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 At 30 minutes FIO2 was increased to 100%, the carotids were released, and the withdrawn blood was reinfused.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.

Myers et al showed that juvenile monkeys with elevated blood glucose levels subjected to hypoxic ischemic brain injury had more severe damage than those who were fasted before hypoxic ischemic brain injury. It has been hypothesized that this injury is due to buildup of higher levels of lactic acid in the brain cells of the glucose-treated animals, causing low intracellular pH levels and thereby disrupting cellular machinery. Despite enhanced cellular energy stores during the hypoxic ischemia in the glucose-treated animals, brain damage was worse and was associated with higher intracellular lactic acid levels. Similar biochemical phenomena occur in newborn piglets. We have shown that glucose administration before hypoxic ischemic brain injury worsens injury in the newborn pig. However, clinically, it is more important to know the effect of glucose administration after hypoxic ischemic injury rather than before injury. We rarely have the luxury in clinical care of controlling what happens to our patients before injury. Thus, we set out to determine if glucose given after hypoxic ischemic brain injury worsens injury in the newborn pig.

In the human newborn, unlike the human adult, most clinical injury to the brain is initiated as an asphyxial or hypoxic event of variable acuity. Injury is usually global rather than focal. There is usually some secondary

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 examination 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 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

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. 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 periosteum 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.
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, 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 P02 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. 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. 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. Arterial whole blood lactate level was measured enzymatically. Arterial serum glucose level was measured by the glucose oxidase technique. Blood gases and pressures were measured by standard techniques. 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. 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
saline group (n=22) weighed 1506±73 g and were 1.9±0.2 days old, which is not significantly different from the values in the glucose group. Although values for arterial pH, arterial PCO₂, and temperature (both rectal and oral) changed significantly with time in both the preintervention (P<.0001) and postintervention (P<.0001) period, there were no statistically significant differences between the glucose and control groups for these variables (Fig 2). Similarly, arterial blood pressure, arterial PO₂, blood lactate, and glucose also changed significantly with time in both the preintervention (P<.0001) and postintervention periods (P<.002) (Fig 3). There were no statistically significant differences between the glucose and control groups for these variables (Fig 3). Serum glucose level was significantly higher (P<.0001) postintervention (after reoxygenation and glucose administration) in the glucose group than in the saline group (Fig 3). Glucose values before 35 minutes were comparable in the two groups.

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...
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>n</td>
<td>Score*</td>
</tr>
<tr>
<td>2 Hours</td>
<td>21</td>
<td>8.5 (8.1, 10.5)</td>
</tr>
<tr>
<td>1 Day</td>
<td>20</td>
<td>15.5 (12.2, 18)</td>
</tr>
<tr>
<td>2 Days</td>
<td>17</td>
<td>18 (13.9, 19)</td>
</tr>
<tr>
<td>3 Days</td>
<td>17</td>
<td>17.1 (15.6, 19)</td>
</tr>
</tbody>
</table>

(n=11), 10 [9, 10]; hippocampus (n=11), 10 [9.5, 10]; sum (n=11), 29 [27.5, 29], as previously reported.

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 lactate levels and decreased intracellular pH cause irreversible injury before damage by energy depletion alone. In contrast, elevating serum glucose levels once oxygen is 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. Hattari and Wasterlain, 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.

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 (8, 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</td>
<td>n=17</td>
<td>n=16</td>
</tr>
<tr>
<td>preservation at death</td>
<td></td>
<td></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>

Values are given as median (25th percentile, 75th percentile). n indicates number of piglets; Sum, sum of the three tissues for each piglet.

*Score of 10 is considered normal; 1 is near total necrosis. There were no significant differences between the two groups.

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.

Acknowledgments

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References


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