Effect of Insulin on Acute Experimental Cerebral Ischemia in Gerbils

Seiji Fukuoka, MD, Hwa-shain Yeh, MD, Thaddeus I. Mandybur, MD, PhD, and John M. Tew Jr., MD

We studied the effects of insulin with and without food deprivation on experimental cerebral ischemia in 197 gerbils. Ischemia was induced by unilateral common carotid artery occlusion for 4 hours. Gerbils were divided into four experimental groups and were studied for up to 1 week of survival: Group A (n=50) was fed but received no insulin, Group B (n=50) was deprived of food for 24 hours before surgery but received no insulin, Group C (n=49) was fed and received daily injections of 0.1 IU lente insulin for 3 days before surgery, and Group D (n=48) was deprived of food and received daily insulin injections. Insulin treatment was continued in Groups C and D after surgery. Blood glucose levels of all gerbils were determined before treatment (overall mean±SEM 88.0±12.4 mg/dl) and before carotid artery occlusion (Group A 92.2±18.3 mg/dl, Group B 81.4±11.7 mg/dl [p<0.05 different from before treatment], Group C 92.8±22.3 mg/dl, and Group D 66.1±24.0 mg/dl [p<0.001 different from before treatment]).

Among the four groups, 52 gerbils died within 1 week. Neurologic deficits were scored and histologic evidence of the infarcts was graded in survivors at 1 week. Group C gerbils had the best stroke index scores. Histologic evaluation revealed that 35.9% of Group A, 21.1% of Group B, 13.9% of Group C (p<0.05 compared with Group A), and 28.1% of Group D survivors developed cerebral infarcts. Our results indicate that Group C, with daily insulin injections but without hypoglycemia, had the most favorable outcome in this study, which suggests that insulin may be beneficial in ischemic stroke. (Stroke 1989;20:396-399)

In general, the brain has been considered an insulin-independent organ; however, insulin receptors have been identified in the rat brain. Insulin also regulates ornithine decarboxylase activity, which is the rate-limiting enzyme in the synthesis of polyamines in the brain. Recently, insulin receptors have been found on the endothelium of cerebral microvessels, on platelets, and throughout the brain. The exact function of these receptors is not known. Honour and Hockaday have shown that daily injections of insulin, resulting in only minimal reduction of blood glucose levels, inhibit the accumulation of platelets at the site of microvascular injury in the pial vessels of diabetic mice. Furthermore, Rosenblum and El-Sabban have demonstrated that pretreatment with insulin delays platelet accumulation in normal mice as well as in diabetic mice.

Mongolian gerbils (Meriones unguiculatus) have been widely accepted as a model of cerebral ischemia. Following unilateral occlusion of the common carotid artery, 35-65% of gerbils develop brain infarcts attributed to their incomplete circles of Willis. We studied the effect of insulin on cerebral infarction in gerbils to determine if pretreatment with insulin enhances recovery from ischemic stroke in normoglycemic gerbils.

Materials and Methods

We used 197 male, 5-6-week-old Mongolian gerbils, each weighing 40-50 g, randomly divided into four groups: Group A (n=50) was fed but received no insulin, Group B (n=50) was deprived of food for 24 hours before surgery but received no insulin, Group C (n=49) was fed and received daily injections of 0.1 IU lente insulin subcutaneously for 3 days before surgery, and Group D (n=48) was deprived of food for 24 hours before surgery and received the same dose of insulin injections daily for 3 days. On Day 1, 15 μl blood was taken from the retro-orbital sinus plexus of each gerbil for determination of blood glucose levels. All gerbils were fed Purina gerbil chow (Richmond, Indiana) ad libitum except for the 24 hours before surgery in which they were fasted. All gerbils were killed at the end of the 1-week follow-up period. All experiments were performed under the guidelines of the National Institute of Neurological Disorders and Stroke for the care and use of laboratory animals.
Groups B and D. Blood glucose concentration was measured again in each gerbil on the days of surgery (Day 0) and sacrifice (Day 7).

On the day of surgery, all gerbils were lightly anesthetized with ether. A ventral midline incision was made on the neck, and the right common carotid artery was clamped with an aneurysm clip. After 4 hours, the clip was removed and patency of the carotid artery was assured by direct visualization; the skin incision was then sutured.

After recovery from anesthesia, the gerbils were observed for 6 hours and, then, daily for neurologic deficits assessed using a method modified from McGraw et al.10 One neurologic deficit point each was given for tremor, hair rough-up, obtundation, or paucity of movement; two points for convulsions; three points each for loss of light reflex, neck or trunk torsion, splayed and rolling hind limbs, rolling seizures, or circling behavior; and six points for coma. The neurologic deficit points were summed and divided by the number of survivors as a stroke index score (SIS) for each group; the higher the score, the more severe the postischemic neurologic deficit.

Insulin treatment was continued for 6 days after surgery in Groups C and D.

No gerbils died before surgery. Those dying after surgery were necropsied; the brains were removed and fixed in 10% buffered neutral formalin (BNF) for histologic examination. On Day 7, the surviving gerbils were killed and necropsied; after removal from the skull, these brains were also fixed in BNF. After fixation for 3 days, a standard 3-mm-thick coronal section was taken from the midportion of the cerebral hemisphere, 6 mm posterior to the tips of the frontal poles in each brain. This section was fixed in BNF for 5 days, then embedded in paraffin. The anterior surface of the tissue block was then cut using a rotary microtome, and the 5-μm sections were stained with hematoxylin and eosin. As far as corticotomy was concerned, the anterior surface of the tissue block was taken from the midportion of the cerebral hemisphere, 6 mm posterior to the tips of the frontal poles in each brain. This section was fixed in BNF for 5 days, then embedded in paraffin. The anterior surface of the tissue block was then cut using a rotary microtome, and the 5-μm sections were stained with hematoxylin and eosin. The infarcts, if present, were easily recognized as unstained areas of edema and ischemic neuronal changes. The infarcted areas involving the cerebral cortex, white matter, hippocampus, and basal ganglia were measured separately. A grading system was developed and is depicted in Table 1.

We assessed the effect of insulin on neurologic signs, mortality, and the extent of cerebral infarction. We used analysis of variance and Student's t test to compare the blood sugar levels in each group before treatment and before surgery and the SIS among the groups. We used the χ² test to compare the percent survivors on Day 7 on frequency tabulations. We used nonparametric analysis of variance and Wilcoxon's rank test to compare infarct grades among groups.

### Results

The blood sugar levels before treatment were quite similar in all groups (Table 2). There was a significant decrease in the blood sugar levels of Groups B and D after treatment before surgery (Table 2).

The SIS for each group is given in Figure 1. Group C gerbils, which received daily injections of lente insulin but were not deprived of food, had the lowest SIS, that is, the fewest neurologic deficits.

Mortality rate during the 7 days after surgery was 22% in Group A, 24% in Group B, 26.5% in Group C, and 33.3% in Group D. Thirty-eight gerbils died within 24 hours after surgery, and another 14 died between Days 2 and 5 (Figure 2). All of these gerbils had Grade 6 cerebral infarcts at necropsy.

Histologic evaluation of the surviving gerbils' brains revealed infarcts in 14 of 39 (35.9%) Group A, eight of 38 (21.1%) Group B, five of 36 (13.9%) Group C (p<0.05 compared with Group A), and nine of 32 (28.1%) Group D gerbils. The infarcts were graded; the results are shown in Figure 3. Gerbils in Group C had the least infarction by histologic grade. No major cortical involvement was demonstrated, most of the ischemic infarcts being confined to the hippocampus and basal ganglia.

### Discussion

Insulin, a hormone secreted by the beta cells of the pancreas, lowers the concentration of glucose in the blood and affects fat and protein metabolism.11

### Table 1. Grades of Cerebral Infarction in Gerbils

<table>
<thead>
<tr>
<th>Grade</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Small hippocampal lesion</td>
</tr>
<tr>
<td>2</td>
<td>Moderate or large hippocampal lesion, or small basal ganglia lesion</td>
</tr>
<tr>
<td>3</td>
<td>Hippocampal and basal ganglia lesions, or hippocampal and small cortical lesion</td>
</tr>
<tr>
<td>4</td>
<td>Hippocampal and basal ganglia and small cortical lesions</td>
</tr>
<tr>
<td>5</td>
<td>Hippocampal and basal ganglia and moderate cortical lesions</td>
</tr>
<tr>
<td>6</td>
<td>Hippocampal and basal ganglia and large cortical lesions (massive hemispheric infarct)</td>
</tr>
</tbody>
</table>

Small, involvement of one-third of region; moderate, involvement of more than one-third but less than two-thirds of region; large, involvement of more than two-thirds of region.

### Table 2. Blood Sugar Levels in Gerbils Before Surgical Induction of Ischemia

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Before treatment</th>
<th>Before surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50</td>
<td>88.5±13.4</td>
<td>92.2±18.3</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>87.2±12.4</td>
<td>81.4±11.7*</td>
</tr>
<tr>
<td>C</td>
<td>49</td>
<td>87.3±8.5</td>
<td>92.8±22.3</td>
</tr>
<tr>
<td>D</td>
<td>48</td>
<td>89.5±13.9</td>
<td>66.1±24.0†</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>88.0±12.4</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean±SEM mg/dl. A, fed but received no insulin; B, deprived of food but no insulin; C, fed but received insulin; D, deprived of food for 24 hours but received insulin for 3 days before unilateral common carotid artery occlusion.

* p<0.05, 0.001, respectively, different from before treatment.
Insulin has been detected in the cerebrospinal fluid, and its chronic administration results in higher brain-to-plasma ratios. Circulating insulin has been shown to alter appetite drive at a presumed satiety center in the brain and to influence net glucose uptake and glycogen formation by the brain. Recent in vitro experiments suggest that insulin is essential for the growth and survival of neurons in serum-free culture medium.

It has been demonstrated that platelet activation and accumulation occur in experimental stroke models although the process of stroke progression remains unknown. Brain edema developed during carotid artery occlusion is inversely proportional to the collateral blood flow and is markedly exacerbated during reperfusion. Theoretically, the area of less ischemia should show less hyperemia and greater edema resolution during reperfusion. It is suggested that the amount of brain edema and hyperemia during reperfusion depends on the severity of cerebral ischemia. Based on its action on platelets, insulin may improve the collateral or residual circulation in the focal ischemic area.

Our experiment demonstrated that there is no difference in blood sugar levels in gerbils with or without insulin injection if there is no food deprivation. However, gerbils that were deprived of food for 24 hours before the surgical induction of ischemia developed significant hypoglycemia after insulin treatment, as seen in Group D (Table 2). These results indicate that gerbils are capable of maintaining a normal range of blood sugar concentration after a small amount of insulin, if there is no food deprivation.

Fifty-two gerbils died of stroke within 5 days after surgery, 73% of these within 24 hours. Group D had the highest mortality (33.3%); no significant difference was found between Groups A, B, and C (Figure 2). Hyperglycemia and hypoglycemia appear greatly to enhance the severity of ischemic brain damage. The outcome of Group D gerbils with significant hypoglycemia during carotid artery occlusion was unfavorable.

Analysis of SIS in survivors on Day 7 revealed significantly better scores in Group C than in Groups A ($p<0.005$) and B ($p<0.01$); there was no significant difference between Groups C and D. Most of the neurologic deficit in survivors was observed in the first 48 hours after carotid occlusion.

Evaluation of histologic grade in survivors indicates that Group C had the fewest cerebral infarcts. There was a significant difference in the proportion of gerbils with infarcts between Group C (13.9%) and control Group A (35.9%). It should be noted that no survivor in Group C suffered cerebral infarction of greater than Grade 4 (Figure 3). The difference in results between Groups C and D was apparently due to the adverse effects of hypoglycemia in Group D.

Our results indicate that insulin has an ameliorative effect on the outcome of the ischemic insult in...
gerbils when given daily if normal blood glucose levels are maintained. Insulin may exercise a beneficial effect on ischemic stroke by enhancing the survival of neurons in the ischemic zone. This is a hypothesis requiring further study.

References

KEY WORDS • cerebral ischemia • glucose • insulin • gerbils
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