Effect of Carotid Artery Ligation and Infusion of Fluosol FC-43 Emulsion on Brain Surface Oxygen Tensions

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SUMMARY In eight rabbits, the common carotid artery was ligated and multiple estimations of brain surface oxygen tension performed using a seven barreled mini-electrode. In five rabbits ligation of the carotid artery resulted in impairment of cortical oxygenation. The remaining three rabbits showed no impairment in the supply of oxygen to the cerebral cortex after carotid occlusion. In the five rabbits who displayed a reduction in oxygen supply after carotid ligation, ventilation with 33% oxygen after the infusion of 15 ml/kg of Fluosol FC-43 produced an improvement in cortical oxygenation in only three of the five rabbits. When these animals were ventilated with 100% oxygen after carotid ligation and Fluosol infusion, oxygen supply in all five was commensurate with or greater than that during control conditions.

This study has used this technique for two purposes. Firstly, to investigate the effect of carotid occlusion on delivery of oxygen to cerebral tissue. Secondly, to study the effect of infusion of a Fluosol emulsion on cerebral oxygen supply after carotid occlusion.

Methods

1. The Rabbit Preparation

In eight half lop male rabbits weighing between 2.5 and 3.4 kg, anesthesia was induced by intramuscular injection of ketamine 15 mg/kg. A tracheostomy was performed, and mechanical ventilation instituted with nitrous oxide/oxygen (2:1) and halothane (0.5%). The level of ventilation was adjusted to maintain an arterial PCO2 as near 40 mm Hg as possible. A peripheral venous line was placed for infusion of Hartmann's solution at 5 ml/kg/hr, and a femoral arterial cannula inserted. Arterial pressure was continuously monitored via a Statham P23 pressure transducer and recorded on a Grass model 5D polygraph. End tidal PCO2 was measured with a Beckmann LB2 CO2 analyser, and arterial blood samples were taken regularly for analysis on an ABL 2 blood gas analyser.

The right common carotid artery was exposed, a suture placed loosely around it, and 1 ml of 0.5% lignocaine was instilled around the artery. A 1 cm diameter right sided craniotomy was performed, the

THE ABILITY OF PERFLUOROCHEMICALS to act as oxygen and carbon dioxide carriers in place of hemoglobin was demonstrated in 1966.1 However it is only more recently that formulations of perfurodecalin and perfurooctrilexipromine emulsified in the non-ionic surfactant Pluronic F-68 (Fluosol-DA) have been found suitable for administration to humans.2 Because of the oxygen carrying capacity and relatively low viscosity of Fluosol compared with blood, there has been speculation whether this compound may improve ischemia in tissues whose perfusion has been reduced to critical levels. Some studies have suggested that it may have beneficial effects on cerebral ischemia in a variety of animal models and in humans. 5,6

If multiple readings of tissue oxygen tension (Pto2) are taken with electrodes small enough to resolve the gradients of oxygen tension between individual capillaries, it is possible to build up a picture of tissue oxygenation within the cerebral microcirculation.6

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dura reflected, and the defect in the skull covered with plastic sheeting during periods when no PtO2 estimations were being made. Brain surface temperature was monitored by means of a 2 mm bead thermistor positioned at the edge of the bone flap. After surgical preparation the animal was allowed to stabilize for three quarters of an hour before commencing measurements of PtO2.

2. Determination of Brain Oxygen Tensions

The design and operation of the seven barrelled oxygen electrode using intermittent sweep potential polarography has been described elsewhere.7, 8 The electrode itself is of a standard membrane-covered design,9 and consists of seven 25 micron platinum wires fused into glass and supported in a stainless steel casing. At the working surface of the probe the platinum wires are ground to a plane surface so that the electrode can measure oxygen tensions at tissue surfaces. The assembly was first calibrated in saline solutions equilibrated with nitrogen and 10% oxygen in Adams tonometers. These solutions were maintained at the temperature recorded at the cortical surface by a circulating water bath. The electrode assembly was then applied to the cortical surface of the rabbit by means of a counterbalanced arm modified from one previously described for use with a single oxygen probe.10 This arm allowed the electrode to float upon the surface of the brain without exerting more than 0.5 gm/cm pressure on actual brain substance, while the probe was maneuvered across small areas of the cortex by adjustments to the counterbalancing mechanism. After measurements in the animal the electrode was returned to the calibrating solutions and recalibration performed before the next series of measurements.

3. Carotid Ligation and Fluosol Infusion

Control measurements for brain surface PtO2 were first obtained when the animal was ventilated with either 33% or 100% oxygen. The snare which had been placed around the carotid artery was then tightened and the measurements repeated at both inspired oxygen concentrations. Following the post occlusion PtO2 estimations, Fluosol FC-43 (Perfluorotributylamine 25% in Pluronic F-68) (Green Cross Corp., Japan), 15 ml/kg was infused over 15 minutes. The oxygen electrode was then reapplied to the cortex and measurements again performed with inspired oxygen concentrations of 33% and 100%.

4. Analysis of Results

For each experimental steady state, i.e., control, after carotid occlusion, or after Fluosol infusion, the PtO2 values obtained for each rabbit were compared using the Mann-Whitney U Test for large samples. For ease of visual presentation in this paper, the results from those rabbits who showed similar trends were pooled and then plotted in histogram form. The results from blood gas analysis were compared using Student's t test.

**Table 1**

Mean PtO2 (mm Hg), Std Deviation, Modal PtO2 and the Percentage of Values less than 10 mm Hg during Control Conditions, after Carotid Occlusion, and after Infusion of Fluosol FC-43 15 ml/kg

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean PtO2</th>
<th>Std Dev</th>
<th>Modal PtO2</th>
<th>% Freq &lt; 10 mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (33% O2)</td>
<td>30</td>
<td>14</td>
<td>30-35</td>
<td>7</td>
</tr>
<tr>
<td>Occlusion (33% O2)</td>
<td>30</td>
<td>14</td>
<td>25-35</td>
<td>6.6</td>
</tr>
<tr>
<td>Fluosol (33% O2)</td>
<td>36</td>
<td>17</td>
<td>35-40</td>
<td>6.0</td>
</tr>
<tr>
<td>Control (100% O2)</td>
<td>91</td>
<td>45</td>
<td>60-70</td>
<td>0.1</td>
</tr>
<tr>
<td>Occlusion (100% O2)</td>
<td>72</td>
<td>39</td>
<td>30-40</td>
<td>1.1</td>
</tr>
<tr>
<td>Fluosol (100% O2)</td>
<td>112</td>
<td>51</td>
<td>140-150</td>
<td>0.3</td>
</tr>
</tbody>
</table>

**Results**

Pooled frequency histograms for PtO2 in each of the six experimental situations are shown in figures 1 to 7. The salient features of each histogram are summarised in table 1. Figure 1 shows the normal histogram of PtO2 values obtained across the cerebral cortex when the animals were ventilated with 33% oxygen (8 animals). Following acute occlusion of the right common carotid artery, the measured response in cortical tissue oxygen tensions was variable. In five rabbits (fig. 2) a reduction in the supply of oxygen to the cerebral cortex compared with control conditions was demonstrated, with a shift of the oxygen histogram to the left (p < .05), and an increase in the frequency of PtO2 values less than 10 mm Hg. In the remaining three animals no such reduction in the supply of oxygen to cerebral tissue was shown (fig. 3). Figure 4 refers to the five rabbits who displayed reductions in PtO2 after carotid occlusion, and shows the pooled cortical oxygen histogram after infusion of 15 ml/kg of Fluosol FC-43 emulsion and during ventilation with 33% oxygen. Of these five rabbits, three showed significant shifts of the histogram to the right as compared with measurements after carotid occlusion (p < .05), such that the oxygen...
histogram was now similar to control. In the other two rabbits no right shift of the histogram towards control values was demonstrated.

In figures 5 to 7 pooled histograms for the five rabbits who exhibited a left shift after carotid occlusion are depicted now, while the animals were ventilated with 100% oxygen. Compared with control conditions (fig. 5), all these rabbits showed a significant reduction in $\text{PtO}_2$ ($p < .05$) after carotid occlusion (fig. 6). After infusion of Fluosol (fig. 7) all the rabbits showed a significant shift to the right of the histogram ($p < .05$), and it generally became bimodal with some areas showing a large increase in oxygenation above control levels.

Table 2 shows the results from blood gas analysis during the different experimental steady states. The groups are comparable save for significant rises in arterial oxygen tension after Fluosol infusion.

**Discussion**

This study has used the technique of sweep potential polarography with 25 micron electrodes to investigate oxygen tension fields on the rabbit cerebral cortex. The factors which influence oxygen tension at any given point in the tissues are complex and include the pattern of the capillary network, the oxygen tension at the capillary wall, the rate of oxygen uptake by the tissues, the diffusion coefficient for oxygen within the tissues and the distance which oxygen has to be transported. The oxygen tension at the capillary wall will in turn depend on the flow of blood within capillaries and the capacity of blood flowing within the capillaries for transporting oxygen. By taking multiple readings of $\text{PtO}_2$ with probes small enough to resolve the oxygen
gradients between capillaries it is possible to describe an "oxygen tension field" by constructing histograms of tissue oxygenation. This will give direct information about the adequacy of the microcirculation and the supply of oxygen to cerebral tissue.

In rabbits we found histograms of brain surface PtO\textsubscript{2} similar to those in guinea pigs, cats, and sheep. Tissue PtO\textsubscript{2}'s are generally within the range 20 to 60 mm Hg, but with tensions in some areas approaching that of arterial blood, and some areas in the watershed zones between capillaries displaying tensions near zero. A change to 100% oxygen in the inspired gas produced large increases in measured PtO\textsubscript{2}, confirming the findings of Leniger-Follert et al. The increase in arterial oxygen tension provides a steeper gradient for oxygen diffusion within the tissues, which far outweighs any hindrance to oxygen transfer brought about in the capillary network by reflex hyperoxic vasoconstriction.

Changes in cerebral blood flow and in the electroencephalogram are well documented after carotid occlusion. Measurements of tissue oxygen tension have been made after acute middle cerebral artery occlusion in baboons, but not after carotid occlusion. Three animals in this study showed no impairment in oxygen supply and presumably possessed sufficient collateral circulation to maintain cerebral blood flow at levels which did not impair oxygen delivery. Unfortunately no cerebral blood flow measurements were performed to determine at what flow levels the five other rabbits displayed shifts in their oxygen histograms. In these rabbits the increase in the number of areas displaying extremely low PtO\textsubscript{2}’s is important. Although individual micro-areas of cortex can tolerate a low PtO\textsubscript{2}, any increase in the number of such areas will result in the coalescence of some and thus potential hypoxic cerebral damage.

Fluosol could benefit ischemic cerebral tissue by any of the following mechanisms. Firstly, infusion of Fluosol will result in blood volume expansion which may increase flow within the systemic circulation. Secondly, addition of Fluosol to whole blood reduces viscosity, and this together with a reduced propensity for red cell sludging could improve microcirculatory flow. Increases of the order of 12% have been noted in cerebral blood flow after the infusion of Fluosol-DA. Furthermore, because oxygen dissociates from Fluosol in accordance with Henry's law, it can unload a significant amount of oxygen to the tissues at a high oxygen tension, thus providing a steep gradient for oxygen diffusion.

Changes in oxygen supply caused by microcirculatory as opposed to the oxygen carrying properties of Fluosol, can to some extent be differentiated by examining its effects under normoxic and hyperoxic conditions. This is so because under normoxic conditions

### Table 2: Mean Values with SEM for Arterial PO\textsubscript{2} (mm Hg), PCO\textsubscript{2} (mm Hg), and pH, during Control Conditions, after Carotid Occlusion, and after Infusion of Fluosol FC-43 15 ml/kg

<table>
<thead>
<tr>
<th>Condition</th>
<th>PO\textsubscript{2}</th>
<th>PCO\textsubscript{2}</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (33% O\textsubscript{2})</td>
<td>113 (7.7)</td>
<td>40 (5.8)</td>
<td>7.18 (.042)</td>
</tr>
<tr>
<td>Occlusion (33% O\textsubscript{2})</td>
<td>114 (7.4)</td>
<td>42 (4.5)</td>
<td>7.18 (.047)</td>
</tr>
<tr>
<td>Fluosol (33% O\textsubscript{2})</td>
<td>144 (11.6)*</td>
<td>44 (6.3)</td>
<td>7.20 (.048)</td>
</tr>
<tr>
<td>Control (100% O\textsubscript{2})</td>
<td>242 (24.6)</td>
<td>39 (5.4)</td>
<td>7.20 (0.39)</td>
</tr>
<tr>
<td>Occlusion (100% O\textsubscript{2})</td>
<td>256 (27.1)</td>
<td>41 (6.1)</td>
<td>7.19 (.045)</td>
</tr>
<tr>
<td>Fluosol (100% O\textsubscript{2})</td>
<td>351 (28.1)†</td>
<td>45 (7.9)</td>
<td>7.19 (.052)</td>
</tr>
</tbody>
</table>

*P < .05; †p < .01.
Fluosol itself will contribute little to oxygen delivery, and therefore any effect seen on the oxygen histogram will probably be caused by changes in microcirculatory flow. Under these conditions we found that the effect of Fluosol was variable, three animals showing improvements in oxygen supply, and two showing no improvement.

When Fluosol was administered with 100% oxygen after carotid occlusion, tissue oxygen supply always improved and in fact exceeded that found during control conditions. The ability of Fluosol to unload oxygen at high tensions must have provided a sufficient gradient for oxygen diffusion to all areas. The oxygen histogram itself changed, becoming bimodal. A possible explanation is that at the arterial end of capillaries Fluosol unloads oxygen at high tensions so producing microareas with high P\textsubscript{O\textsubscript{2}}'s, whereas at the venous end of capillaries oxygen transfer to tissues is effected by haemoglobin at more normal oxygen tensions.

There are certain limitations in applying these observations to the clinical situation. Firstly, the cerebral metabolic rate for oxygen is generally reduced under anaesthesia, and therefore improvements in oxygen supply produced by any agent could be magnified as compared with the awake state. Secondly, tissue oxygen measurements are only one measure of cerebral metabolism, and while the supply of oxygen may be adequate, that of other substrates may not. However our data demonstrates that following carotid occlusion in rabbits, Fluosol infusion and 100% oxygen administration is capable of returning oxygen delivery to levels seen with 100% oxygen administration before occlusion. If 33% oxygen is administered some improvement in oxygen supply with Fluosol following carotid occlusion may occur, but this is not guaranteed.

Acknowledgments

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References

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