Protective Effect of Fluosol-DA in Acute Cerebral Ischemia

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SUMMARY Fluosol-DA (Perfluorochemical Blood Substitutes) are small particle fluorocarbons suspended in an emulsion and have a high propensity for carrying oxygen and carbon dioxide. Fluosol-DA was investigated for the modification of acute focal cerebral ischemia and compared to mannitol. A total of 36 adult cats were divided into 3 groups and had transorbital microtourniquet ligation of the middle cerebral artery (MCA). Control animals were given intravenous isotonic saline. Animals in the experimental groups were treated with either intravenous Fluosol-DA or mannitol. All animals were nursed in an oxygen chamber and 4 cats from each group were sacrificed at 1, 3 and 6 hours after MCA occlusion. The results of macroscopic and histological examination of the brain suggested Fluosol-DA had a definite protective effect on acute focal cerebral ischemia which was in keeping with the observed neurological outcome. It is suggested that Fluosol-DA may support flow in the microcirculation and that the small particles of Fluosol-DA may be able to reach the area of ischemia via collaterals by decreasing blood viscosity, preventing narrowing of the arteriolar and capillary lumen and increasing the cerebral blood flow.

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WORK BY CLARK in 1966 pioneered the investigation of perfluorochemicals as artificial blood substitutes.1 Since then, much progress has been made both in the U.S. and Japan in refining these chemicals and minimizing their toxicity.

The most effective of these substances to date, Fluosol-DA, has recently been developed in Japan. The oxygen-carrying component is a mixture of perfluorodecalin and perfluorotripropylamine which is emulsified (to concentrations of 20% and 35%) using Pluronic F-68, a non-ionic surfactant and detergent. Intralipid with glycerol and a small amount of fatty-acid are added and the mixture is homogenized with an iso-osmolar fluid to an average particle size of 0.1 μ.2 The total surface area for gas exchange per unit of volume is about 100-170 times that of blood and exchange occurs twice as fast as with hemoglobin.3 Elimination from the body occurs primarily through the lungs, with small amounts being excreted in urine and feces, and it follows a logarithmic time course. Some Fluosol particles undergo phagocytosis by cells of the reticulo-endothelial system.4 Although Fluosol collects in some body tissues, primarily in liver and feces, and it follows a logarithmic time course.

Some Fluosol particles undergo phagocytosis by cells of the reticulo-endothelial system.5 Although Fluosol collects in some body tissues, primarily in liver Kupffer cells and lung, it seems to produce no ill effects. Rats and rabbits have been exchange transfused with Fluosol to a hematocrit of 1% for longer than 24 hours and gone on to have a normal life span.6

With its small size and high propensity for carrying oxygen and carbon dioxide, Fluosol-DA may have a beneficial effect in the modification of cerebral ischemia. The purpose of this study was to test the efficacy of Fluosol-DA in the treatment of acute focal cerebral ischemia after occlusion of the middle cerebral artery (MCA) and to compare the protective effect with that of mannitol.6,7

Materials and Methods

Implantation of Occlusive Microtourniquet

A total of 36 adult mongrel cats, with an average weight of 2.9 kg, were anesthetized with ketamine-HCL (30 mg/kg, IP) and atropine sulfate (0.2 mg, 1P). Each cat was intubated and a 20 gauge polyethylene catheter was inserted into the cephalic vein for infusion of lactated Ringer’s solution. The head was fixed on the operating table using skull pins. Using an operating microscope, the left proximal MCA was exposed via the supero-medial transorbital approach modified from a previously described method.6 A 7.0 polypropylene (Prolene) thread was placed around the MCA and then passed through a polyethylene tube (1D; 0.015″). A xylocaine (1%) soaked cotton pad was applied to the MCA to relieve any spasm of the vessel when it occurred. The dural opening was sealed easily with a small piece of gelatin sponge (Gelfoam). The polyethylene tube, which protruded 5 mm from the orbital edge, was covered completely by the skin during closure.

Occlusion of MCA

Each animal was anesthetized lightly with ketamine-HCL (10 mg/kg, IP) 4 or 5 days after implantation of the microtourniquet. The sutured incision was opened over the polyethylene tube. The microtourniquet was tightened to occlude the MCA by gently pulling on the thread while holding the tube. Hemostatic clips (small Weck hemoclips) were applied across the polyethylene tube to secure the tightened microtourniquet. The skin was then sutured closed over the microtourniquet for the remainder of the experiment. No CSF leakage occurred in any of the animals.

Acute Experiments

The cats were divided into 3 groups of 12 animals each; a control group, a Fluosol-DA treated group and a mannitol treated group. Immediately after

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occlusion of the MCA, the control group was given intravenous isotonic saline solution (15 ml/kg) and the treatment groups received either Fluosol-DA 35% (15 ml/kg) (The Green Cross Corporation, 1-47, Chuoh 1-Chome, Joto-ku, Osaka, Japan) or 20% mannitol (1.2 gm/kg) intravenously. All animals were transferred into an oxygen chamber (95% oxygen and 5% carbon dioxide) and observed closely for signs of neurological deficit or dysfunction. Four animals from each group were sacrificed at 1, 3 and 6 hours after the MCA occlusion. Hematocrit was measured immediately before and at 5 minutes, 30 minutes, 1, 3 and 6 hours after the MCA occlusion. Arterial blood gas analysis was done in all animals at the time of sacrifice. A carotid angiogram was performed before and after occlusion of the MCA to document adequacy of the occlusion.

Perfusion of Cerebral Vessels

At sacrifice, each animal was anesthetized with intravenous injection of pentobarbital (20 mg/kg). The heart was exposed by thoracotomy and a large perfusion cannula inserted into the ascending aorta via a left ventriculostomy. The cannula was secured with a ligature. The descending aorta was clamped and the superior vena cava cut vertically at the level of the heart. Perfusion of the cerebral vessels was carried out initially by flushing with 100 ml of isotonic saline solution, followed by 500 ml of 20% phosphate buffered formalin (pH 7.3) formalin. The brain was removed and fixed in 500 ml of 20% phosphate buffered formalin for 4 days.

Examination of the Brain

Five mm coronal slices taken 3 mm posterior to the temporal lobe tip were inspected and photographed. The degree of brain swelling was evaluated by blind measurements of the mid-line shift of the third ventricle. The tissue was fixed in 10% phosphate buffered formalin and 7 μ paraffin sections, stained with hematoxylin/eosin/cresyl violet, were prepared and photographed. The sections were examined microscopically and all cortical ischemic changes were noted on the corresponding photographs. Ischemic neuronal alteration was classified according to severity as previously described by Little: grade 1 indicated slightly shrunken neurons with or without cytoplasmic vacuolation; grade 2 indicated moderately shrunken neurons with cytoplasmic eosinophilia and increased nuclear basophilia or swollen neurons with pale vacuolated cytoplasm and a pale vesicular nucleus; and grade 3 indicated severely shrunken nucleus and/or incrustations (fig. 1). The percentage area of cortical neurons showing ischemic alteration in the cerebral hemisphere was determined in identical slices of brain, 3 mm posterior to the temporal lobe tips. Each grade was given scores to evaluate severity of ischemic neuronal damage. A Hewlett-Packard 9815A desk-top calculator in conjunction with a Hewlett-Packard 9864A digitizer was then used on each photograph to determine the percentage area of ischemic damage. Comparisons of data were made using Student's t-test.

Results

Observation After Left MCA Occlusion

The clinical neurological response to the insult of MCA occlusion was variable. Initially, all animals were difficult to arouse and hemiplegic, likely due to
the combination of anesthesia and ischemic effect. However, at 3 hours, 3 Fluosol treated animals and one mannitol treated animal were able to stand and walk. At 6 hours, 3 Fluosol and one each of mannitol and control cats could walk.

**Hemodynamic and Cardiorespiratory Effects**

The serial percentile change of hematocrit after the MCA occlusion is shown in figure 2.

The changes in hematocrit seen in both experimental and control animals were not statistically different from each other (p > 0.05). In each animal there was an initial drop in hematocrit which corresponded, in the control animals, to the expected hemodiluting effect of adding saline and in the experimental animals, to a combination of hemodilution and osmotic effect. In all animals, the initial hematocrit drop at 5 minutes was statistically significant (p < 0.01); thereafter, the hematocrits returned to levels not statistically different from baseline (p > 0.05).

The mean arterial blood gas analysis is shown in the table. There was significant elevation of PO₂ in animals treated with Fluosol (p < 0.05). The PO₂ levels obtained in the Fluosol animals were almost double those in control and mannitol treated animals. The Fluosol treated animals had an elevation of PCO₂ which is not significantly different from that of control and mannitol treated animals (p > 0.05).

**Angiography**

A left common carotid angiogram confirmed the complete occlusion of the left proximal MCA.

**Macroscopic Findings**

All animals showed complete occlusion of the MCA at the time of necropsy. This was confirmed by checking entrapment of arterial wall in the tip of the polyethylene tube. One mannitol treated cat was excluded from this study because of slight subarachnoid hemorrhage due to a traumatic procedure.

Brain swelling with left to right mid-line shift was observed in 10 control, 6 Fluosol, and 5 mannitol treated animals. Fluosol and mannitol treated animals showed less severe mid-line shift than those in the control group (p < 0.05). The mean mid-line shifts were, control cats 1.0 ± 0.8 mm (SD), Fluosol treated cats 0.5 ± 0.5 mm (SD) and mannitol treated cats 0.4 ± 0.5 mm (SD).

**Microscopic Findings**

Histologic methods were used to evaluate the degree of ischemic damage. The mean ischemic neuronal alteration is shown in figure 3. The area of ischemic change increased progressively with time. In the control animals, this change ranged from 23% at one hour to 57% of the hemisphere showing ischemic change at 6 hours. By contrast, the Fluosol and mannitol treated animals had approximately 50% less ischemic neuronal change. Of particular interest were animals in both the Fluosol and mannitol treated groups which showed minimal neuronal change at 3 hours and 6 hours. One cat in the mannitol treated group had extensive neuronal damage at 6 hours, while none of the Fluosol treated cats had this extensive damage (fig. 4). The scores of severity which were given to the extension of each grade of neuronal damage are shown in figure 5. There was a significant difference between experimental and control groups (p < 0.01) but there was no significant difference between the Fluosol and mannitol statistics (p > 0.05).

**Table. Mean Arterial Blood Gas Analysis**

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>FLUOSOL</th>
<th>MANNITOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.28 ± 0.06</td>
<td>7.23 ± 0.09</td>
<td>7.31 ± 0.07</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td>254 ± 46</td>
<td>408 ± 123</td>
<td>237 ± 107</td>
</tr>
<tr>
<td>PCO₂ (mmHg)</td>
<td>39 ± 8</td>
<td>46 ± 14</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>O₂ - Saturation %</td>
<td>99.0 ± 1.2</td>
<td>99.7 ± 0.2</td>
<td>99.2 ± 0.7</td>
</tr>
<tr>
<td>Base Excess</td>
<td>-8.4 ± 1.3</td>
<td>-8.2 ± 0.9</td>
<td>-7.5 ± 2.6</td>
</tr>
</tbody>
</table>
Discussion

Twelve cats were given intravenous Fluosol-DA immediately after acute occlusion of the left MCA and the effect of Fluosol-DA was evaluated and compared with the same number of control and mannitol treated cats. The left MCA was occluded by tightening a previously implanted microtourniquet. The animals were observed in a 95% oxygen chamber for periods up to 6 hours after MCA occlusion. Results of macroscopic and histologic examination suggested Fluosol-DA had a definite protective effect on acute focal cerebral ischemia in keeping with the minimal neurologic findings in the animals.

The initial responses after acute occlusion of the MCA in conscious cats were agitation, forced circling movement to the side of the occlusion and tonic deviation of the head and neck followed by weakness of the contralateral limbs. The improvement in neurologic deficit observed in Fluosol and mannitol treated animals was more prominent in Fluosol treated animals, suggesting this drug had a favorable effect on the progression of the acute focal ischemic insult.

Impairment of microcirculation following acute focal ischemia may be a major factor in the pathogenesis of cerebral infarction. Microcirculatory failure is considered to be a result of a) increased blood viscosity causing stasis and intravascular clotting and b) narrowing of the arteriolar and capillary lumina. Blood viscosity relates directly to a concentration of plasma protein and of the formed elements of the blood. The concentrations of these osmotically active substances are increased during ischemia by the shift of inorganic ions, especially Na+ from the extracellular to intracellular space. Because ischemia impairs the active transport mechanism of electrolytes, water leaves the intravascular compartment causing an increase in viscosity and increased tissue pressure. When cerebral and vascular metabolism is severely impaired by ischemia, narrowing of the arteriolar and capillary lumina occur. This has been determined to be a result of compression by swollen astrocytic processes and by the increasing tissue pressure of the resulting cerebral edema.
this increase in blood viscosity and vessel narrowing persists, the "no reflow phenomenon" may occur with progression and irreversibility of the ischemic process and eventual complete breakdown of the blood-brain barrier.

Hemodiluting agents such as low molecular weight dextran and serum albumin have been shown to provide some protection against cerebral infarction by decreasing blood viscosity and by influencing the surface charges on the erythrocytes. Moreover, mannitol, a hyperosmotic agent, has been shown by Little and confirmed in our study, to prevent ischemic damage to the neurons. It was determined that increased plasma osmolality produced by mannitol helped to prevent the migration of fluid from the intravascular to extravascular space. Data from the present investigation showed that Fluosol treated animals had significantly less brain swelling compared to mannitol treated and control animals. The degree of ischemic neuronal alteration in the Fluosol and mannitol treated groups was statistically less severe and less extensive than that of the control group. The Fluosol treated animals showed a slight benefit over the mannitol treated animals at 6 hours. Extensive and severe neuronal damage, which was seen in one mannitol treated animal in this study, was also demonstrated in a previous study. This suggests individual variations in collateral circulation.

Fluosol-DA is a biologically inert substance and elimination from the body occurs mainly through the lungs following a logarithmic time course. The total surface area for gas exchange per unit volume is approximately 100 to 170 times that of blood and the exchange occurs twice as fast as that of hemoglobin. The importance of the known gas-carrying properties of Fluosol-DA in the modification of cerebral ischemia is, however, uncertain. The oxygen dissociation curve of the Fluosol-DA is linear, making oxygen transport poor at low Po2 values. However, when the Po2 drops from 550 mm Hg (which can be achieved by providing an 85% O2 environment) to 50 mm Hg (tissue pressure) Fluosol-DA can give up 6.9 vol% O2. Blood at a hematocrit of 45% can only release 5.9 vol% O2 over the same pressure drop.

The viscosity of Fluosol-DA plus blood is lower than whole blood. When Fluosol-DA is given intravenously, the osmolality of Fluosol falls from 410 mOsm to 320 mOsm. At 320 mOsm, there is a net movement of water from the extracerebral to the intravascular space. However, the resultant hemodilution was shown to be temporary and normal hematocrit level was restored in 30 minutes.

The possible mechanisms of protection by Fluosol-DA in focal cerebral ischemia are; a) it decreases blood viscosity, b) it prevents narrowing of the arteriolar and capillary lumina by decreasing fluid migration from the intravascular to extravascular space, c) it increases the availability of O2 for the tissues, d) it may increase the cerebral blood flow by raising local PCO2 and, most significantly, e) it is a small particle oxygen and carbon dioxide carrier which can reach the area of ischemia via the collateral circulation.

Although the underlying mechanism is not yet completely defined, this study suggests Fluosol-DA may be useful in the treatment of ischemic stroke and cerebrovascular spasm after subarachnoid hemorrhage occurs.

Acknowledgment

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