Streptokinase Treatment Versus Calcium Overload Blockade in Experimental Thromboembolic Stroke

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Thromboembolic brain ischemia was produced in dogs using an autologous blood clot model. The effect of postembolic treatment with flunarizine and streptokinase on hemispheric cerebral metabolic rate for oxygen (CMRO₂), oxygen extraction ratio (OER), and cerebral blood flow (CBF) was studied by positron emission tomography (oxygen-15 technique) 24 hours after the insult. We studied five groups of experimental dogs and compared them with a control group of nonembolized dogs. Group I received no treatment, Group II was treated locally with 500,000 IU streptokinase starting 30 minutes after the insult, Group III received streptokinase locally 30 minutes after the insult and 0.1 mg/kg i.v. flunarizine immediately after the insult and 2 hours later, Group IV received flunarizine as Group III, and Group V was orally pretreated with 0.5 mg/kg/day flunarizine during 2 weeks preceding embolization. Compared with the contralateral hemisphere, in the embolized hemisphere a significant reduction of CMRO₂ (-25% to -40%) and CBF in normocapnia (-35%) and hypercapnia (-50%) was observed in Groups I, II, and V. In Groups III and IV, CMRO₂, OER, and CBF of the embolized hemisphere were within the normal range during normocapnia and hypercapnia; the extent of the ischemic lesions was markedly less than in the other groups of experimental dogs. We conclude that flunarizine treatment after experimental thromboembolic stroke had a favorable influence on brain tissue. Chronic preventive flunarizine treatment failed to have a beneficial effect. (Stroke 1989;20:357-361)

Fibrinolysis by the administration of streptokinase, urokinase, or other plasminogen-activating substances is a powerful tool to restore blood flow in vascular beds after thromboembolic occlusion. These drugs, however, have been used only sporadically in the treatment of acute thromboembolic brain ischemia.1-3 The possible beneficial effect of therapeutic fibrinolysis in ischemic stroke undoubtedly depends on the delay between the onset of ischemia and the initiation of therapy. Recently, encouraging reports have been published concerning the use of thrombolytic drugs in experimental cerebral ischemia.4-6 Calcium entry blockers may also be of value in the treatment of cerebral ischemia because they are potentially able to protect ischemic tissue from intracellular calcium overload and to relax constricted brain vessels,7 but well-controlled experimental studies have given conflicting results.8-13 Recent critical reviews suggest that both fibrinolytic drugs and calcium entry blockers may have a place in the therapy of cerebrovascular disease in selected patients.14,15 Encouraged by the results obtained with immediate therapeutic thrombolysis,6 we investigated the possible usefulness of the calcium overload blocker flunarizine in experimental thromboembolic stroke in dogs.

Materials and Methods

Our procedure has been described in detail elsewhere8 and can be summarized as follows. The experiments were carried out on mongrel dogs of either sex weighing 16–36 kg. Under 10 mg/kg i.v. pentobarbital anesthesia and after premedication with 2 mg/kg i.m. xylazine, catheters were implanted into the left internal carotid artery and a superficial neck vein. The catheters were filled with 5,000 IU/ml heparin and tunneled subcutaneously toward the dorsal region. All dogs received antibiotics and were allowed to recover for 3 days before embolization.
Three hours before embolization, blood was drawn from the cephalic vein into a PE-240 polyethylene catheter (i.d. 1.67 mm) and kept at 37°C for 3 hours to allow clot formation. A 10-cm-long piece of the clot was flushed into the internal carotid artery with 10 ml saline at 39°C at a rate of 15 ml/min.

Ninety minutes before the measurements, anesthesia was induced by 30 mg/kg i.v. thiopental; the dogs were paralyzed with 10 mg/kg i.v. gallamine and artificially ventilated with a mixture of 33% O₂ and 67% N₂O. One femoral artery and one femoral vein were cannulated for blood pressure measurement, arterial blood sampling, and intravenous infusion. The dog's head was fixed in a stereotactic apparatus and placed in the gantry of a positron emission tomography (PET) scanner (Neuro-ECAT, Ortec, Oak Ridge, Tennessee) for measurement of cerebral metabolic rate for oxygen (CMRO₂), oxygen extraction ratio (OER), and cerebral blood flow (CBF). Body temperature was kept at 39°C. End-tidal CO₂ concentration was kept at the desired level by electronic feedback adjustment of tidal volume. The dogs received an intravenous infusion of Tyrode's solution with 1.5 mg/kg/hr gallamine at a rate of 1.9 ml/min.

CMRO₂, OER, and CBF were measured using the oxygen-15 steady-state method as described earlier and calculated pixel-by-pixel in three coronal slices 35, 19, and 3 mm in front of the intermeatal line and evaluated in elliptic regions of interest covering each cerebral hemisphere, using the appropriate formulas. CMRO₂, OER, and CBF were measured during normocapnia (Paco₂ approximately 33 mm Hg); CBF was also measured during hypercapnia (Paco₂ approximately 58 mm Hg, achieved by addition of 5% CO₂ to the inspired gas mixture). From the three tomographic planes, hemispheric weighted average values were calculated and compared statistically.

There were five experimental groups, each comprising seven dogs. Dogs in Group I were embolized and received no further treatment. In Group II, fibrinolytic therapy was started 30 minutes after embolization by infusion of 500,000 IU streptokinase (Behringwerke AG, Marburg, FRG) through the carotid catheter as described earlier. Dogs in Group III received 0.1 mg/kg i.v. flunarizine dissolved in 0.1 ml physiological saline within the first few minutes after embolization, followed by fibrinolytic therapy started 30 minutes later (as in Group II) and a second identical flunarizine injection 2 hours after the insult. Dogs in Group IV received the same flunarizine treatment but no fibrinolytic therapy. Dogs in Group V were pretreated during the 2 weeks preceding embolization by oral administration of 0.25 mg/kg flunarizine twice daily. The dogs were studied 24 hours after the embolization and compared with a control group of seven nonembolized dogs.

In four dogs receiving the same treatment as those of Group IV, the concentration of flunarizine was determined in blood taken 5 minutes after the first and second injections and 22 hours later. In the Group V dogs a blood sample for determination of the plasma flunarizine concentration was taken at the time of embolization.

All experiments were undertaken according to the "Guiding Principles in Care and Use of Animals" (American Physiological Society), the Belgian law for animal protection and welfare, and the Guidelines of the European Community for the use of animals in laboratory investigations.

After the PET measurements, the dogs were killed by an intravenous injection of an overdose of barbiturate and saturated KCl and the brains were immediately immersed in a formalin solution. After fixation, the brains were cut in 7-mm-thick slices for morphologic inspection of the lesions.

The experimental data were compared with normal control values using Dunnet's test after analysis of variance.

Results

Values for arterial blood pressure, Paco₂, and hematocrit were all within the normal range; arterial oxygenation was adequate, with Paco₂ between 100 and 140 mm Hg. Values for hemispheric CMRO₂, OER, and CBF during normocapnia are summarized in Figure 1. In the control group, mean±SEM values for CMRO₂, OER, and CBF were 3.34±0.180 ml/100 ml/min, 41.6±2.10%, and 38.8±1.84 ml/100 ml/min, respectively. During hypercapnia, control CBF increased to 83.5±5.60 ml/100 ml/min. Interhemispheric differences were never observed.

In the experimental dogs, interhemispheric asymmetry was seen; the ratios of values for the embolized vs. normal hemispheres are illustrated in Table 1.

In the contralateral hemisphere, CMRO₂ was never significantly different from control. In Groups III and IV, OER was not different from control, whereas in Groups I, II, and V OER in the contralateral hemisphere was significantly increased. CBF during normocapnia and hypercapnia was lower than control in Groups I, II, and V (but not significantly so), and in Groups III and IV CBF was within the control range (Figure 1).

In the embolized hemisphere, CMRO₂ was significantly decreased (~25% to ~40%) in Groups I, II, and V; CMRO₂ was not different from control in Groups III and IV. In all five experimental groups, OER in the embolized hemisphere was not significantly different from control. In Groups III and IV, CBF during normocapnia was within the control range, whereas during hypercapnia CBF was lower than control (but not significantly so). In Groups I, II, and V, CBF was decreased by approximately 35% and 50% under normocapnic and hypercapnic conditions, respectively.

Mean±SEM plasma flunarizine concentration in four dogs, treated as Group IV, was 26.6±1.5 and 39.7±4.7 ng/ml immediately after the first and second injections, respectively, and decreased to unde-
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**Figure 1.** Bar graphs, mean±SEM results of positron emission tomography studies showing cerebral metabolic rate for oxygen (CMRO₂), oxygen extraction ratio (OER), and cerebral blood flow (CBF) under conditions of normocapnia (upper right) and hypercapnia (lower right) in nonembolized control and experimental dogs 24 hours after insult. EMB(I), embolized only; STR(II), treated with streptokinase; FLU-STR(III), treated with flunarizine and streptokinase; FLU(IV), treated with flunarizine; FLU-pre(V), pretreated with flunarizine; hatched columns, embolized hemisphere; open columns, contralateral hemisphere; n=7 in each group. *p<0.05, **p<0.01 different from CONTR.

Detectable levels 22 hours later. In Group V, mean ±SEM plasma flunarizine concentration at the moment of embolization was 14.6±3.3 ng/ml.

Embolization provoked clinical signs of stroke, usually within the first few minutes after the insult. Typical behavior consisted of circling gait and head deviation toward the side of the embolization, extensor hypotonia with defective placing of the contralateral forepaw, and mild to severe incoordination.

Twenty-four hours after the insult, the dogs from Groups I and II remained in practically the same clinical condition as immediately after embolization, except for two of 14 dogs that spontaneously improved. In Groups III and IV, 10 of 14 dogs showed definite clinical improvement. The clinical behavior of the dogs in Group V was very heterogeneous and did not allow us to draw any conclusions.

Gross morphologic observation of the brain slices revealed unilateral lesions. In Groups I and II, the lesions involved mostly the caudate nucleus, the internal capsule, the lateral cortex, and the thalamic region and generally extended over four or five slices. In Groups III and IV, the observed lesions were rather discrete and in general extended over only one or two slices (five of 14 brains showed no macroscopic damage). In Group V, the diffuse lesions generally covered five consecutive slices.

Hemorrhagic infarcts were observed in six of 12 investigated brains from Groups I and II. In Group IV, one of seven dogs had a hemorrhagic infarct while two other brains showed discrete hemorrhagic lesions; in Group III, none of the seven dogs showed such lesions.

**Discussion**

The clinical signs observed with our model are compatible with an acute obstruction of the middle cerebral artery (MCA) as was confirmed by digital

**Table 1.** Ratios of CMRO₂, OER, and CBF Values in Embolized vs. Contralateral Hemispheres in Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMRO₂ (ml/100 ml/min)</td>
<td>0.99±0.02</td>
<td>0.75±0.07*</td>
<td>0.71±0.05*</td>
<td>0.84±0.05†</td>
<td>0.84±0.03*</td>
<td>0.73±0.06*</td>
</tr>
<tr>
<td>OER (%)</td>
<td>0.99±0.01</td>
<td>0.84±0.05†</td>
<td>0.87±0.04*</td>
<td>0.97±0.04</td>
<td>1.00±0.04</td>
<td>0.85±0.06†</td>
</tr>
<tr>
<td>CBF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (ml/100 ml/min)</td>
<td>0.99±0.02</td>
<td>0.87±0.05</td>
<td>0.83±0.07†</td>
<td>0.86±0.05†</td>
<td>0.85±0.03†</td>
<td>0.86±0.06†</td>
</tr>
<tr>
<td>H (ml/100 ml/min)</td>
<td>1.01±0.04</td>
<td>0.72±0.09†</td>
<td>0.69±0.06*</td>
<td>0.79±0.07†</td>
<td>0.68±0.06*</td>
<td>0.67±0.09†</td>
</tr>
</tbody>
</table>

Data are mean±SEM. CMRO₂, cerebral metabolic rate for oxygen; OER, oxygen extraction ratio; CBF, cerebral blood flow; N, normocapnia; H, hypercapnia. Control, nonembolized; I, embolized only; II, treated with streptokinase; III, treated with flunarizine and streptokinase; IV, treated with flunarizine; V, pretreated with flunarizine.

*tp<0.01, 0.05, respectively, different from CONTR.
subtraction angiography. Twenty-four hours after the insult, part of the involved hemisphere was infarcted as indicated by the lowered CMRO2. In spite of the fact that spontaneous clot lysis may have occurred by that time, tissue perfusion was clearly depressed.

In recent years much attention has been given to the possible deleterious effect of intracellular calcium accumulation in brain ischemia and to the possible protective effect of calcium entry blockers. Our results indicate that flunarizine (a calcium overload blocker) may decrease damage to cerebral tissue after experimental blood clot embolism (as indicated by normalized CMRO2) and may prevent delayed hypoperfusion (as indicated by lower OER). This favorable outcome is most likely the result of a protective effect of flunarizine early after embolization since the plasma concentrations of flunarizine were lowered to undetectable levels after 24 hours.

The positive effects of flunarizine on the evolution of experimental thromboembolic stroke are also reflected in our morphologic observations, which showed that flunarizine alone (and especially when combined with streptokinase) substantially reduced the extent of ischemic lesions and largely prevented their hemorrhagic transformation. Early reopening of occluded blood vessels by therapeutic fibrinolysis nevertheless appears to be important in addition to cerebroprotective medication since a tendency toward hemorrhagic transformation of the lesions persists when flunarizine is administered without subsequent streptokinase therapy.

Normally, the cerebrovascular system has an important dilatation capacity that allows maintenance of a normal metabolic supply to the brain under stressful conditions. Measurement of carbon dioxide reactivity of the CBF to evaluate cerebrovascular reserve is a known procedure in clinical practice. Figure 2 shows the carbon dioxide reactivity of CBF in the embolized hemisphere of control and Group I, III, and IV dogs. The carbon dioxide reactivity of Group I dogs was markedly lowered. Although carbon dioxide reactivity was also lowered in Groups III and IV, the values are not significantly different from control, indicating better preservation of cerebrovascular reserve under flunarizine treatment. We emphasize that very early treatment (within 5 minutes) with streptokinase alone (see Reference 6) completely prevents the lowering of CMRO2 in the embolized hemisphere but leaves carbon dioxide reactivity greatly impaired (bar STR I in Figure 2).

Chronic oral pretreatment with a rather high sustaining dose of flunarizine did not have a beneficial effect on the course of ischemia although plasma flunarizine concentration at the moment of embolization was in the same range as after its intravenous administration. About the reason for this "failure" we can only speculate, but consideration must be given to the observations that calcium antagonists may also have some adverse effects because they may lower systemic blood pressure and cerebral perfusion or produce generalized vasodilation of the cerebrovascular bed, which may further lower perfusion in the threatened zone (intracerebral steal). Flunarizine presented as a calcium overload blocker is reported not to lower blood pressure, but the effect of prolonged administration on CBF reactivity needs further examination.

In conclusion, our results indicate that calcium overload blockade, alone and combined with fibrinolysis, may contribute to the treatment of acute cerebral ischemia. Preventive administration of flunarizine, however, failed to have a beneficial effect on the outcome of thromboembolic brain ischemia under our experimental conditions. More work is needed to further elucidate the possibilities, drawbacks, and limitations of the use of such pharmacologic approaches.

Acknowledgments

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