Protection from Cerebral Air Emboli with Perfluorocarbons in Rabbits

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SUMMARY Cerebral air emboli have been implicated in the transient neurological deficits seen in patients after cardiovascular surgery. Since perfluorocarbon emulsions such as FC-43 have greater solubilities for gases compared to plasma, it was decided to investigate their potential in providing protection against transient ischemia produced by such emboli. Forty rabbits were divided into three groups of ten, twenty, and ten. They were anesthetized with acepromazine and ketamine and were given an infusion of either hetastarch or FC-43, 10 ml/kg i.v. In Group I, which received a bolus air injection into the internal carotid artery, survival rates were: 2/5 for the hetastarch group and 5/5 for the FC-43 group (p < .05). In Group 2, which received an air infusion until the EEG was flat bilaterally, 3/10 hetastarch-treated rabbits survived while 10/10 FC-43-treated rabbits survived for 24 hours (p = .035). In Group 3, analysis of blood viscosity and osmolality over a four hour period following either hetastarch or FC-43 infusion resulted in no significant differences between the two treatments but a significant decrease in both variables after 30 minutes which returned to baseline values at 60 minutes. The results of the study demonstrate a protective effect from cerebral air emboli delivered into the internal carotid artery as either a bolus or slow infusion in rabbits pretreated with a perfluorocarbon emulsion in terms of survivability and recovery from transient neurological deficits.

METHODS

Forty New Zealand White rabbits, weighing 2 to 3 kg, were divided into groups of ten (Group 1), twenty (Group 2), and ten (Group 3). All experiments followed the guidelines for the care and use of laboratory animals published by the National Research Council. In Group 1 anesthesia was induced with a mixture of acepromazine 0.7 mg/kg, and ketamine 30 mg/kg, i.m. An endotracheal tube was placed and anesthesia was maintained with 0.4% halothane in 100% oxygen.

The animals were mechanically ventilated to maintain $P_{CO_2}$ at 35 ± 5 mm Hg. Atracurium, 3 mg/kg i.v., was administered for muscle relaxation. Catheters of 5 and received an infusion of FC-43 (PFE; Alpha Therapeutic Corp., Los Angeles, CA) or hetastarch (H; Hespan; American Critical Care, McGaw Park, IL), 10 ml/kg i.v., respectively over 30 minutes. Hestastarch is the major component of the liquid phase of FC-43. After 15 min a bolus of air, 0.25 ml/kg was injected into the internal carotid artery. Ten min after the bolus, inhalational anesthesia was discontinued and the animals were observed for one hour. Ventilation was maintained until either the animals resumed spontaneous ventilation or one hour had lapsed. During the recovery period, catheters were removed and the skin was closed. The animals were then extubated and returned to their cages. Twenty-four hours after the air injection, the animals were evaluated for major neurologic deficits and, after anesthesia with aceprom-
mazine and ketamine as before, their EEGs were recorded.

In Group 2, seven days prior to the experiment, the twenty rabbits were anesthetized as in Group 1 and permanent skull EEG electrodes were placed over the frontal and occipital regions. On the day of the experiment, using the same anesthetic protocol, the right external carotid artery was ligated while the right internal carotid artery was catheterized via the common carotid artery using 27 ga Teflon tubing. Sodium thiopental, 25 mg/kg i.v., was used as a test for transient flattening of the EEG. The rabbits were then given 6 ml/kg of lactated Ringers and allowed 30 min for stabilization. They were randomly divided into two subgroups of 10, one receiving FC-43 (PFE) the other hetastarch (H), 10 ml/kg i.v. over 30 minutes. After 10–15 minutes, the CAE was administered via the internal carotid artery using a syringe pump at a rate of 0.1 ml/kg/min until bilateral flattening of the EEG was observed. After 10 min, inhalational anesthesia was discontinued but ventilation with 100% oxygen was maintained until spontaneous breathing resumed. Catheters were removed, the incisions closed, and after extubation, the animals were returned to their cages. At 24 hours post-CAE, the EEGs were recorded with the animals anesthetized as before.

In Group 3, ten rabbits were anesthetized as before and breathed spontaneously. Catheters were placed in the marginal ear vein and femoral artery; a 2 ml baseline arterial sample was taken. The animals then received an infusion of either FC-43 or hetastarch, 10 ml/kg i.v. over 30 minutes. Additional blood samples were taken at 15, 30, 60 min, and 4 hours after the infusion. Duplicate samples were analyzed for whole-blood osmolality on a vapor pressure osmometer (Wescor 5100C; Logan, UT) and for whole-blood viscosity at four different shear rates on a viscometer (Brookfield LVT-D; Brookfield, MA).

The EEG was graded for symmetry and the presence or absence of activity; differences between the subgroups were tested using Chi-square analysis. Fisher's Exact Test was used to test differences in survival between subgroups. Differences in interval data between subgroups were tested using group t-tests or analysis of variance for repeated measures; significance was accepted at the 0.05 level of probability.

**Results**

In Group 1, EEG frequency and amplitude decreased bilaterally in both subgroups following CAE. Four min after CAE, frequency and amplitude were below baseline in the H subgroup while in the PFE subgroup, the frequency was decreased on both sides but the amplitude increased bilaterally above baseline. At 10 min, the H group still demonstrated decreased frequency and amplitude but the PFE group values were within 20% of control values. Heart rate decreased in both groups immediately following CAE but returned to baseline by 10 min. However, blood pressure increased by 20 and 5 mm Hg for H and PFE-treated rabbits, respectively, and remained elevated for the duration of the study.

Survivability markedly differed for the two subgroups. In the H subgroup, one rabbit died following extubation and two rabbits died within 24 hours of CAE. In the PFE subgroup, all rabbits survived 24 hours following CAE ($p < .05$). Neurological evaluation revealed that the right pupil was greater in size than the left one-hour post-CAE in four rabbits, two from each subgroup. However, pupil size returned to normal after 24 hours in all but one H rabbit. Other neurologic deficits noted in the two subgroups at one

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PFE</th>
<th>Hetastarch</th>
<th>$p$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol air infused (cc)</td>
<td>4.28 ± 2.17t</td>
<td>3.03 ± 1.26</td>
<td>.076</td>
</tr>
<tr>
<td>Time to flat EEG (min)</td>
<td>13.7 ± 8.1</td>
<td>9.2 ± 3.4</td>
<td>.071</td>
</tr>
<tr>
<td>Time to recovery (min)</td>
<td>15.2 ± 7.0</td>
<td>24.8 ± 20.0</td>
<td>.095</td>
</tr>
<tr>
<td>Baseline BP-syst (mm Hg)</td>
<td>88.5 ± 11.0</td>
<td>90.6 ± 13.7</td>
<td>.36</td>
</tr>
<tr>
<td>Baseline BP-diast (mm Hg)</td>
<td>53.0 ± 9.0</td>
<td>50.0 ± 15.2</td>
<td>.31</td>
</tr>
<tr>
<td>Post CAE BP-syst (mm Hg)</td>
<td>85.0 ± 12.8</td>
<td>79.0 ± 26.2</td>
<td>.27</td>
</tr>
<tr>
<td>Post CAE BP-diast (mm Hg)</td>
<td>53.0 ± 9.0</td>
<td>50.0 ± 15.2</td>
<td>.31</td>
</tr>
<tr>
<td>Baseline HR (b/min)</td>
<td>229.0 ± 23.4</td>
<td>234.0 ± 31.6</td>
<td>.35</td>
</tr>
<tr>
<td>Post CAE HR (b/min)</td>
<td>208.0 ± 25.3</td>
<td>205.5 ± 31.0</td>
<td>.43</td>
</tr>
<tr>
<td>Baseline PaO2 (mm Hg)</td>
<td>501.1 ± 58.4</td>
<td>434.7 ± 84.9</td>
<td>.035</td>
</tr>
<tr>
<td>Baseline PaCO2 (mm Hg)</td>
<td>33.5 ± 5.4</td>
<td>34.0 ± 5.0</td>
<td>.42</td>
</tr>
<tr>
<td>Baseline pH</td>
<td>7.391 ± 0.031</td>
<td>7.383 ± 0.041</td>
<td>.32</td>
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<tr>
<td>Post-CAE PaO2 (mm Hg)</td>
<td>444.4 ± 98.0</td>
<td>335.9 ± 161.3</td>
<td>.05*</td>
</tr>
<tr>
<td>Post-CAE PaCO2 (mm Hg)</td>
<td>41.7 ± 12.5</td>
<td>40.7 ± 13.5</td>
<td>.44</td>
</tr>
<tr>
<td>Post-CAE pH</td>
<td>7.312 ± 0.085</td>
<td>7.314 ± 0.099</td>
<td>.48</td>
</tr>
</tbody>
</table>

*Statistically different.
† Mean ± standard deviation.
TABLE 2 Viscosity at 115 per Second and Osmolality before and after Infusions of either Hetastarch (n = 5) or Perfluorocarbon

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>4 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity (centipoise)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFE</td>
<td>3.446*</td>
<td>3.124</td>
<td>3.004†</td>
<td>3.288</td>
<td>3.400</td>
</tr>
<tr>
<td></td>
<td>0.613†</td>
<td>0.315</td>
<td>0.523</td>
<td>0.391</td>
<td>0.423</td>
</tr>
<tr>
<td>Hetastarch</td>
<td>3.120</td>
<td>3.108</td>
<td>2.906‡</td>
<td>3.116</td>
<td>3.048</td>
</tr>
<tr>
<td></td>
<td>0.372</td>
<td>0.210</td>
<td>0.358</td>
<td>0.372</td>
<td>0.168</td>
</tr>
<tr>
<td>Osmolality (milliosmols)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFE</td>
<td>348</td>
<td>338</td>
<td>325‡</td>
<td>346</td>
<td>341</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>6</td>
<td>12</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Hetastarch</td>
<td>336</td>
<td>333</td>
<td>327‡</td>
<td>327‡</td>
<td>325‡</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>12</td>
<td>4</td>
<td>3</td>
<td>11</td>
</tr>
</tbody>
</table>

(PFE; n = 5).

*Mean.
†Standard deviation
‡Significantly different from baseline.

hour were irregular breathing (1H), diminished pain perception (1PFE; 2H), diminished muscle tone or motor deficit (1H), and inability to maintain posture (2H; 2PFE).

In Group 2, both subgroups PFE (n = 10) and H (n = 10) demonstrated comparable bilateral flattening of the EEG 10 min after the air infusion was discontinued. At 60 min, 8 of 10 PFE and 6 of 10 H rabbits had complete recovery of EEG activity. Twenty-four hours post CAE, 8 of 10 PFE rabbits had normal EEG activity while only 3 of 10 H rabbits had normal activity. In addition, the other two PFE rabbits had survived 24 hours but had asymmetrical EEG activity and signs of neural deficits (right pupil dilated) while the remaining seven H rabbits had died; survivability was significantly greater in the PFE treated group (p = .035).

No significant differences were found in the heart rate, arterial blood pressure, pH, and pCO₂ between the two subgroups before and after CAE (table 1). However, the arterial pO₂ was significantly increased in the PFE rabbits before and after CAE (table 1). The volume of air infused and the time to isoelectric EEG activity was greater in the PFE subgroup compared to the H subgroup but the differences were not statistically significant.

In Group 3, the whole-blood viscosity and osmolality were not significantly different between the two treatments (table 2). However, there was a significant decrease in both variables during the first 30 minutes; these values returned to baseline by 60 minutes except for the osmolality in the hetastarch-treated group.

Discussion

Perfluorocarbons combined with 100% oxygen ventilation have previously been shown to provide protection against focal cerebral ischemia in cats after transient occlusion of the middle cerebral artery. In this laboratory it has previously been shown that similar pre-treatment will protect rabbits against massive venous air embolization. The results of this study demonstrate a protective effect from CAE delivered as either a bolus or a slow infusion into the internal carotid artery in rabbits pretreated with PFE in terms of survivability and recovery from transient neurological deficits.

The protective effect observed with PFE may be the result of one of several postulated mechanisms. Nitrogen in the CAE may be absorbed by the PFE which has been denitrofied with the rabbits breathing 100% oxygen, the PFE would have to absorb the nitrogen rapidly so as to prevent blockages of fine vessels, which probably occurs in the untreated rabbits. Since the emulsion has a large surface area and a high solubility for nitrogen, this mechanism is possible. Enhanced oxygen delivery because of an increase in oxygen-carrying capacity of blood with FC-43 (see table 1) may increase the tissue resistance to CAE-induced pathology in animals pretreated with PFE. Changes in blood rheology may also contribute to better oxygen delivery in areas of partial arteriolar blockage by CAE. In an effort to learn more toward this last hypothesis blood viscosity and osmolality were measured. No significant differences were found between the PFE treatment and the hetastarch treatment that could be explained by improved flow around a partial blockage as a result of PFE treatment. These results do not exclude the possibility that the small particles in the emulsion may flow around partial blockages or restricted collaterals to provide improved oxygenation compared to plasma alone. However, our results suggest that nitrogen absorption or enhanced oxygen delivery may account for the increased survivability of the PFE-treated group.

Cerebral air embolism is a complication of coronary artery bypass grafting, cardiac valvular repair and congenital cardiac surgery, Venous air embolism always carries some risk of transfer of the air to the systemic circulation. Pre-treatment of rabbits with PFE has been shown to provide protection from experi-
BRAIN EDEMA is an important pathological state to investigate as it interferes with cerebral circulation. The condition is thought to comprise two types of edema: cytotoxic and vasogenic. The effect of brain edema is complicated not only because of the resulting mass effect but also due to its effect on cerebral metabolism. Until recently, most experiments on brain edema have been carried out employing morphological and biochemical methods; however, these experimental methods have not provided a complete understanding of water characteristics in the brain. The introduction of nuclear magnetic resonance (NMR) techniques in the field of biophysics as a non-invasive method should provide important information. Using NMR, the state of water in the tissue can be evaluated by measuring relaxation times.

Materials and Methods

1) The Model of Ischemic Brain Edema

One hundred and fifty Mongolian gerbils (Meriones unguiculatus) weighing about 80g each were used. After ether anesthesia, right cerebral hemispheric ischemia was induced by ligating the right carotid artery under a surgical microscope. Sixty percent of the animals showed rolling seizures or severe suppression of motor activity immediately after the operation, and they were judged to be suitable for further experiments. Gerbils were divided into three groups: normal
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