism. Several studies of the effect of postischemic glucose on brain injury were carried out in adult rats. Pulsinelli et al., using 20 minutes of four-vessel occlusion, found that giving glucose after injury had no effect on brain pathology. Voll and Auer, using 10.5 minutes of bilateral carotid occlusion with hypotension in hyperglycemic rats, found that restoring normoglycemia with insulin after injury reduced pathological damage. Interestingly, this effect was seen even when the effect of the insulin on blood glucose was prevented by glucose administration, suggesting that the protection was caused by insulin rather than reduced blood glucose. Although giving glucose before injury will cause a dramatic increase in brain lactic acid levels, giving glucose after injury causes little increase in brain lactate, since mitochondria can then metabolize the accumulated lactic acid, and cellular acid balance can be restored by the ion pumps and the outward diffusion of lactic acid into the reestablished circulation.

The use of normal saline rather than hyperosmolar saline as a placebo for 50% dextrose would be expected to produce a difference in osmolality between the groups. Although osmolality was not measured, the difference in glucose concentration seen in the groups would be expected to produce a difference of 10 mOsm/L at 35 minutes and approximately 6 mOsm/L thereafter. Laptook et al. found that an infusion of hyperosmolar lactate or lactic acid that increased serum osmolality by 35 mOsm/L in uninjured newborn pigs increased cerebral blood flow by about 50%. In contrast, Duckrow et al. found that increasing serum osmolality by 20 mOsm/L with mannitol or glucose reduced cerebral blood flow in uninjured adult rats by 10% and 24%, respectively. The effect of hyperosmolality on cerebral blood flow in injured brain is also controversial.

Thus, although a difference in osmolality between the groups may have had an effect on cerebral blood flow, how this could have affected the results of this experiment is speculative.

How are the results of this experiment clinically applicable? To the extent that the physiology of the piglet with regard to glucose metabolism after hypoxic ischemic brain injury is similar to human newborns, the results might have applicability. Glucose metabolism in the newborn pig's brain is more like that of the human infant than that of the newborn rat. The maturational stage of the newborn pig's brain is similar to that of the human newborn. Similarly, to the extent that the protocol used in this experiment mimics circumstances occurring in the clinic, the results might be applicable. Clinical circumstances surrounding hypoxic ischemic injury to the brain of the human newborn vary. Glucose administration after hypoxic ischemic brain injury as induced in this protocol does not affect the severity of injury in the newborn pig.

### Table 1. Neurological Examination Scores for the Glucose-Treated and Control Groups by Time After Reoxygenation

<table>
<thead>
<tr>
<th>Time of Examination</th>
<th>Glucose-Treated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Score*</td>
<td>Score*</td>
</tr>
<tr>
<td>2 Hours</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>1 Day</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>2 Days</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>3 Days</td>
<td>17</td>
<td>16</td>
</tr>
</tbody>
</table>

### Table 2. Pathological Examination Results for the Glucose-Treated and Control Groups

<table>
<thead>
<tr>
<th>Tissue Examined</th>
<th>Glucose-Treated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>All piglets</td>
<td>n=21</td>
<td>n=22</td>
</tr>
<tr>
<td>Cortex</td>
<td>9 (6, 9)</td>
<td>9 (7, 10)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>9 (7, 10)</td>
<td>9 (7, 10)</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>9 (3.2, 10)</td>
<td>7.5 (3.10)</td>
</tr>
<tr>
<td>Sum</td>
<td>26 (18, 28)</td>
<td>25 (16.5, 28)</td>
</tr>
<tr>
<td>Piglets with brain preservation at death</td>
<td>n=17</td>
<td>n=16</td>
</tr>
<tr>
<td>Cortex</td>
<td>9 (8.3, 9)</td>
<td>9 (9, 9)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>9 (7, 10)</td>
<td>9 (7, 10)</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>9 (3.3, 10)</td>
<td>8 (3, 9)</td>
</tr>
<tr>
<td>Sum</td>
<td>27 (18.5, 28)</td>
<td>25 (19, 28)</td>
</tr>
</tbody>
</table>

### Discussion

Elevating glucose levels during injury worsens injury to the brain in newborn and adult animals. The newborn rat, because of the limited ability of its brain to metabolize glucose, is an exception to this generalization. Although increasing blood glucose during hypoxic ischemic injury results in elevated brain energy levels, the resulting increased brain lactic acid levels and decreased intracellular pH cause irreversible injury before damage by energy depletion alone. In contrast, elevating serum glucose levels once oxygen has been restored has no adverse effect on brain injury in the newborn pig. Surprisingly, very few studies of the effect of postischemic hyperglycemia on brain injury have been performed. Hattar and Wasserlain, using bilateral carotid occlusion with hypoxia in the newborn rat, found that postischemic hyperglycemia reduced brain injury. Sheldon et al. using unilateral carotid occlusion with hypoxia in the newborn rat, found that postischemic hyperglycemia had no effect on the area of infarction, but glucose did make microscopic injury worse.

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27. Kuehn DJ, Hoehman WE, Thomas C, Albrecht RF, Miletic MJ. The interaction of nitrous oxide and isoflurane with incomplete cerebral ischemia in the rat. Anesthesiology. 1989;70:767-774.

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