Modification of Cerebral Ischemia With Fluosol

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SUMMARY Fluosol-DA (Perfluorochemical Blood Substitute) was investigated in a previous study and found to provide some protection from ischemia and possible usefulness in limiting the size of infarction. In the present study, larger doses over longer periods of acute focal cerebral ischemia were used. Twenty-four cats had transorbital ligation of the middle cerebral artery (MCA). The 12 experimental animals were given 20% Fluosol-DA. The control group of 12 received isotonic saline solution. Twenty-four hours after the MCA occlusion, the cats were perfused with saline and phosphate-buffered formalin. The brains were removed and immersed in 10% formalin for 2 weeks. The results of macroscopic and histological examination suggested that, although Fluosol-DA did not provide complete protection from ischemic injury to the brains of the cats treated, it may have helped to slow the development of the pathological changes. Stroke Vol 16, No 1, 1985

Fluosol-DA did provide some protection from ischemia and might be useful in limiting the size of infarction. The present report is based on further investigation of the effectiveness of Fluosol-DA, using larger doses over longer periods of acute focal cerebral ischemia.

Material and Methods

Implantation of Occlusive Device (Tourniquet)

Twenty-four cats were anesthetized with intraperitoneal injection of ketamine-HCl (30 mg/kg). Intubation was done for the security of airway and the head was immobilized in an operating apparatus. As described in our previous study, the microtourniquet was surgically placed in position, around the proximal portion of the left MCA. The tourniquet was not tightened at this time. During the recovery period of four or five days following the operation, the cats were kept under observation and showed no apparent neurological deficit. Following this period, each animal was anesthetized...
with intraperitoneal ketamine-HCl (30 mg/kg) and the left femoral artery and vein were catheterized with 18.5 G cutdown-catheters. Twelve of the 24 cats were given 20% Fluosol-DA (15 ml/kg) I.V. and 12 cats received equivalent volumes of isotonic saline solution I.V. All cats were then given 95% oxygen and 5% carbon dioxide through a face mask. The external end of the implanted tourniquet was re-exposed and the tourniquet was tightened to occlude the MCA permanently. After closure of the wound, the animals were transferred to a controlled environment (95% O₂ and 5% CO₂) for a 24-hour period. Arterial blood samples were taken for the measurement of HCT, serum osmolality, and blood gas just before the intravenous administration of fluids, then again 30 minutes, 1 hour, 3 hours, 6 hours, and 24 hours after the MCA occlusion. Six (Group A) out of the 12 Fluosol-treated cats were given an additional injection of Fluosol-DA (15 ml/kg), six hours after occlusion. The remaining six (Group B) were given injections of Fluosol-DA (15 ml/kg) at both three hours and six hours after occlusion. Six cats (Group C) out of the 12 saline-injected animals were given additional doses of saline (15 ml/kg) six hours after occlusion. The other six (Group D) received additional doses of saline (15 ml/kg) both three hours and six hours after occlusion.

**Perfusion-Fixation**

Twenty-four hours after the MCA occlusion, and without releasing the occluding tourniquet, each animal was anesthetized with intravenous pentobarbital (20 mg/kg), and perfused through a left ventriculostomy with 200 ml of normal saline followed by 500 ml of 10% phosphate-buffered formalin as previously described. After perfusion, the brains were removed and immersed in 10% formalin for two weeks.

**Examination of the Brains**

A coronal section 3 mm posterior to the temporal lobe tip was processed and 7 μm histological sections were examined microscopically. All specimens were stained with cresyl violet and Luxol fast blue. On the left hemisphere, all cortical layers of the whole mantle were inspected and classified according to severity of neuronal damage. The categories were: normal, grade 1, grade 2 and grade 3 according to the classification used previously. All observations were transcribed to corresponding photographic prints made from the histological slides. The percentage area of cortex showing each grade of neuronal alteration was determined using a Hewlett-Packard 9815A calculator in conjunction with a Hewlett-Packard 9864A digitizer. This figure was multiplied by the number of the grade and accumulated to show the degree of ischemic damage in every animal. These scores were compared between groups using Student’s t-test.

**Results**

**Observations Following Left MCA Occlusion**

Twenty-two animals demonstrated immediate hemiplegia or monoplegia while two cats from Group B showed no apparent weakness. Over the next 24 hours prior to sacrifice, most of the affected animals had regained part of lost motor function. Immediately before sacrifice, all but three of the Fluosol-treated animals were awake and able to sit and walk. In contrast, all of the control animals were drowsy with signs of a major hemispheric deficit.

**Hematocrit (HCT), Serum Osmolality and Blood Gas Analysis**

Before the administration of Fluosol or saline, at 30 minutes, 1 hour, 3 hours, 6 hours, and 24 hours after occlusion, HCT, serum osmolality and blood gases were determined from blood samples taken from the descending aorta via the femoral catheter. In each animal, consecutive values of HCT were compared to the baseline value taken just before injection of Fluosol or saline. There was no statistically significant change in any group, and no significant difference between groups (p > 0.05) (table 1). Fluorocrit levels were also recorded (table 1).

Serum osmolality was analyzed in the same manner with Fluosol-treated animals showing increased osmolality at 30 minutes, 1 hour, and 3 hours (p < 0.05) (table 2). In the arterial blood gas analysis, Fluosol-treated animals had significantly higher PO₂ values after injection than did the saline-injected groups (p < 0.05). There was no significant difference between groups in PCO₂, pH, HCO₃⁻, and base excess values (p > 0.05) (table 3).

**Autopsy Findings**

Three Fluosol-treated cats were found to have edematous lungs on gross and microscopic examination at sacrifice. One was from Group A and the other two were from Group B. The lungs of all remaining animals were normal on gross and microscopic examination. Midline shift was measured from the brain slices. All animals (control and treatment) except two from Group A, demonstrated measurable shift of midline structures away from the ischemic hemisphere. The midline shift was less in the Fluosol-treated animals. This difference was significant between Groups A and C (p < 0.025) but not significant between Groups B and D (p > 0.05).

**Microscopic Findings of the Brains**

All animals showed ischemic changes in the cortex of the left hemisphere. In the control groups, there was little transitional area (i.e., grades 1 and 2) with grade 3 area being the most prominent. Grade 3 neuronal changes were accompanied by loss of cellular architecture and edema. The distribution of neuronal damage is schematically shown on diagrams (fig. 1). The areas which demonstrated neuronal changes (grades 1, 2, and 3) were accumulated and calculated as a percentage of the total cortical area in each animal (fig. 2). Total ischemic area in Fluosol-treated groups was not significantly different from that in saline-injected groups (p > 0.10).

To measure the degree of ischemic damage, each
area was multiplied by one, two or three according to severity grading, then accumulated again (fig. 3). There was now a significant difference between the new scores of Group A and Group C \((p < 0.05)\), but not between Group B and Group D.

**Discussion and Conclusions**

In a previous report from this Laboratory, animals treated with Fluosol-DA (15 ml/kg) immediately after the MCA occlusion, developed less extensive and less severe infarction than those treated with saline (15 ml/kg).
Table 3  Blood Gas Analysis

<table>
<thead>
<tr>
<th></th>
<th>PO₂</th>
<th>PCO₂</th>
<th>pH</th>
<th>HCO₃</th>
<th>BE</th>
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<tr>
<td>Baseline ± sd</td>
<td>263 ± 87</td>
<td>46 ± 19</td>
<td>7.27 ± 0.11</td>
<td>19 ± 2</td>
<td>-8 ± 3</td>
</tr>
<tr>
<td>30 min</td>
<td>334 ± 63</td>
<td>38 ± 5</td>
<td>7.31 ± 0.04</td>
<td>19 ± 2</td>
<td>-7 ± 2</td>
</tr>
<tr>
<td>1 hr</td>
<td>393 ± 41</td>
<td>41 ± 5</td>
<td>7.28 ± 0.03</td>
<td>19 ± 1</td>
<td>-7 ± 2</td>
</tr>
<tr>
<td>3 hrs</td>
<td>456 ± 39</td>
<td>41 ± 3</td>
<td>7.29 ± 0.02</td>
<td>19 ± 1</td>
<td>-7 ± 1</td>
</tr>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 hrs</td>
<td>451 ± 45</td>
<td>41 ± 3</td>
<td>7.31 ± 0.04</td>
<td>20 ± 1</td>
<td>-6 ± 2</td>
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<tr>
<td>24 hrs</td>
<td>162 ± 55</td>
<td>35 ± 4</td>
<td>7.35 ± 0.11</td>
<td>19 ± 3</td>
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<tr>
<td>Group B</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 hrs</td>
<td>439 ± 66</td>
<td>39 ± 3</td>
<td>7.34 ± 0.04</td>
<td>21 ± 1</td>
<td>-4 ± 1</td>
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<tr>
<td>24 hrs</td>
<td>124 ± 28</td>
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<td>7.37 ± 0.03</td>
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<td>Control ± sd</td>
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</tr>
<tr>
<td>Baseline ± sd</td>
<td>229 ± 62</td>
<td>37 ± 3</td>
<td>7.32 ± 0.04</td>
<td>19 ± 1</td>
<td>-7 ± 2</td>
</tr>
<tr>
<td>30 min</td>
<td>262 ± 45</td>
<td>34 ± 5</td>
<td>7.32 ± 0.04</td>
<td>17 ± 1</td>
<td>-8 ± 2</td>
</tr>
<tr>
<td>1 hr</td>
<td>274 ± 52</td>
<td>35 ± 5</td>
<td>7.32 ± 0.05</td>
<td>17 ± 2</td>
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<tr>
<td>3 hrs</td>
<td>368 ± 50</td>
<td>41 ± 5</td>
<td>7.30 ± 0.05</td>
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<tr>
<td>6 hrs</td>
<td>363 ± 33</td>
<td>41 ± 4</td>
<td>7.29 ± 0.02</td>
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<td>-7 ± 2</td>
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<tr>
<td>24 hrs</td>
<td>89 ± 59</td>
<td>30 ± 1</td>
<td>7.39 ± 0.04</td>
<td>19 ± 1</td>
<td>-6 ± 2</td>
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<td>Group D</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>6 hrs</td>
<td>418 ± 36</td>
<td>40 ± 1</td>
<td>7.33 ± 0.03</td>
<td>21 ± 1</td>
<td>-5 ± 2</td>
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<td>26 ± 6</td>
<td>7.43 ± 0.03</td>
<td>19 ± 2</td>
<td>-3 ± 2</td>
</tr>
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</table>

kg I.V.) up to six hours after occlusion. In the present study, six cats (Group A) were given two injections of Fluosol-DA while the other six cats (Group B) were given three injections. From observation of fluorocrit changes, Fluosol appeared to be lost from circulation faster following the first injection than after subsequent injections. This could be explained by the fact that the initial drop in Fluosol levels may be due to saturation of the reticuloendothelial system followed by a less dramatic elimination through the lungs.

Model

Of the various animal models for experimental cerebral ischemia, middle cerebral artery occlusion via...
transorbital approach is one of the most frequently applied. This model has several useful features. Brain damage can be minimized by avoiding large craniotomy.4 The enlarged optic foramen is easily sealed with pieces of Gelfoam, which allows the animal to return to good physiological condition in one week when MCA occlusion is performed. However, the model has some disadvantages. When the artery is occluded by a clip or suture, this may disrupt the sympathetic nerve conduction in the arterial wall.5 Also, in contrast with primates, the cat has abundant and variable collateral circulation; thus occlusion of MCA proximally may be expected to result in cerebral ischemia of ranging degree and area.

Osmolality

The osmolality of Fluosol-DA is 410 mOsm and falls to 320 mOsm following the almost instantaneous removal of glycerol from circulation.6 This is still slightly hyperosmotic compared with blood.

In the present study, we found a significant increase in serum osmolality in Fluosol-treated animals, but when compared to saline-treated groups, no significant difference was detected. We can conclude that increase in osmolality is apparent but minimal.

Hyperosmotic agents have been used to modify cerebral ischemia. Among them, mannitol appears to have been the most effective in delaying the course of ischemic change. In the report by Little,7 plasma osmolality after administration of mannitol (0.5 mg/kg) showed marked increase lasting for at least two hours. In order to maintain raised osmolality, mannitol was injected intermittently. The protective effect of a hyperosmotic agent may be due to several factors: expansion of blood volume, decrease in viscosity, or prevention of water movement from circulation to extravascular space.

Although osmolality in our study did not vary as much as in the mannitol study, this modest increase may still affect the course of ischemic change.

**O₂ Carrying Capacity**

Fluosol-DA, 20% dissolves O₂ according to Henry's law. At a PO₂ of 550 mm/Hg, it will dissolve about 6 vol% of oxygen, and about 0.8 vol% at a PO₂ of 50 mm/Hg. When arterial PO₂ is raised to 550 mm/Hg, O₂ availability from Fluosol-DA is equivalent to blood of 45% HCT. Moreover, Fluosol-DA will exchange O₂ at twice the rate of whole blood so that rapid oxygenation of hypoxic tissue is possible.

In this study, PO₂ in Fluosol-treated animals was significantly higher than in the control group. There was no statistically significant difference in hematocrit between treated and control groups, and therefore no difference in hemodilution resulting from fluid administration. The hemodilution that occurs after Fluosol administration is temporary; the Hct is known to return to pre-infusion level as rapidly as 30 minutes. After 30 minutes, the HCT in our experimental animals had returned to baseline values, where they remained throughout the experimental period. Consequently, Fluosol had merely augmented plasma and increased O₂-carrying capacity. High oxygen availability in the brain tissue of Fluosol-treated cats was confirmed by colleagues, using implanted oxygen electrodes in the same model to measure cerebral oxygen tension.9

**Ischemic Changes**

Group A, treated with two injections of Fluosol, developed smaller ischemic changes than Group C, treated with two injections of saline (p < 0.05). The ischemic changes in Group B (3 × Fluosol) seemed less severe than in Group D (3 × saline), but there was much variation in this treatment group and the difference was not statistically significant (p > 0.05). These observations suggest that, at least in Group A, Fluosol-DA has a protective effect on cerebral ischemia by delaying the neuronal alteration. Because of prompt
restoration of HCT and osmolality levels following injection of Fluosol, the beneficial effect is probably due to its ability to carry O$_2$, rather than to alter viscosity, osmolality, or blood volume. Although Fluosol-DA exhibited a certain protective effect on cerebral ischemia, this effect was not so marked as found in the previous study, in which neuropathologic observations were only made up to six hours after MCA occlusion.\textsuperscript{3}

This may be explained as follows:
1. Cerebral edema and associated pathological changes in brain tissue following ischemia usually reach their peak 24–48 hours after insult, which means that any form of treatment may appear progressively less effective over longer experimental periods.\textsuperscript{10}

2. While HCT in cats did not indicate significant hemodilution, one cat in Group A and two cats in Group B had edematous lungs at autopsy. This indicates multiple injections of Fluosol-DA (15 ml/kg) may have overloaded the vascular system in those animals, resulting in less efficient cardiopulmonary function.

3. During the experiments, the animals were kept in a chamber of 95% O$_2$ and 5% CO$_2$. Artificial ventilation was not available. The data obtained demonstrate slight elevation in PCO$_2$ and mild acidosis. This may have created an increase in intracranial pressure (ICP) which may in turn have affected the microcirculation over the 24-hour duration of the experiment.

Although Fluosol-DA did not provide complete protection from ischemic injury to the brains of the cats in this study, the results do suggest that Fluosol may retard the development of the pathological changes. Recently reported evidence indicates that treatment with both Fluosol-DA and 20% mannitol can restore normal EEGs in ischemic dogs.\textsuperscript{11} Further evaluation of perfluorochemicals is needed to determine optimum levels and combination effects with other drugs.

**Editor’s Note:** In accordance with *Stroke* policy this article was guest-edited by Murray Goldstein.

**References**
Modification of cerebral ischemia with Fluosol.
S J Peerless, R Nakamura, A Rodriguez-Salazar and I G Hunter

Stroke. 1985;16:38-43
doi: 10.1161/01.STR.16.1.38

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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