Optimal blood glucose levels while using insulin to minimize the size of infarction in focal cerebral ischemia

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Object. Insulin has been shown to ameliorate cerebral necrosis in global and, more recently, in focal cerebral ischemia. The goal of this study was to determine the relationship between this neuroprotective effect and blood sugar levels in a rat model of focal ischemia.

Methods. Thirty-four rats were subjected to 80 minutes of transient middle cerebral artery occlusion at a mean arterial blood pressure of 60 mm Hg and a temperature of 37°C. Insulin (3.5 IU/kg) was administered 1 hour before (12 rats) and 20 minutes after (12 rats) ischemia; 10 animals served as controls. A quantitative histopathological study conducted after 1 week of survival showed that insulin was not beneficial in reducing the size of the infarction or selective neuronal necrosis in the penumbra when administered before or after ischemia. In addition to infarction, six animals from the insulin-treated groups had bilateral selective neuronal necrosis in the hippocampus or the neocortex. A nonlinear regression analysis in which glucose levels were compared with both cortical necrosis and total infarction yielded a U-shaped curve with a nadir for cerebral necrosis that lay in the 6- to 7-mM blood glucose range. The increased brain damage induced by insulin occurred in animals with very low blood sugar values in the range of 2 to 3 mM.

Conclusions. These results in rats indicate that if insulin is used following ischemia, blood glucose levels should be maintained at approximately 6 to 7 mM. From these data one can infer that hypoglycemia of less than 3 mM should be avoided in situations of focal cerebral ischemia in which insulin is used. Additional animal studies and clinical trials in humans are needed to study the effects of insulin on ischemia.

KEY WORDS • insulin • ischemia • focal • hypoglycemia • normoglycemia • rat
Insulin in focal brain ischemia

periment. To determine the maximum tolerable dosage of insulin, 7 IU/kg and 5 IU/kg of insulin were given to groups of 25 rats on separate occasions in pilot experiments. Both these doses proved to be lethal for all rats, whereas the dose of 3.5 IU/kg, which we had used in a previous study, produced only mild hypoglycemia. On the basis of these findings we selected a dose of 3.5 IU/kg insulin for the present study in which we used 34 rats divided among three groups.

Experimental Groups

The insulin that we used consisted of bovine crystalline zinc insulin (CZL Novo Nordisk Canada Inc., Mississauga, ON, Canada) and longer acting zinc insulin suspension (NPH; Novo Nordisk Canada Inc.), which were administered intravenously and intraperitoneally, respectively. The rats were randomly allocated to one of three groups and were allowed free access to rat chow and water before and after the ischemic insult.

The first group did not receive insulin and served as a control group (10 animals). The second group was treated with insulin 60 to 65 minutes before ischemia (12 animals) and was named the insulin_ group. A third group was treated with insulin 20 minutes after ischemia and was named the insulin_ group (12 animals). Both insulin-treated groups received 3.5 IU/kg insulin (a ratio of 1:2 CZL/NPH). Afterward, all rats were allowed to experience a spontaneous level of glycemiam, with neither insulin nor glucagon administered subsequently. The resulting blood glucose levels are shown in Table 1.

Ischemia Model

After a 4- to 5-minute inhalation of 3 to 4% halothane added to a mixture of 40% O2 and 60% N2O, each rat was intubated and given mechanical ventilation. Anesthesia was maintained using a 1% halothane mixture.

The ventral tail artery was cannulated for both blood pressure monitoring and sampling of blood gas and glucose levels (Table 1). Blood pressure was automatically recorded by a computer every 5 seconds. A lateral tail vein was dissected and connected with P-50 polyethylene tubing to an infusion of 0.9% normal saline given at a rate of 4 ml/kg/hr to avoid dehydration and changes in the hematocrit level. The animal's body temperature was controlled using a thermistor-regulated feedback-controlled heating blanket with the core temperature set at 37°C. The temperature of the animal's head was monitored separately and controlled by an overhead heating lamp, which was located symmetrically over the skull vertex. A thermometer was placed in the external auditory meatus and the head temperature was maintained at a mean of 37°C ± 0.2°C. Blood glucose was measured using glucose oxidase reagent strips and a reflectance glucometer (Ames Co., Mishawaka, IN). A pH/blood gas analyzer (model 1304; Instrumentation Laboratories, Milan, Italy) was used to monitor blood gases and pH before, during, and 15 minutes after MCA occlusion. Glucose was monitored at 3, 12, and 24 hours and postischemia.

A neurological assessment was conducted at 3, 12, and 168 hours (1 week) after the ischemic insult by a researcher blinded to the experimental groups to which the animals were assigned. The Bedarson neurological grading criteria were used to rank the severity of the neurological deficit, with the resulting grades of 0, 1, 2, and 3, respectively, for normal, mild, moderate, and severe neurological deficit.

In a previous study we examined blood pressure and the duration of MCA occlusion in this reperfusion model. Based on the results of that study, we chose 80 minutes of occlusion at a blood pressure of 66 mm Hg to obtain an infarction of the desired size, which was smaller than 100 mm3. To produce ischemia, the sternomastoid, diaphragmatic, and omohyoid muscles were separated by a right ventrolateral incision in the neck. With the aid of an operating microscope, the common carotid artery was isolated and the occipital and superior thyroid artery branches of the ECA were isolated and coagulated. The ECA was cut with microsurgical bipolar forceps and tied loosely with a 6-0 silk suture. A microvascular clip was placed temporarily at the origin of the ECA. The ECA stump was cut with fine surgical scissors, and a 22-mm 3-0 monofilament nylon thread, the tip of which was smoothed using fine-grade sandpaper, was introduced into the ICA through the ECA. The silk suture surrounding both the ECA stump and the intralaminar thread was tightened to prevent bleeding, and the microclip was removed. A 22-mm nylon thread was advanced into the ICA until a slight resistance was encountered and a sharp decrease of blood pressure was displayed on the computer screen, indicating that the tip of the thread had reached the proximal segment of the anterior cerebral artery. The length of the nylon thread introduced into the ICA was approximately 20 mm, blocking the ICA and the anterior and posterior cerebral arteries to the MCA.

The rats in all groups were allowed to survive for 1 week after the operation. Animals that died before 1 week were excluded from the histopathological data analysis, because it was possible that their infarctions had not completely matured and were only included in the calculation of the mortality rate. After anesthesia had been induced by administration of a 2% halothane mixture, transcardiac perfusion was performed using saline followed by 4% phosphate-buffered formaldehyde, pH of 7.3. The brains were removed and cut into 3-mm coronal slices. After the tissue had been processed, embedded in paraffin, serially sectioned at 8-µm thickness, and stained with hematoxylin and eosin, we prepared slides of brain tissue cut at 2.50-µm intervals from 5.2 mm anterior to the bregma to 6.8 mm posterior to that structure. On each of these sections, the areas of infarcted cortex and striatum were traced by an operator blinded to the experimental groups, who used a video analysis system (Jandel Scientific, San Rafael, CA). The total area of each hemisphere was also measured separately because obvious atrophy was seen in the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group (10 rats)</th>
<th>Insulin_ Group (12 rats)</th>
<th>Insulin_ Group (12 rats)</th>
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<tr>
<td>mean weight (g)</td>
<td>263.6 ± 21.9</td>
<td>273.2 ± 15.4</td>
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<td>mean weight loss (g)</td>
<td>6.7 ± 65.6</td>
<td>21.5 ± 48.7</td>
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<td>5.0 ± 1.3</td>
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<tr>
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<td>10.8 ± 1.9</td>
<td>4.0 ± 2.0</td>
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<td>10 min postischemia</td>
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<td>36.92 ± 0.08</td>
<td>36.91 ± 0.12</td>
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<td>36.98 ± 0.11</td>
<td>36.98 ± 0.09</td>
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<td>37.01 ± 0.07</td>
<td>37.00 ± 0.05</td>
<td>37.00 ± 0.13</td>
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ischemic hemisphere compared with the contralateral one; atrophy was calculated by subtracting the area of the ischemic hemisphere from the nonischemic hemisphere. Areas of cortical and striatal necrosis plus atrophy were summed to provide the total area of lost cerebral tissue. Volumes of infarction in the cortex and striatum, and the total tissue loss in the hemisphere were then determined using a three-dimensional reconstruction program (PC3D; Jandel Scientific), which aligns the sections along a reference point (the center of the corpus callosum) and integrates the area measurement in three dimensions to calculate the volume accurately.

Quantification of Selective Neuronal Necrosis

In addition to infarction, selective neuronal necrosis was found within 1 mm of the sharply demarcated rim of the infarction and, hence, was quantitated with the aid of a microscope by counting the absolute number of dead neurons in this histological penumbra. The absolute numbers of dead neurons were counted in the entire section and the sum from three standardized coronal levels was used in the statistical analysis. Neuronal necrosis in the ipsilateral and contralateral hippocampus and any contralateral hemispheric infarction were also noted qualitatively.

Statistical Analysis

Measures of physiological parameters were subjected to a one-way analysis of variance, with the Scheffé test for multiple comparisons. A two-way analysis of variance was used to determine the effect of hypotension and the duration of MCA occlusion on the volume of the infarction and on selective neuronal necrosis. The rate of mortality and the Bederson neurological scores were analyzed using nonparametric chi-square and Wilcoxon rank-sum tests, respectively. Second-order regression was used to analyze the relationship between blood glucose and infarction size. All data were summarized as means ± SDs. Differences were considered significant at a probability level lower than 0.05.

Results

Physiological variables are presented in Table 1. Blood glucose levels before, during, and 10 minutes after ischemia were lower in the insulin² group. In the insulin¹ group, the postischemic glucose level at 3 hours was considerably lower than the control group. The rate of mortality was greater in both insulin-treated groups—eight rats in the insulin² group, and five in the insulin¹ group compared with only two rats in the control group—but failed to reach statistical significance (p > 0.05, chi-square test). The majority of rats died within 12 hours after the initiation of ischemia and displayed the typical hypoglycemic shock pattern, progressing from stupor to coma, convulsion, and respiratory failure. At the time of death the animals' blood glucose levels were below 1.5 mM. Neurological assessment was impossible in most insulin-treated rats at 3 hours posts ischemia because they were in a comatose or stuporous state. Neurological grades did not differ significantly between groups at 12 hours or at 7 days posts ischemia (p > 0.05, data not shown).

The histopathological findings showed no apparent benefit of insulin treatment when a groupwise analysis was performed. The area of striatal infarction in the control group was 26 ± 12 mm³, compared with 27 ± 12 mm³ in the insulin² group and 26 ± 8 mm³ in the insulin¹ group (Fig. 1). The area of cortical infarction was insignificantly larger in the insulin² group (55 ± 49 mm³), followed by the control (33 ± 24 mm³) and insulin¹ (26 ± 42 mm³) groups. The total extent of cerebral tissue loss, including atrophy, was 70 ± 22 mm³ in the control group, 93 ± 51 mm³ in the insulin¹ group, and 58 ± 53 mm³ in the insulin² group (p > 0.05, Fig. 1).

Visual counting of selective necrotic neurons in the ischemic penumbra (Fig. 2) showed that the number of necrotic neurons (mean ± SD) in the control group was 3975 ± 2347, compared with 2368 ± 2063 cells in the insulin¹ group and 2944 ± 2466 cells in the insulin² group (p > 0.05, Fig. 2). Six rats from insulin-treated groups had selective neuronal necrosis in the hippocampus (including the CA1 and CA3 sectors and the dentate gyrus). Four of these animals had ipsilateral cell loss and two had bilateral cell loss. One insulin-treated rat had selective neuronal necrosis in the contralateral neocortex. No control animals had any necrosis outside the territory of infarction either in the hippocampus or anywhere in the contralateral hemisphere.
Insulin in focal brain ischemia

Because our earlier findings that insulin reduces the damage of focal ischemia in the rat were apparently contradicted by the present findings, we analyzed the blood glucose level in relation to the infarction volume, in an attempt to determine a possible link between blood glucose levels and quantitative neuropathology. A graph showing a comparison of the blood glucose level and the volume of cortical necrosis and total infarction demonstrated a U-shaped curve, and nonlinear regression analysis yielded a nadir for cerebral necrosis at a blood glucose level of approximately 7 mM (Fig. 3).

Discussion

It is widely accepted that hyperglycemia accentuates the brain damage produced by cerebral ischemia in animals and humans. Stroke-related damage is augmented by hyperglycemia regardless of whether diabetes mellitus is present. The mechanism likely involves the enhanced accumulation of lactic acid in the tissue and glucose-sensitive acidosis in the ischemic penumbra. The idea behind initial experiments with insulin was to mitigate this hyperglycemic–acidotic mechanism of ischemic damage. Another possibility is that corticosteroids play a detrimental role in hyperglycemic ischemia.

In cases of global cerebral ischemia, either profound hypoglycemia or hyperglycemia is detrimental. Warner and colleagues found that insulin-induced normoglycemia (7–9 mM) occurring immediately before the onset of forebrain ischemia reduced brain damage. Strong and colleagues demonstrated improved cognitive function and brain mitochondrial function in rats rendered mildly hypoglycemic with insulin (3 mM) before they experienced global ischemia. At the upper end of normal glucose values, a step function for worsening seizures and histological outcomes was found in rats at a hyperglycemic threshold of 10 to 13 mM. The lower limits of the effects of blood glucose, and insulin in focal ischemia have been less well studied.

In the situation of focal ischemia, coadministration of glucose to raise the blood glucose level from 3.4 ± 0.2 mM to 10.1 ± 0.5 mM effectively cancelled most of the neuroprotective effect of insulin, when compared with untreated control animals in which the mean blood glucose level was 8.4 ± 0.2 mM. The results of these experiments thus indicated that lowering blood sugar by administering insulin is desirable in cases of focal ischemia, but the lower limit of effectiveness was not studied to determine whether the curve relating blood glucose levels to brain damage is linear or U-shaped. Similar experiments of MCA suture occlusion model led to early death and larger infarction. The present series of concurrently performed experiments confirms the findings of de Courten-Myers, et al., in cats, and indicates that too low a blood glucose level, on the order of 2 mM, is harmful in rats. Some experimental results have shown that glucose can be beneficial in some circumstances, perhaps by fueling glutamate uptake.

Only results in humans can delineate any negative effects of clinical hypoglycemia, and the threshold at which such effects occur in humans when insulin is used to treat focal ischemia. In the past we conducted a pilot study on the use of insulin in humans who undergo cardiac bypass surgery and in whom high glucose levels are seen in combination with the possibility of air or other embolism; we found no detrimental effects, even in patients with glucose levels of 1 to 2 mM who were clinically well while their blood glucose levels were this low (Borger and Auer, unpublished observations).

Conclusions

In the present study conducted in rats, our results indicate that blood glucose levels should be maintained at approxi-
mately 6 to 7 mM when insulin is used following ischemia. From these data one can infer that hypoglycemia of less than 3 mM should be avoided in cases of focal cerebral ischemia in humans in which insulin is used. Our findings underscore the need to study insulin in human ischemia in surgical as well as medical settings.

References


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