A Novel Treatment for Ischemic Intracranial Hypertension in Cats

Rodney D. Bell, MD; Glenn D. Frazer, PhD; Jewell L. Osterholm, MD; and Serge W. Duckett, MD, PhD, DSc

There is no accepted efficacious treatment for ischemic cerebral edema. We show in a cat model of focal cerebral ischemia that infarct volume can be reduced (p<0.05) by ventriculocisternal perfusion with an oxygenated fluorochemical emulsion (bis-perfluorobutylethylene). An accompanying effect of such ventriculocisternal perfusion is a reduction in intracranial pressure. At 18 hours following the start of the perfusion, there was a significant (p<0.05) difference in intracranial pressure between nonperfused controls (mean 11.4 [range 2.3-23.0] torr, n=6) and cats perfused with an oxygenated nutrient solution not containing fluorochemical (mean 11.3 [range 3.0-29.0] torr, n=8) or animals perfused with the oxygenated fluorochemical emulsion (mean 2.21 [range 0-3.5] torr, n=7). Perfusion with this oxygenated fluorochemical emulsion warrants further study as a treatment for elevated intracranial pressure. (Stroke 1991;22:80-83)

Although there is active investigation into many aspects of the treatment of acute stroke, there is currently no accepted treatment for a patient so afflicted. Death during the acute phase occurs due to intractable brain swelling and herniation.1 Brain swelling and intracranial pressure (ICP) elevation become maximal in 2-4 days. The brain swelling and edema associated with transtentorial herniation are responsible for the death of approximately 10% of patients with ischemic stroke.2 For these patients there is no uniformly acceptable treatment that reduces or blocks the development of cerebral edema and the subsequent rise in ICP. Surgical decompression and medical treatment with hyperosmolar agents have had limited success in the treatment of ischemic cerebral edema,3-4 and corticosteroids have not been shown to be of benefit in ischemic vascular disease.5

We have shown in a cat model of focal cerebral ischemia that we can reduce the volume of infarction by ventriculocisternal perfusion with various oxygenated fluorochemical nutrient emulsions.6-10 A separate, but perhaps related, effect of such ventriculocisternal perfusion is the reduction of ICP.

Materials and Methods

We used 21 male closed-colony cats (Liberty Laboratories, Reading, Pa.) weighing 3-5 kg. The animals were fasted overnight and each was premedicated with 0.05 mg/kg i.m. atropine. Anesthesia was induced with 50 mg methohexital sodium, and maintained with 50 mg α-chloralose-urethane every 8 hours supplemented with 70% N2O in 30% O2. All surgical sites were infiltrated with 0.5% lidocaine before incision.

Focal cerebral ischemia was produced by application of an aneurysm clip on the middle cerebral artery as close as possible to its origin off the internal carotid artery through a retro-orbital exposure. The orbit was then filled with absorbable gelatin sterile powder, and the eyelid was sutured shut. In all cats, an inflow catheter was placed in the left lateral ventricle using a stereotaxic instrument (David Kopf Instruments, Tujunga, Calif.) and in 15 cats an outflow catheter was placed in the cisterna magna under microscopic visualization. The cat remained in the stereotactic frame for the remainder of the experiment. The other six cats were not perfused, did not have outflow catheters placed, and served as controls. Of the 15 cats with catheters, eight were perfused with an oxygenated nutrient solution not containing fluorochemical and seven were perfused with oxygenated fluorochemical emulsion.

Arterial blood samples were obtained every 2 hours or as necessary for monitoring and maintaining the following physiologic parameters throughout the experiment: PaO2 at >100 torr, PaCO2 at 25-35 torr, pH at 7.35-7.45, total CO2 concentration at 17-24
mmol/l, and glucose concentration at 70–200 mg/dl. In addition, rectal temperature, urine output, end-tidal $CO_2$, inspired $O_2$, inspired $N_2O$, respiratory rate, blood pressure, and ICP were monitored continuously and recorded hourly.

The stem emulsion (bis-perfluorobutylethylene) was provided by the Jackson Laboratory Group, Chemicals and Pigments Division, E.I. du Pont de Nemours & Co. (Inc.) (Wilmington, Del.) and combined with annex solutions containing electrolytes, glucose, amino acids, and albumin to produce a finished emulsion compatible with intrathecal perfusion in cats. The emulsion particle size was monitored and found to be stable over the experimental period.

The nutrient solution and the fluorochemical emulsion were equilibrated at 37°C with a mixture of $O_2$ and $CO_2$ (medical grade, analyzed and blended on site) using a CAPIOX II 08 membrane oxygenator (Terumo Corp., Tokyo, Japan). The final gas tensions were $PCO_2$ 28–32 torr and $PO_2$ 650–700 torr. The nutrient solution and the fluorochemical emulsion were pumped through the oxygenator by a peristaltic pump capable of accurate delivery at flow rates from 6 to 900 ml/hr (Critikon, Tampa, Fla.). A fluid level manometer was placed between the oxygenator and the cat, with a port for aseptic sampling and an air-fluid ballast to dampen fluctuations in pressure due to the peristaltic pump. Perfusion was started no earlier than 90 minutes after the ischemic insult, and the cat was perfused to a maximum rate of 3 ml/min. The oxygenated nutrient solution or fluorochemical emulsion exited the cisterna magna through the outflow catheter, which was equipped with a sampling port with a septum and an aseptic air break to eliminate any siphon effect.

At the time of sacrifice with an overdose of intravenous pentobarbital, 20.5 hours after occlusion, the brain was removed and the cerebrum was cut into 5-mm-thick coronal sections. Sections were stained with 2,3,5-triphenyltetrazolium chloride (TTC) for 40 minutes at 40°C. The tissue was then fixed in 10% formalin for 24 hours, the slices were weighed, and the total volume of the cerebrum was determined by fluid displacement. Each cut surface was photographed along with a metric scale. The photographs were projected, and the areas of the cut surface and the areas of infarction were measured with a computer digitizer. The sections were then embedded in paraffin, cut, and stained with hematoxylin and eosin and Heidenhain's stain. To ensure objectivity, infarct volume was estimated twice independently, by using a macroscopic technique (TTC staining) and microscopic examination by a neuropathologist. Both methods use a truncated cone mathematical model, using the areas determined by the computer digitizer. The neuropathologic evaluation was performed without knowledge of the cat's treatment group. To correct for the normal variation in brain volume, infarct volume was expressed as a percentage volume of the cerebrum.

We used two-way repeated-measures analysis of variance (ANOVA) to compare physiological parameters among the three treatment groups over the 18 hours of the experiment. We compared infarct volumes using one-way ANOVA, using the Student-Newman-Keuls test for pairwise comparisons. Since the data did not have a normal distribution, we compared ICP using Kruskal-Wallis nonparametric ANOVA, using the Mann-Whitney $U$ test for pairwise comparisons. The results are expressed as mean±SEM; $p<0.05$ is the level of significance.

Results

There were no significant differences among the groups with regard to arterial pH, $PCO_2$, or $PO_2$ (Table 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control (n=6)</th>
<th>Nutrient solution (n=8)</th>
<th>Fluorochemical emulsion (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>7.39±0.01</td>
<td>7.41±0.01</td>
<td>7.40±0.02</td>
</tr>
<tr>
<td>After treatment</td>
<td>7.43±0.02</td>
<td>7.40±0.01</td>
<td>7.40±0.01</td>
</tr>
<tr>
<td>$PCO_2$ (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>29.7±0.8</td>
<td>28.9±1.1</td>
<td>30.9±0.8</td>
</tr>
<tr>
<td>After treatment</td>
<td>26.3±0.1</td>
<td>29.0±1.1</td>
<td>27.3±1.4</td>
</tr>
<tr>
<td>$PO_2$ (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>165.7±2.1</td>
<td>170.3±7.9</td>
<td>173.3±4.9</td>
</tr>
<tr>
<td>After treatment</td>
<td>169.9±3.8</td>
<td>165.0±7.7</td>
<td>167.9±3.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Before treatment, 1–1.5 hours after occlusion; after treatment, 19–20 hours after occlusion.
The ICP in the three groups is shown in Figure 1. The ICP was comparable in all groups at the onset of the ischemic insult. Three and one-half hours after the onset of ischemia (2 hours after the onset of perfusion), the ICP of the control group began to rise; this rise continued through the termination of the experiment. In contrast, ICP of the nutrient solution-perfused group initially rose more slowly than that of the control group, but by 15 hours ICP equaled that of the control group. The ICP of the fluorochemical emulsion-perfused group remained stable throughout this period. At 18 hours ICP of the control group (mean 11.4 [range 2.3–23.0] torr) and the nutrient solution-perfused group (mean 11.3 [range 3.0–29.0] torr) were significantly higher than that of the fluorochemical emulsion-perfused group (mean 2.21 [range 0.0–3.5] torr).

Discussion

We have extensive experience with middle cerebral artery occlusion in cats, which is why we chose this model. Cats possess a bony tentorium, which makes them less tolerant of increases in ICP than other species. Small rises in ICP can cause transtentorial herniation. This anatomic novelty in cats probably accounts for the early rise in ICP seen in these studies. The wide range of ICP seen in the control cats reflects variation in the model with respect to infarct volume. This wide variation in ICP also parallels that seen clinically in patients suffering from middle cerebral artery occlusion.

There are several mechanisms whereby ventriculocisternal perfusion of ischemic brain with oxygenated fluorochemical emulsion may exert its beneficial effect. The pathophysiology of cerebral ischemia is extremely complex; however, several concepts have evolved that pertain to the pattern of injury following an ischemic event. These include 1) selective vulnerability, 2) an ischemic threshold, 3) a maturation phenomenon (i.e., progressive development of the lesion following an ischemic insult), and 4) peripheral expansion of the necrotic core of an infarct into the adjacent areas of an ischemic penumbra. This fourth mechanism could lead to a synergistic, reciprocal relation between a regional increase in tissue pressure due to necrosis and edema and a reduction of the regional cerebral blood flow to below levels critical for survival of the tissue of adjacent areas. Ventriculocisternal perfusion has the potential for altering the ischemic threshold, the maturation phenomenon, and the peripheral expansion of the ischemic core.

We considered that ventriculocisternal perfusion with artificial cerebrospinal fluid alone or with high osmotic/oncotic pressures could reduce ICP. However, in previous experiments we have found that brains perfused with osmotically adjusted vehicle do not in fact show smaller infarcts. In addition, many (but not all) vehicle-perfused animals could not be perfused for the full treatment period because of an intractable rise in ICP. In our current study, inclusion in the nutrient solution of an oncotic agent (which was also present in the fluorochemical emulsion) did not significantly lower the ICP at 18 hours, although there is the suggestion (not statistically significant) that the early rise in ICP might have been delayed (Figure 1).

We have postulated that ventriculocisternal perfusion with an oxygenated fluorochemical emulsion delivers O₂ and oxidizable substrate to ischemic tissue that has had its blood supply compromised. The cerebral blood flow threshold below which irreversible neuronal death occurs (12 ml/100 g/min) no longer holds since substrate is being supplied independently of cerebral blood flow. This is one proposed mechanism whereby ventriculocisternal perfusion reduces infarct volume.

The only difference in composition between the fluorochemical emulsion and the nutrient solution is the fluorochemical. The data from this study indicate that the increased oxygen-carrying capacity of the former compared with the latter has a significant
impact on reducing the volume of cerebral infarction and preventing the rise of ICP.

It is possible that the lack of a rise in ICP in the fluorochemical emulsion–perfused cats merely reflects the fact that their infarcts were small and therefore produced much less cerebral edema. It is also possible (and likely) that the reductions in the rise of ICP and infarct volume represent a modification of the maturation phenomenon and a prevention of the peripheral expansion of the necrotic core (i.e., treatment of the ischemic penumbra). The fact that ventriculocisternal perfusion effectively washes the brain, removing ischemic metabolic products such as CO₂ and other metabolic waste products such as lactate, supports this hypothesis. In addition, ventriculocisternal perfusion with oxygenated fluorochemical emulsion may remove excess excitatory and potentially destructive neurotransmitters (especially glutamate/aspartate), which may cool the brain (thereby reducing the metabolic demand), and may restore the ionic composition of the extracellular fluid toward normal concentrations, and may remove other mediators of secondary brain damage (such as bradykinin and free fatty acids). Studies are currently being planned using intracerebral dialysis probes to address these possibilities.

Regardless of its mechanism of action, ventriculocisternal perfusion with an oxygenated fluorochemical emulsion following cerebral ischemia offers the possibility of an effective therapy for the prevention of elevated ICP. Further research is needed to ascertain the time course whereby this mode of therapy might be effective and to determine the effect of varying osmotic and oncopressures in the perfusate.

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References


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