Failure of Flunarizine to Improve Cerebral Blood Flow or Neurologic Recovery in a Canine Model of Complete Cerebral Ischemia

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SUMMARY Ten minutes of cerebral ischemia was produced in 12 dogs by temporary ligation of the venae cavae and aorta. After reperfusion the dogs received the calcium entry blocker, flunarizine, 6 μg/kg infused over a ten minute period. Cerebral blood flow (CBF) and metabolism (CMRO₂) were measured pre-ischemia and for 2 h post-ischemia in 6 dogs. At the end of the study brain biopsies were analyzed for cerebral metabolites. Neurologic recovery was evaluated for up to 48 h post-ischemia in an additional 6 dogs. The results of each study were compared to those previously obtained in untreated animals. The cerebral blood flows (when expressed as a percent of the pre-ischemic control value) of the flunarizine-treated and untreated groups were similar throughout the post-ischemic period. Following an initial hyperemia, the CBF fell to significantly less than the pre-ischemic control values, and remained approximately 26% of control during the final 90 min in both groups. The CMRO₂ was also the same for both groups. Cerebral metabolites were similar although abnormal in both groups. Flunarizine produced pulmonary edema in 5 of 6 dogs studied for neurologic recovery. Four of these dogs died within 12 h and another dog demonstrated severe neurologic damage. None of the untreated dogs developed pulmonary edema, but 6 of 7 dogs evidenced severe neurologic damage or were dead at 48 h. Thus, flunarizine failed to improve either cerebral blood flow or neurologic outcome when given after complete cerebral ischemia in the dog. A cardiodepressive effect of flunarizine might have contributed to the poor neurologic outcome. These results are compared to those obtained following treatment with another calcium entry blocker, nimodipine, in the same animal model.

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Methods

Twelve unmedicated fasting adult mongrel dogs, weighing 10–15 kg were studied. Six dogs were used to study the effect of flunarizine on CBF and CMR0 2, after 10 min of complete cerebral ischemia. Anesthesia for the surgical preparation was 1% halothane in 60–70% nitrous oxide and oxygen. Succinylcholine, 40 mg, was given intravenously to facilitate endotracheal intubation and continued thereafter at an infusion rate of 150 mg/h to maintain muscle paralysis. Ventilation was controlled with a Harvard pump, adjusted to maintain normocarbia during the control period and thereafter kept constant. Cannulae were inserted into a femoral artery for pressure measurements and blood sampling and into a femoral vein for fluid and drug administration. Blood collected during the CBF measurements was returned via this femoral vein. Through a right-sided thoracotomy in the fourth intercostal space, umbilical tapes were placed around the ascending aorta, inferior vena cava, and superior vena cava above the ayzygos vein. After heparinization (300–400 units/kg intravenously), the sagittal sinus was exposed, isolated, and cannulated as previously described15 for direct measurement of CBF by a square-wave electromagnetic flow meter (EP 300 API, Carolina Medical Electronics).16 Esophageal and parietal epidural thermistor probes were placed to monitor temperature which was maintained at 37°C ± 0.1°C by the use of heat lamps. A four lead biparietal electroencephalogram (EEG) was recorded continuously from electrodes cemented to the skull. Arterial and sagittal sinus blood gases were determined by electrodes at 37°C (Instrumentation Laboratory). Blood oxygen contents were calculated from measurement of oxyhemoglobin concentration (CO oximeter, IL 282) and oxygen tension.17 CMR0 2 was calculated as the product of CBF and the arterial-sagittal sinus blood oxygen content difference.

After the surgical preparation was completed, the halothane was discontinued for at least 20 min before taking pre-ischemic control measurements of arterial blood gases, mean arterial pressure (MAP), temperature, CBF, and CMR0 2. Complete cerebral ischemia lasting exactly 10 min was achieved as previously described4. The ascending aorta and venae cavae were simultaneously occluded, confining the cardiac output to the coronary and pulmonary circulation. The sagittal sinus cannula was also occluded during this time to avoid aspiration of air. Once the EEG became isoelectric, within 45 s of occlusion, the nitrous oxide in the inspired gas mixture was replaced with nitrogen for the remainder of the study. After 10 min the tape around the aorta was released, the tapes around the venae cavae having been released 15 s earlier.

Immediate resuscitative efforts consisted of administration of intravenous fluids and sodium bicarbonate. MAP returned to normal within 1 min. Ten minutes after release, flunarizine, 6 μg/kg, was given as a slow infusion over 10 min as described by White et al.12 Blood gases, MAP, CBF and CMR0 2 were measured for a 120 min period post-ischemia. At the completion of the study the dura overlying the cerebral hemispheres was excised and cortical biopsies were taken by a technique that deposits a sample of brain (200–400 mg) into liquid nitrogen within one second.19 The tissue was stored at −76°C and prepared for analysis in a refrigerated box (−25°C) as described by Folsbergrova et al.20 The cerebral tissue was analyzed by enzymatic fluorometric techniques for phosphocreatine (PCr), ATP, ADP, AMP, glucose21 and lactate and pyruvate.22 The energy state of the brain was expressed as the energy charge, \[ EC = \frac{[ATP] + 0.5 [ADP]}{[ATP] + [ADP] + [AMP]} \]

An additional six dogs were used to evaluate neurologic recovery following 10 min of complete cerebral ischemia. As in our other functional studies9, 10, 14 these dogs were maintained on 70% nitrous oxide and oxygen for the surgical preparation. In addition, eight ml of 0.5% procaine was infiltrated into the chest wall and 12 ml 0.25% bupivacaine was used for intercostal blockade. Intubation, ventilation, femoral venous and arterial cannulations and thoracotomy were performed as in the previous group. A four lead EEG was recorded from biparietal needle electrodes. Body temperature was maintained at 37.5 ± 0.4°C. The inspired gas was changed to 100% oxygen 5 min pre-ischemia and to room air 20 min post-ischemia if arterial oxygenation was adequate (PaO2 > 80 mmHg). After the ischemic period, any metabolic acidosis was treated with sodium bicarbonate. A flunarizine infusion, 6 μg/k, was begun 10 min following the ischemic period and completed within another 10 min. MAP returned to normal within 1 min in all dogs. The thoracotomy was closed with chest tube drainage of residual air in the pleural cavity, and the lungs were reexpanded. Arterial blood gases were measured pre-ischemia and at 5–10 min intervals post-ischemia until stable, thereafter at 30 min intervals. Ventilation was controlled until spontaneous ventilation was considered sufficient (PaCO2 < 45 mmHg). The dogs were then extubated and observed.

The dogs were evaluated neurologically up to 48 h post-ischemia and assigned to one of four groups.18 Grade 1 (no damage) dogs ate and behaved normally with fully coordinated movements. Grade 2 (moderate damage) dogs could stand alone, but were ataxic, or exhibited partial or complete blindness. Grade 3 (severe damage) dogs could not stand alone or were comatose. Grade 4 (dead) dogs died within 48 h. Dogs that survived 48 h were then killed. In all dogs the thorax was examined at necropsy to assess any gross complications of thoracotomy.

Comparison of CBF and CMR0 2, between flunarizine-treated and untreated dogs4 was accomplished using Student's t test for unpaired data. Pre-ischemic values were compared to post-ischemic values by Student's t test for paired data. Cerebral metabolites from the flunarizine-treated group were compared with cerebral metabolites from normal4 and untreated4 groups using the Bonferroni t test for comparison of three groups (p < 0.01). The Fisher exact test was used to compare neurologic recovery of the flunarizine-treated
dogs with untreated dogs. Pre-ischemic values are expressed as mean ± SEM while post-ischemic values for CBF and CMRO₂ are expressed as percent of their respective pre-ischemic control value.

Results

During the studies of cerebral blood flow and metabolism there were no significant differences in blood pressure or blood gases between flunarizine-treated and untreated dogs at any time pre- or post-ischemia except for a lower arterial oxygen tension (76 ± 7 mmHg) in the flunarizine-treated dogs occurring 1 min post-ischemia (table 1). In both the untreated and flunarizine-treated dogs the PaCO₂ was significantly increased and the pH and buffer base significantly decreased immediately following the ischemic period. The PaCO₂ normalized within the first 10 min post-ischemia but the pH and buffer base remained abnormally low in both groups throughout the post-ischemic period. In the untreated group the mean arterial pressure gradually decreased to 98 ± 6 mmHg at 90 min post-ischemia, significantly less than the pre-ischemic value (124 ± 8 mmHg). In the flunarizine-treated group the MAP was lower than control 1 min post-ischemia (95 ± 12 mmHg) and then decreased gradually to 88 ± 6 mmHg at 90 min post-ischemia. The 10 min infusion of flunarizine had no obvious effect on blood pressure since there was no significant difference in MAP between the two groups throughout the remainder of the post-ischemic period.

Cerebral blood flow for the flunarizine-treated and untreated groups is presented in figure 1. CBF increased proportionately in both groups during the first 10 min post-ischemia. Infusion of flunarizine during the 10th-20th min had no obvious immediate effect on CBF. Between 10 and 15 min post-ischemia the CBF of both groups plummeted to significantly less than pre-ischemic values and remained so throughout the post-ischemic period. When expressed as a percent of the pre-ischemic control value for each group, there were no significant differences in CBF between the two groups at any time during the post-ischemic period. During the final 90 min of the study the CBF averaged 26% of control in each group.

The CMR₀₂ values during the post-ischemic period for both the flunarizine-treated and untreated groups are presented in figure 2. As with the CBF values, there was no difference in CMR₀₂ between the two groups. In each group, CMR₀₂ reached its nadir 10 min post ischemia (40% of the pre-ischemic value) and then increased to approximately 70% of the pre-ischemic value with return of EEG activity.

After circulatory arrest the EEG became isoelectric within 35 ± 2 s in the untreated group and 23 ± 3 s in the flunarizine group, significantly sooner for the flunarizine group. EEG activity returned at 18 ± 3 min post-ischemia in the untreated group and 18 ± 2 min in the flunarizine group.

There were no significant differences in cerebral metabolite levels between untreated and flunarizine-treated and untreated dogs.
FIGURE 2. CMRO2 values (percent of pre-ischemic control value ± SEM) during 120 min after 10 min of complete ischemia for dogs with (filled circles) and without (open circles, dashed line) flunarizine treatment. There was no significant difference in CMRO2 between the two groups.

Treated groups (table 2). The ATP, glucose and lactate concentrations of the flunarizine-treated group and the glucose of the untreated group were significantly different from normal values.

The functional neurologic status of the dogs 48 h post-ischemia was similar in both groups. Six of seven untreated dogs and 5 of 6 flunarizine-treated animals were severely damaged or dead 48 h post-ischemia (table 3). However, all of the abnormal flunarizine-treated animals developed severe pulmonary edema immediately after receiving flunarizine. Four of these died within 12 h. The one normal dog in the flunarizine-treated group did not develop gross pulmonary edema although there was some evidence of it at necropsy. None of the untreated dogs developed pulmonary edema. The mean arterial pressures and arterial blood gases taken pre-ischemia and prior to extubation were presented in table 4. These values did not differ significantly except that at the time of extubation the flunarizine-treated dogs were hypoxic presumed due to the pulmonary edema.

Discussion

Following a period of complete cerebral ischemia, there is a transient 5 to 10 min period of reactive hyperemia followed by a prolonged period of marked hypoperfusion. It is speculated that this post-ischemic hypoperfusion state may have a major influence on the magnitude of cerebral recovery. Until recently, there was no supporting evidence for this hypothesis because standard therapeutic interventions had little or no effect on this hypoperfusion state. Using the same animal models as in the present study, we reported that nimodipine, a calcium entry blocker, when given before and after complete cerebral ischemia, favorably modified the post-ischemic hypoperfusion state (mean CBF = 46 ml/100g min⁻¹, 47% of pre-ischemic control during the last hour of study) and improved neurologic outcome in dogs following ten minutes of complete ischemia. Since the only measured variable that was improved by nimodipine was CBF, it was concluded that the post-ischemic hypoperfusion state does appear to contribute to the ultimate neurologic damage. In an identical study except that nimodipine was given only after the ischemic event, CBF was again higher during the hypoperfusion period in the nimodipine group (CBF = 51 ml/100g min⁻¹, 34% of pre-ischemic control) than the untreated group. However, in the neurologic function studies, when nimodipine was given only after ischemia, neurologic outcome was intermediate and not significantly different from either the untreated group or the pre-treated nimodipine group.

Flunarizine has been reported to be a potent inhibitor of calcium-induced constriction of isolated arteries, being most effective in cerebral arteries. If post-ischemic hypoperfusion is solely due to an increase in calcium influx into potassium depolarized vascular smooth muscle as suggested by Hoffmeister et al, flunarizine might prevent it. In addition, the increase in blood viscosity which occurs following ischemia and contributes to microcirculatory failure is reported to be prevented by flunarizine.

Recently, White et al reported that flunarizine not only improved but even increased cerebral cortical blood flow above pre-ischemic levels for 90 min following a 20 min period of circulatory arrest in dogs

| Table 2 Cerebral Metabolite Values for Normal Dogs and for Dogs Exposed to 10 min of Complete Cerebral Ischemia |
|-----------------|-----------------|---------|-----------------|-----------------|
| Treatment       | n               | PCr (μmol/g) | ATP (μmol/g) | EC              | Glucose (μmol/g) | Lactate (μmol/g) | Lactate/Pyruvate |
| Normal*         | 7               | 3.07 ± 0.17  | 2.14 ± 0.10  | 0.92 ± 0.01     | 2.21 ± 0.18     | 1.04 ± 0.14     | 17 ± 1           |
| Untreated*      | 4               | 2.88 ± 0.16  | 1.97 ± 0.15  | 0.92 ± 0.01     | 4.48 ± 0.44†    | 1.80 ± 0.50     | 18 ± 1           |
| Flunarizine     | 6               | 2.71 ± 0.24  | 1.69 ± 0.07† | 0.89 ± 0.01     | 4.07 ± 0.51†    | 2.20 ± 0.23†    | 22 ± 2           |

Mean ± SEM.

*Data taken from study by Steen et al (1978).

†Significantly different from normal value (p < 0.01, Bonferroni t-test).

Abbreviations: PCr = phosphocreatine; EC = energy charge.

FLUNARIZINE IN COMPLETE CEREBRAL ISCHEMIA/Newberg et al 669

TABLE 3 Neurologic Status 48 Hr Following 10 Min of Complete Ischemia in Dogs

<table>
<thead>
<tr>
<th>Grade of neurologic damage</th>
<th>Untreated*</th>
<th>Flunarizine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (none)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2 (moderate)</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>3 (severe)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>4 (dead)</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

maintained on cardiopulmonary bypass. Because of this improvement in CBF post-ischemia the possibility that flunarizine might therefore be more effective than nimodipine in improving neurologic outcome prompted us to examine its effects in our animal models using the same dose and timing (6 μg/kg ten minutes after reperfusion) as that used by White et al.12

Unfortunately our results demonstrate that flunarizine had little or no effect on the post-ischemic CBF. Certainly the post-ischemic CBF values (after the initial 10–15 min of reactive hyperemia) never approached the pre-ischemic value but rather remained at a level approximately 26% of the pre-ischemic value. This CBF is similar to that of the untreated animals but significantly less than that observed in nimodipine treated animals.9,10

Therefore, our results clearly do not confirm those reported by White et al.12 That study differed from our study in several major ways. Their dogs were continuously supported by cardiopulmonary bypass; the method of complete cerebral ischemia was cardiac standstill which persisted for 20 min; and anesthesia was maintained with a ketamine infusion, known to increase CBF.27 Perhaps, most importantly, they measured CBF by a new thermal technique whereby an epidurally placed thermistor records the washout of a cold injectate.28 The CBF calculated from this technique yielded control values for the dog two to three times those reported by other investigators.13,29–30 Since validation of this technique by comparison with conventional methods of CBF measurement has not been reported, their results are difficult to evaluate further. The studies of neurologic function also confirmed the ineffectiveness of flunarizine therapy post-ischemia in our animal model. This is in agreement with the study of Hossmann et al31 which reported the failure of flunarizine to prevent post-ischemic tissue accumulation of calcium or to improve cortical electrical function or biochemical recovery. Unexpectedly, five of six dogs in the present study developed severe pulmonary edema shortly after receiving flunarizine and the resulting hypoxia might have contributed to the ultimate poor outcome. We have not previously encountered the development of pulmonary edema in this model of cerebral ischemia studying untreated animals or animals treated with either pentobarbital14 or nimodipine.9,10 Although flunarizine is reported to have little effect on isolated myocardium,11 we believe that left ventricular failure was the basis for the pulmonary edema in our dogs. Presumably the effects of cerebral ischemia, surgical stress with catecholamine release (secondary to light anesthesia consisting of 70% nitrous oxide and local anesthetic infiltration), and flunarizine therapy combined to produce this complication. Interestingly, no pulmonary edema developed in the dogs given nimodipine for the post-ischemic CBF studies. These dogs differed only in that 1% halothane anesthesia was used during the surgical preparation. Halothane, unlike nitrous oxide, suppresses the catecholamine response to surgical stress.32–33

It can be concluded that, using this model of complete cerebral ischemia, flunarizine does not appear to be useful for post-ischemic therapy. It failed to improve post-ischemic cerebral blood flow and, in our animal model, it failed to favorably influence neurologic functional recovery. The latter may have been accounted for, at least in part, by the occurrence of severe pulmonary edema.

References

TABLE 4  Arterial Pressure (MAP) and Arterial Blood Gases Pre-ischemia and at the Time of Extubation for Dogs Followed up to 48 hr in Neurologic Studies

<table>
<thead>
<tr>
<th></th>
<th>Untreated group* (n = 6)</th>
<th>Flunarizine group (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre-ischemia</td>
<td>pre-ischemia</td>
</tr>
<tr>
<td></td>
<td>time of extubation</td>
<td>time of extubation</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>161 ± 5</td>
<td>158 ± 11</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>328 ± 62</td>
<td>425 ± 15</td>
</tr>
<tr>
<td>PaCO2 (mm Hg)</td>
<td>37 ± 1</td>
<td>39 ± 1</td>
</tr>
<tr>
<td>pH</td>
<td>7.36 ± 0.02</td>
<td>7.32 ± 0.01</td>
</tr>
<tr>
<td>Buffer base (mEq/L)</td>
<td>42 ± 1</td>
<td>41 ± 0</td>
</tr>
</tbody>
</table>

*Mean ± SEM.

*Data from Steen et al, 1979.

†Not otherwise reported.

‡Significantly different from untreated group (p < 0.05).
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