Effect of Fluosol on Oxygen Availability, Regional Cerebral Blood Flow, and Infarct Size In a Model of Temporary Focal Cerebral Ischemia

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SUMMARY Twenty-four cats had an intravenous infusion of either Fluosol or saline and then were subjected to 2 hours of middle cerebral artery occlusion. All the animals infused with Fluosol and one-half the animals infused with saline were ventilated with 100% O2. Tissue oxygen availability and regional cerebral blood flow were measured by platinum electrodes using direct voltage recordings for oxygen measurements and hydrogen clearance curves for measurements of cerebral blood flow. With 100% oxygenation tissue oxygen availability increased significantly in the Fluosol treated animals, however, during the time of ischemia oxygen availability decreased below baseline values to about an equal level whether the animals were treated with Fluosol or saline. Regional cerebral blood flow fell to a similar value in all groups during the time of occlusion. One hour after reperfusion blood flow increased considerably above baseline values in all groups and oxygen availability also increased in all groups but particularly in the Fluosol treated animals. Overall mortality and the size of infarct 1 week after the ischemic insult were not significantly different in the three groups. Mortality was directly related to the size of the infarct which, in turn, was related to the degree of ischemia during the time of occlusion.

PERFLUOROCHEMICALS (PFC), or fluorocarbons, are inert organic compounds capable of substituting for the gas transport functions of blood because of their high affinity for O2 and CO2.1,2 In acute experiments in other laboratories, as well as in ours, they have been shown to be useful in protecting the brain from the effects of ischemia.3-5 In another study where cats were sacrificed 24 hours after occlusion of one middle cerebral artery (MCA), PFC also seemed of value although their effect was not as beneficial as in the acute experiments.6 However, in a more "chronic" study where cats were treated either with PFC or with dextran and kept alive for one week after a 4-hour period of MCA occlusion, we failed to show any benefit from either agent and there was a high mortality from cerebral infarction in both groups (unpublished data). We concluded that the 4-hour period of MCA occlusion followed by reperfusion in the cat is probably too severe an ischemic insult to allow the protective effect of any agent, if such effect was only moderate, to manifest itself. We are reporting here the results of a study of the effects of Fluosol DA 20% (developed by the Green Cross Corporation, Osaka, Japan) on brain tissue oxygen availability (O2a), regional cerebral blood flow (rCBF), extent of brain infarction, and survival in a model of cerebral ischemia in the cat. The surviving animals were kept alive for a week for better histologic delineation of the infarct. The ischemic insult was limited to 2 hours of temporary MCA occlusion in view of the high morbidity and mortality in our previous study with a 4-hour period of occlusion.

Materials and Methods

Mongrel cats of either sex, weighing between 3 and 3½ kg, were anesthetized with ketamine (20 mg/kg) and pentobarbitone (20 mg/kg) given intraperitoneally. The animals were then given antibiotics and their heads were fixed in a stereotactic frame (David Koft Instruments, California). Especially conducted electrodes (Rhodes Medical, California) were implanted in the area supplied by the MCA on the right side (four electrodes) and on the left side (one electrode). The electrodes were constructed of 90% platinum and 10% iridium and were bipolar with two separate recording, individually isolated, tips. The electrodes were inserted stereotactically following the coordinates of Snider and Niemer.8 The first electrode on the right side was inserted in the caudate nucleus and the rest of the electrodes were 2, 6, and 9 mm from the medial one going laterally in a coronal plane. The single electrode on the left side was placed in the caudate nucleus. A silver-silver chloride reference electrode was placed 6 mm lateral to the fourth electrode on the left side. The electrodes were fixed to the skull with dental cement and the electrode heads were protected with a polyethylene ring. The right femoral artery and vein were exposed and catheterized with a #19 gauge catheter which was filled with heparin and buried in a subcutaneous pouch for future use.

Three days later the animals were again anesthetized in the same manner and then were paralyzed with curare and ventilated using a Harvard ventilator to maintain PCO2 about 30 torr. The arterial catheter was then connected to a transducer for constant monitoring of blood pressure. The right MCA was exposed by a slight modification of the technique described by

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O'Brien and Waltz. At this point the animals were divided into 2 groups of 12 cats. The first group of cats were given an intravenous infusion of Fluosol (20 ml/kg). All of these animals were then ventilated with 100% O2 (Group 1). The second group of 12 cats were infused with normal saline (20 ml/kg). This group was subdivided into 2 subgroups. The first of these subgroups of 6 cats was ventilated with 100% O2 (Group 2) and the second subgroup of 6 cats was ventilated with room air (Group 3).

Immediately after the infusion of either Fluosol or saline, O2a and rCBF studies, which had initially been obtained to give a baseline value, were repeated again. After these studies were completed, the MCA was occluded with a microaneurysm clip (Scoville). The orbital defect was then covered with gelfoam and closed temporarily. One hour after occlusion O2a and rCBF studies were conducted again. Two hours after placement of the clip reperfusion was established by removing the clip from the MCA. Flow was confirmed in all cases by direct observation under the microscope. One hour after removal of the clip O2a and rCBF studies were repeated. Arterial blood gases were drawn frequently during the experiment and at least once immediately before each rCBF determination. The ventilator was readjusted, if necessary, to insure a pCO2 of about 30 torr at the time of each rCBF determination.

After the last set of oxygen availability and blood flow studies was completed, the animals were allowed to awaken spontaneously and then were returned to their cages.

One week after temporary occlusion of the MCA the surviving animals were reanesthetized and then were perfused with 30 ml of normal saline and 30 ml of 2% tetrazolium chloride (TTC) at 30°C through both carotid arteries. The venous blood was allowed to drain freely through the severed jugular veins. The brains were then removed and cut immediately in the coronal plane at the level of the mammillary bodies and incubated in 2% TTC at 37°C for 30 minutes. The cut surfaces of the brain were then photographed to delineate the infarcted area as indicated by lack of staining with TTC. This indirect method of grossly demonstrating infarction has been studied extensively in our laboratory. The brains were then fixed with 10% phosphate buffered formalin and were saved for future histologic examination. Histologic examination was carried out after staining with Luxol fast blue and counter-staining with hematoxilin and eosin without knowledge of the experimental group to which the brain belonged. The infarcted area was delineated and mapped out on a sheet of paper after fixed magnification. The area of infarction was then measured by planimetry and was expressed as a percentage of the total coronal section of the brain at that level.

Regional cerebral blood flow was measured using hydrogen clearance technique. The electrodes were polarized at 0.36 volts and the reference electrode was placed subcutaneously in the neck. Hydrogen gas and air mixture (4 to 6%) was fed into the ventilator until all the electrodes displayed at least 0.2 volt deflections. Hydrogen inhalation was then discontinued and the hydrogen washout curves were recorded. After the electrodes' voltage returned to baseline values, rCBF at each electrode was calculated by computer. Only mono-exponential flow curves were used for analysis. Blood flow was measured before and after the infusion of either Fluosol or saline and then again after exposure to 100% O2 in those animals that were so treated. Blood flow determinations were then repeated 1 hour after arterial occlusion and then again 1 hour after reperfusion. For convenience in the interpretation of results, the rCBF in the right hemisphere was expressed as the average of the recordings in the 4 electrodes placed on that hemisphere.

To measure O2a the electrodes were polarized at −600 millivolts. At this voltage the electrode tip acts as an electron donor and in this situation oxygen is the chief recipient. The current flow through the electrode is directly proportional to oxygen concentration in the region and changes in oxygen delivery to the tissue are reflected as a change in the current at the electrode tip. Since there are inherent limitations in calibrating the electrodes in situ, the changes in O2a were measured as deviations from the baseline control which was obtained under normoxic conditions before any therapeutic manipulations.

Results

Baseline hematocrits, arterial blood gases and systemic blood pressure were comparable in all three groups. There were no changes after the infusion of Fluosol or saline except for an average fall in the hematocrit to 28 ± 5.2% and 31.5 ± 4.5% from 35.6 ± 4.2% and 34.8 ± 4.8% in the Fluosol and saline groups respectively. pO2 increased to an average of 629 ± 64 torr in the Fluosol group and 524 ± 62 torr in the saline group (Group 2) after ventilation with 100% O2.

Baseline rCBF values in the right hemisphere, when expressed as an average of the 4 electrodes in that hemisphere, were not significantly different in the 3 groups (fig. 1). However, there was substantial variability between the animals in each group and, as expected, flows were consistently higher in electrodes 1 and 4 (caudate nucleus and probable cortical grey respectively) than in electrodes 2 and 3 (deep white matter). After infusion of Fluosol or saline, average rCBF in the right hemisphere increased slightly in Groups 1 and 2 and decreased slightly in Group 3, but these changes did not reach statistical significance. With 100% O2 ventilation rCBF decreased slightly in Groups 1 and 2. One hour after MCA occlusion there was a significant fall in rCBF in all groups. With reperfusion there was a marked average rise in rCBF in all groups (fig. 1). However, the variability between animals was substantial and some animals had flow values below 20 ml/100 gm/minute whereas in others the flow was over 80 ml/100 gm/minute (fig. 2). The mean rCBF after reperfusion was not significantly different in the 3 groups (solid line, fig. 2).
Results of $O_2a$ studies in the right hemisphere are summarized in figure 3. In this figure the changes in voltage at each of the 4 electrodes in the right side are averaged for each animal and then for all animals in each group and are expressed as a percentage change from the average baseline voltage for the group. There was no significant change in the average $O_2a$ in any of the 3 groups with infusion of Fluosol or saline. With 100% oxygenation (Groups 1 and 2) there was a significant rise in $O_2a$ in the animals in Group 1 (Fluosol treated). With MCA occlusion $O_2a$ fell below baseline in all groups. With reperfusion $O_2a$ increased above the baseline in all groups probably reflecting the increase in rCBF with reperfusion (fig. 1). In Groups 1 and 2 (100% $O_2$) the increase in $O_2a$ over baseline was significant and it was particularly marked in Group 1 (Fluosol and 100% $O_2$). Figure 4 is a composite of the actual recordings in an animal in Group 1. The fluctuations in voltage, or "oxygen cycles" noted by other investigators are clearly shown. These cycles become damped in electrodes 1 to 3 as $O_2a$ decreases abruptly with MCA ligation and then increases abruptly with reperfusion (clip removal). This record is typical of Group 1. Of note is the fact that electrode 4 does not show a fall in $O_2a$ with occlusion and a rise with reperfusion. This is probably because in this cat this electrode (the most lateral) was outside the area of ischemia produced by MCA occlusion which indeed is confirmed by rCBF recordings from this electrode (no fall or rise in rCBF with MCA occlusion and reopening). This was the case in several animals which reflects the variability in collateral circulation in the cat's brain (i.e.: in some animals electrode 4 was clearly

[Figure 1: Average rCBF in the right hemisphere for each group at different times during the experiment. The rCBF value for each animal is given by the average of the values calculated for each of the four electrodes in the right hemisphere. There are no significant differences between the average values for each group at any time.]

[Figure 2: rCBF for each animal in each group after reperfusion. The mean for each group is indicated by the solid line. There is no significant difference between the mean value for each of the three groups.]

[Figure 3: Percentage change from baseline value in oxygen availability in the right hemisphere for each of the three groups at different times. The oxygen availability for each animal is given by the average value calculated for each of the four electrodes in the right hemisphere.]

[Figure 4: Composite of actual recordings of oxygen availability at the four electrodes in the right hemisphere in one of the animals in Group 1 (Fluosol + 100% $O_2$).]
within the area of ischemia and in others outside it or in the "border zone" area showing only minimal changes in rCBF with clip application and removal).

The area of infarction in each animal expressed as a percentage of the total cross-sectional area of a coronal slice through the mammillary bodies is shown in figure 5. The differences in average size of infarction between the 3 groups (solid line, fig. 5) did not reach statistical significance although there is a trend favoring the animals treated only with saline and 100% O₂ ventilation. Figure 5 indicates a relatively strong correlation between size of infarction and mortality. The differences in mortality between the 3 groups did not reach statistical significance although there is a trend favoring the animals treated only with saline and 100% O₂ ventilation. Figure 5 shows that there was some correlation between size of infarction and mortality and mean rCBF during the time of MCA occlusion. All but one of the animals that died had an average rCBF in the right hemisphere during the time of occlusion of less than 12 ml/100 gm/minute. The correlation between mortality and rCBF during reperfusion was not as strong (fig. 2).

Discussion
These results are consistent with our recent, as yet unpublished observations, comparing the effects of Fluosol and dextran in a similar chronic model of cerebral ischemia in cats. In that study the period of temporary MCA occlusion was 4 hours and mortality from massive infarction and edema was very high (66%) and was not significantly different in any group. We felt that with a lesser insult (2 hours of temporary occlusion) we might be able to demonstrate a beneficial effect of Fluosol as suggested by earlier acute studies.⁵,⁶ Even though mortality was indeed lower in the present study (33%), we could not demonstrate a benefit for Fluosol and indeed we found a trend indicating that Fluosol may have been harmful in this model. In this study, as in our previous one, the animals that died almost certainly died from cerebral herniation secondary to large edematous infarcts. All dead animals showed evidence of infarction in more than 20% of the total cross-sectional area of the coronal plane at the level of the mammillary bodies. The main difference between the present study and other studies which have suggested a beneficial effect for Fluosol,⁴,⁵,⁷,₁⁶,₁₉ is that in the present study the cats were allowed to survive for 1 week, whereas in the previously reported studies the animals were sacrificed within a maximum of 24 hours from the time of the onset of ischemia. It is possible that Fluosol indeed helps protect the brain during the early hours of ischemia but that it does not prevent delayed ischemic edema and secondary cerebral damage. It is well-known that it takes several hours for ischemic cerebral edema to develop.²⁰ Indeed, in the study by Peerless and collaborators in which the cats were kept alive for 24 hours, the beneficial effect of Fluosol was not as clear as in their earlier study⁵ or in our acute study⁶ where the animals were sacrificed within 6 hours from the onset of ischemia. It is also likely that reperfusion, even after only 2 hours of ischemia, exacerbated brain edema. The deleterious effect of reperfusion after 2 hours of ischemia was also suggested in another report by Peerless and co-workers²¹ and more recently in a
study by Latchaw et al using a model very similar to the one used in the present study.  

The oxygen availability studies showed that, as expected, $O_2a$ in normal brain increases significantly after Fluosol infusion when the animal is ventilated with 100% $O_2$ (fig. 3). When brain is rendered ischemic, however, the $O_2a$ is not different in animals ventilated with 100% $O_2$ whether they are treated with Fluosol or saline; in both groups the $O_2a$ fell below baseline values although it did not decrease as much as in saline treated animals that were ventilated only with room air (fig. 3). In this respect our results differ from those of Sutherland et al.  

They found that in Fluosol treated animals ventilated with 100% $O_2$, the $O_2a$ remained at values above baseline during the period of ischemia. In that study, as in ours, $O_2a$ rapidly rose substantially above baseline after reperfusion, particularly in the animals treated with Fluosol and ventilated with 100% $O_2$ (figs. 3, 4). This rise in $O_2a$ paralleled the rise in rCBF (hyperperfusion) noted after clip removal in the majority of our animals (fig. 1). Unfortunately, this postischemic hyperperfusion and markedly increased $O_2a$ did not consistently signify lack of infarction or guaranteed survival since, as seen in figure 2, over one-half of the animals that died from massive infarction had increased rCBF in the right hemisphere 1 hour after clip removal. Since we did not continue to measure rCBF chronically, we do not know whether later, with progressive brain edema, rCBF in the animals with large infarcts fell gradually to ischemic levels as one would expect.

In summary, in cats subjected to 2 hours of temporary occlusion of the right MCA and kept alive for 1 week after the ischemic period, Fluosol did not improve rCBF during the time of occlusion and did not protect the animals against infarction. Oxygen availability in the ischemic hemisphere during the period of occlusion was not different in animals ventilated with 100% $O_2$ whether they were treated with Fluosol or saline.

References

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