The Perfluorocarbon Fluoromethyloadamantane Offers Cerebral Protection in a Model of Isovolemic Hemodilution in Rabbits

Damianos E. Sakas, MD; Robert M. Crowell, MD; Kwantae Kim, MD, PhD; Kasuyoshi Korosue, MD; Nicholas T. Zervas, MD

Background and Purpose
Perfluorocarbons (PFCs) are considered promising cerebral protection agents because they could combine the beneficial effects of decreased blood viscosity with enhanced oxygen-carrying capacity and oxygen tissue delivery, but trials of PFCs as hemodiluents have been very limited. We evaluated fluoromethyloadamantane (FMA), a new perfluorocarbon compound, as an isovolemic hemodiluant and compared it with low-molecular-weight dextran 40 (D40) and a control group.

Methods
Through a transorbital craniectomy, the internal carotid, anterior, and middle cerebral arteries were coagulated to create a cerebral infarction in anesthetized, mechanically ventilated rabbits. No other experimental procedure was performed in control animals. In the two other groups, hemodilution was commenced 30 minutes after the arterial occlusion with either D40 or FMA. Hemodynamic parameters and brain and systemic temperature were monitored throughout the experiments. All animals were killed 6 hours after the arterial occlusion.

Hemodilution has been investigated extensively as therapy for cerebral ischemia and infarction, but the results have remained controversial. Many investigators have noted that hemodilution reduces blood viscosity and increases cerebral blood flow (CBF). However, hemodilution decreases the "local tissue" red cell mass and oxygen-carrying capacity and thus can potentially harm the ischemic brain. Several investigators have attempted to identify the optimal hematocrit that achieves improved microcirculatory flow without compromising the oxygen-carrying capacity of blood. Others have proposed an alternative approach, namely, the addition to the hemodiluant of a chemical substance with oxygen-carrying capacity; such a compound could compensate for the reduction in oxygen transport and thus might improve the results of treatment.

Perfluorocarbons (PFCs) are inert organic compounds that have a high affinity for oxygen and carbon dioxide. They can substitute for both red cells, by providing oxygen transport and delivery, and plasma proteins, by contributing to colloid osmotic pressure for volume maintenance.

Results
Hemodynamic and metabolic parameters and blood oxygen content were not affected by the infusion of either FMA or D40. Brain and systemic temperature remained constant. The ratio of infarct volume to the hemispheric volume was 19.6±3.7% in the FMA group (n=17), 19.9±4.6% in the D40 group (n=16), and 40.3±5.7% in the control group (n=17). The difference in infarct volume of both FMA and D40 animals compared with controls was statistically significant (P<0.01) when tested with Student's t test. There was no significant difference between FMA and D40 groups.

Conclusions
These results suggest that FMA has cerebral protective properties and should be purified, optimized, and further tested experimentally to develop a stable, efficient, and safe oxygen carrier, potentially suitable for clinical trials.

Key Words: cerebral infarction • cerebral ischemia • hemodilution • perfluorocarbons

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Materials and Methods
Experiments were performed on 50 New Zealand White rabbits of either sex weighing 3.0 to 4.5 kg. The guidelines of the National Institutes of Health for treatment and care of experimental animals were followed.

Preparation of Animals
Anesthesia was induced with 4% halothane in oxygen and maintained with 1% halothane in oxygen (FiO2, 1.0). After a tracheotomy the animals were mechanically ventilated with a Harvard respirator (Harvard Apparatus, S Natick, Mass) to maintain normocarbia (PCO2, 28 to 32 mm Hg). Temperature was held constant at 37°C to 38°C with a heating blanket. Mean arterial blood pressure (MAPB) was monitored using a 16-gauge femoral arterial catheter and a Grass polygraph (Grass Instruments, Quincy, Mass). The same catheter was used for serial blood gas analysis with a model 170/pH blood
gas analyzer (Corning Glass Works, Medfield, Mass). The femoral veins were also cannulated with 16-gauge catheters for hemodilution and blood sampling. The end-tidal CO₂ was monitored with an end-tidal CO₂ monitor (Accucap, Datasonics Corp, Paramus, NJ).

**Surgical Procedure**

After catheterizations, the animal was placed in a head-holder, and after collapsing the eye globe, a small craniectomy was made just above and lateral to the optic foramen without section of the optic nerve. After microsurgical incision of the dura and dissection of the underlying arachnoid membrane, the intracranial portion of the left internal carotid (ICA), middle cerebral (MCA), and anterior cerebral (ACA) arteries were identified and coagulated in a “Y” fashion from the bifurcation of ICA up to a length of 5 mm along the trunk of each vessel. A low-power bipolar coagulation was used under continuous irrigation with normal saline to prevent heat dissipation to the adjacent brain tissue. Brain and temporal muscle temperature were monitored throughout the experiments. The probe, a 23-gauge needle thermistor (model 524, YSI, Yellow Springs, Ohio), was inserted into the temporal lobe and FMA, fluoromethyloadamantane group.

**Table 1. Mean Arterial Blood Pressure, Blood Gases, and pH Values in the Three Groups of Animals**

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>CG</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
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<td>MABP, mm Hg</td>
<td></td>
<td></td>
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<td>C</td>
<td>93.8±2.4</td>
<td>96.2±0.9</td>
<td>102.0±3.6</td>
<td>97.6±3.5</td>
<td>99.4±4.8</td>
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<td>D40</td>
<td>95.8±2.8</td>
<td>100.9±2.8</td>
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<td>FMA</td>
<td>91.5±2.3</td>
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<td>93.8±2.0</td>
<td>99.4±1.6</td>
<td>98.8±1.6</td>
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<tr>
<td>Po₂, mm Hg</td>
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<tr>
<td>C</td>
<td>411.7±24.5</td>
<td>437.4±23.5</td>
<td>467.6±21.0</td>
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<tr>
<td>D40</td>
<td>408.4±22.1</td>
<td>430.9±19.4</td>
<td>442.3±17.8</td>
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<td>439.3±18.3</td>
<td>430.0±25.7</td>
<td>467.1±21.9</td>
<td>446.4±23.1</td>
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<td>Pco₂, mm Hg</td>
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<td></td>
<td></td>
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<tr>
<td>C</td>
<td>29.4±0.8</td>
<td>31.2±1.0</td>
<td>30.9±0.8</td>
<td>30.9±0.9</td>
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<tr>
<td>pH</td>
<td></td>
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<td></td>
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<td>C</td>
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</tr>
<tr>
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<td>7.349±0.03</td>
<td>7.309±0.01</td>
<td>7.300±0.22</td>
<td>7.308±0.02</td>
</tr>
</tbody>
</table>

Values are mean±SE. CC indicates commencement of craniotomy; CG, coagulation of arteries; MABP, mean arterial blood pressure; C, control group; D40, dextran 40 group; FMA, fluoromethyloadamantane group.

Animals hemodiluted with dextran 40 (n=16). Thirty minutes after occlusion, stepwise isovolemic hemodilution was performed by withdrawal of blood and simultaneous replacement of an osmotically equivalent volume of FMA (Adamantech Co, Lynwood, Pa) with bovine albumin added to achieve a hematocrit of 29%. FMA is a perfluorochemical hemoglobin substitute. It is prepared in an egg yolk phospholipid emulsion. For hemodilution and maintenance of isovolemia, the FMA solution should be rendered isotonic by the addition of NaCl. Each 60 mL of blood was replaced with a 50-mL solution containing 42.5 mL FMA and 7.5 mL albumin (bovine albumin 30% solution in approximately 0.85% sodium chloride, Sigma Chemical Co, St Louis, Mo). The FMA was administered through a membrane filter (1.2 Micron Filter Set, Kabivitrum, Inc, Alameda, Calif) at a rate of 3 mL/min. To avoid interaction between FMA and other materials, the solution was prepared just before the hemodilution, and the albumin was infused via a separate venous catheter.

Both D40 and FMA were prewarmed to room temperature by keeping them out of the refrigerator. Before administration, the temperature of the fluids was measured and raised to 37°C if necessary with the aid of an electric heater. The administration of the fluids did not cause any alterations in systemic or brain temperature. FMAcrit and hematocrit were measured concurrently. The blood sample was placed in a capillary hematocrit tube and centrifuged at 12 000 rpm for 6 minutes. After centrifugation, three layers—plasma, red cells, and an intermediate layer—were separately measurable. Serum Na⁺, K⁺, Ca²⁺, Cl⁻, and HCO₃⁻ were measured before and after hemodilution. Five hours after hemodilution (6 hours after arterial occlusion), animals were killed by sodium pentobarbital overdose. Brains were removed immediately for measurement of infarct volume.
Results

General Data

Arterial blood pressure, PO₂, PCO₂, and pH were comparable in the three groups of animals (Table 1). After the craniotomy, the hematocrit was 38.0±0.8%, 37.5±0.6%, and 37.2±0.5% in the control, D40, and FMA groups, respectively. After hemodilution the hematocrit was decreased to 29.0±0.6% and 28.9±0.3% in the dextran and FMA groups, respectively. Brain and systemic temperature remained constant throughout the experiments and were unaffected by the administration of fluids.

Hemodilution Data

In D40 animals, venesection of 30 to 50 mL was necessary to decrease the hematocrit from a mean value of 37.5% to 29.0±0.6%. After hemodilution, the hematocrit tended to increase but did not exceed 30.0±0.5%.

In FMA animals, as in the D40 group, venesection of 30 to 50 mL was necessary to decrease the hematocrit from a mean value of 37.2% to 28.9±0.3%. After hemodilution, the hematocrit tended to increase but did not exceed 30.0±0.7%. Electrolyte levels (Na⁺, K⁺, Cl⁻, Ca²⁺, HCO₃⁻) remained unaffected by the FMA infusion.

FMAcrit ranged from 3.0±0.4 to 3.5±0.4 (Table 2).

Infarct Size

In control animals, the infarct size ranged from 17.3% to 86.5% with a mean±SE value of 40.3±5.7%. One animal did not develop an infarct (Tables 3 and 4). In the D40 group, the infarct size ranged from 1.7% to 53.8% with a mean±SE value of 19.9±4.6%. Five animals did not develop an infarct (Tables 3 and 4). The difference in mean value of infarct size compared with that of control animals was statistically significant (unpaired Student's t test, P<.01). The difference in infarct size between D40-treated and FMA-treated animals was not significant.

Discussion

In the present study, FMA and D40 yielded similar reduction of infarct size. Hemodilution does not reverse cerebral ischemic changes unless the hemodilutant has acceptable microcirculatory properties. The beneficial effect of D40 has been attributed primarily to a reduction of viscosity,1,3 plasma volume expansion,4 and increase of CBF.4,10,11 Conversely, isovolemic hemodilution with compounds such as crystalloids11 or hydroxyethyl starch12 has been detrimental. In the present experimental model, however, our results suggest that FMA, like D40, has beneficial microhemodynamic characteristics for hemodilution treatment of cerebral infarction.

Experimental Model

We chose a model of isovolemic hemodilution rather than FMA infusion without blood withdrawal to assess the suitability of FMA as hemodilutant. The hematocrit was lowered to 30% to 32% because this level is comparable in the three groups of animals (Table 1).

Hematocrit Levels

D40 indicates dextran 40 group; FMA, fluoromethyloadamantane group.

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Hematocrit Levels

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considered the optimal range for tissue oxygen delivery.\textsuperscript{5,10} We administered Fio\textsubscript{2} of 1.0 because previous studies have suggested that a potential benefit from PFCs could only be manifested in a high oxygen environment.\textsuperscript{13} Instead of ligating one cerebral artery at a single point, we chose to extend the area of arterial occlusion. To achieve larger infarcts and decrease the effect of anatomic vascular variability,\textsuperscript{14} the thrombectomy started at the bifurcation of ICA and was extended to ACA and MCA for 5 mm in a Y-shaped fashion. Permanent occlusion of the cerebral arteries was selected because earlier studies of PFCs in our laboratory failed to show significant benefit in models of temporary ischemia followed by reperfusion.\textsuperscript{13,15} In relation to these data, PFCs could enhance the generation of oxygen free radicals by delivering higher tissue oxygen levels.\textsuperscript{8} Finally, the reliability of TTC to delineate cerebral infarction has been demonstrated under similar experimental conditions\textsuperscript{2,10} and further confirmed when the same infarct model was used for acute and chronic experiments.\textsuperscript{10}

**Perfluorocarbons and Cerebral Protection**

Our data correlate well with a number of other studies suggesting cerebral protection by PFCs. Experimental animals survived in fluorocarbon fluid equilibrated with oxygen at atmospheric pressure\textsuperscript{7} or after liquid ventilation with oxygenated PFC.\textsuperscript{17} Exchange-transfusion with PFCs, to a hematocrit of 1\% to 12\%, improved the metabolic cerebral function\textsuperscript{18} and showed CBF increase by 100\%\textsuperscript{19} with acute cerebral infarcts markedly decreased.\textsuperscript{16} Ventilobular subarachnoid perfusion with oxygenated PFCs significantly improved central nervous system oxygen delivery in cerebral hypoxia\textsuperscript{20} and focal\textsuperscript{21} and global\textsuperscript{22} cerebral ischemia. The beneficial effects of PFCs have been attributed to small particle size,\textsuperscript{19} decrease in viscosity,\textsuperscript{23} and brain edema,\textsuperscript{24} resulting in increases in CBF\textsuperscript{23} and particularly collateral flow\textsuperscript{25} and ultimately inducing increases of local tissue oxygen availability\textsuperscript{24,26} and amelioration of metabolic impairments.\textsuperscript{24}

**Fluoromethyloadamantane and Other Perfluorocarbons**

Previously tested PFCs such as Fluosol significantly reduced the experimentally induced acute cerebral ischemia\textsuperscript{16,24} and infarction in cats\textsuperscript{25} and offered cerebral protection during the early ischemic period,\textsuperscript{9,16,25} but in chronic experiments there was small\textsuperscript{27} or no benefit.\textsuperscript{13,15} Thus, it is unclear whether PFCs can prevent delayed cerebral damage. In ischemia followed by reperfusion, tissue PO\textsubscript{2} was not higher in Fluosol-infused animals compared with controls,\textsuperscript{24} and no benefit or advantage of Fluosol compared with D40 was seen.\textsuperscript{13} The promising results of a human trial of Fluosol\textsuperscript{28} were not supported by subsequent studies. It should be noted, however, that the PFC concentration in the human trials was low to demonstrate efficacy under clinical conditions.\textsuperscript{8}

**Does FMA have advantages over other PFCs?** FMA particles exchange oxygen at a much higher rate than blood. Since there is no oxygen binding constant involved, the dissolved oxygen freely moves into the surrounding tissues. Furthermore, the small size of FMA particles (<0.2 \(\mu\)m) facilitates their passage into and through capillaries, partially blocked vessels, and via collateral circulation. Thus, FMA may protect by increased oxygen delivery as well as improved erythrocyte and plasma microflow. Previously investigated PFCs affect the motility of macrophages\textsuperscript{26} and immune responses.\textsuperscript{29} These adverse effects were probably further exacerbated by Pluronic F68 (PF68), the surfactant used in the emulsions of Fluosol and most PFCs. PF68 causes alterations in fibrin structure and fibrin-cell interactions,\textsuperscript{30} inhibits cell growth,\textsuperscript{31} affects polymorphonuclear leukocyte traffic,\textsuperscript{32} and potently activates complement.\textsuperscript{33} All these factors may decrease the circulating oxygen tension and adversely affect chronic cerebral ischemia. Conversely, FMA is emulsified with phospholipids instead of PF68 and has very little complement activating potency.\textsuperscript{4} Intravenous phospholipid-FMA emulsion significantly increases circulating oxygen tension compared with PFCs containing PF68.\textsuperscript{8} By using FMA, which is free from PF68, higher concentrations of PFC can be administered. Thus, an Fio\textsubscript{2} of 0.6, close to clinical situations, may be adequate. Incorporation of antioxidants and free radical scavengers in the FMA solution may overcome problems related with reperfusion. Our results suggest that FMA has beneficial microhemodynamic and cerebral protective properties and should be further tested and developed for possible future use in clinical trials.

**Acknowledgment**

This study was supported in part by the A.S. Onassis Foundation.

**References**

In the interesting accompanying article, fluoromethyloadamantane (FMA), a new fluorocarbon compound, was evaluated as an isovolemic hemodiluent and compared with a control group with a low-molecular-weight dextran (D40) in a model of acute cerebral infarction in rabbits. The authors demonstrate a marked reduction (46%) in infarct volume of both the FMA and D40 animals compared with controls, and therefore the authors suggest that FMA has cerebral protective properties under these circumstances. Perfluorocarbons have been purported to be promising cerebral protection agents because they potentially combine the beneficial effects of decreased blood viscosity with enhanced oxygen-carrying capacity and oxygen delivery. However, their effectiveness has not been clarified properly.

The new FMA agent investigated here has a small particle size, a low viscosity, and a high fluorocarbon content. However, the major mechanism of its action to reduce infarct volume is not clarified in this article. The authors also show that D40 (low-molecular-weight dextran) was also effective in reducing infarct volume, but again the mechanism of action is unclear. The effect of D40 could be attributed to the influence on surface charges of inhibition of erythrocytes, platelet aggregation, interactions with fibrinogen, reduction of viscosity, reduction of edema, plasma volume expansion, and increases in cerebral blood flow. The positive effect of FMA may work by increased oxygen delivery or improved erythrocyte and plasma microflow. Whatever the mechanisms involved, these are two additional agents that must be added to the list of having neuroprotective effects in a model of isovolemic hemodilution in rabbits.

Richard J. Traystman, PhD, Guest Editor
Department of Anesthesiology/Critical Care Medicine
The Johns Hopkins University
School of Medicine
Baltimore, Md

Editorial Comment
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*Stroke*. 1994;25:197-201
doi: 10.1161/01.STR.25.1.197

*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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