I

N RECENT years much interest has focused on
finding treatments that, when administered after
a period of complete cerebral ischemia, will de-
crease the incidence and severity of postischemic neu-
rologic deficits. Factors contributing to the ultimate
injury may include not only the period during which
cerebral blood flow (CBF) is absent but also the early
recirculation period when CBF is altered. In the clini-
cal setting, the immediate definitive treatment of an
ischemic event is to restore CBF. Thereafter, optimal
treatment during the recirculation period is not well
defined, thus accounting for much of the current re-
search. A number of phenomena have been observed
during the recirculation or postischemic period, some
or all of which may contribute to the ultimate neuro-
logic injury. These include 1) transient postischemic
reactive hyperemia followed by a prolonged period of
low flow (the delayed postischemic hypoperfusion
state), which has also been referred to as the no-reflow
phenomenon,1-3 2) decreased intracellular ATP with
resultant influx of calcium into neurons, 3) release of
free fatty acids (FFA) from phospholipids, and 4) in
vitro evidence suggesting that, with the hyperoxia oc-
curring during reperfusion, FFA may lead to the for-
mation of free radicals, with ensuing membrane de-
struction.4,5 It is this last observation that forms the
basis of our current study.

White et al6 and Babbs7 have postulated that forma-
tion of free radicals proceeds via iron-catalyzed re-
thons and that presence of an iron chelator during reper-
fusion following complete cerebral ischemia may
provide cerebral protection by blocking or slowing
such reactions. Kompala et al8 found improved surviv-
al in rats treated with deferoxamine, an iron chelator,
following 6 minutes of cardiac arrest. The current
study was designed to determine if deferoxamine ad-
mnistered intravenously prior to an episode of com-
plete cerebral ischemia would improve postischemic
neurologic outcome in an established dog model.

Materials and Methods

Seventeen unmedicated fasting adult mongrel dogs,
weighing 10-17 kg were studied. The protocol was
approved by the institutional Animal Care Committee.
Analgesia was induced and maintained with 70% ni-
trous oxide and oxygen. Succinylcholine 40 mg i.v.
was given to facilitate tracheal intubation. Ventilation
was controlled using a Harvard pump set to deliver a
tidal volume of 15-20 ml/kg and a rate adjusted to
maintain Paco2 between 35 and 40 mm Hg. Cannulae
were inserted percutaneously into a femoral artery for
blood pressure monitoring and blood sampling and
into a peripheral vein for fluid and drug administration.
After infiltration of the chest wall with 20 ml of pro-
caine 0.5%, a thoracotomy was performed in the right
fourth intercostal space, and umbilical tapes were
placed around the ascending aorta, inferior vena cava,
and superior vena cava above the azygos vein. An
electrocardiogram and a two-channel bifrontal, bipa-
rietial electroencephalogram (EEG) were recorded
using needle electrodes. Arterial blood gasses were
determined by electrodes at 37°C (Instrumentation
Laboratory 1303). Body temperature was maintained
at 36.5-37.5°C using heat lamps and blankets. End-
tidal CO2 was monitored with a mass spectrometer
(Perkin-Elmer 1100 Medical Gas Analyzer). Blood
glucose was determined by a membrane-bound en-
zyme technique (Yellow springs Instruments Model
23A Glucose Analyzer). All dogs received penicillin
600,000 units im and streptomycin 500 mg i.m. preis-
chemia.

Failure of Deferoxamine, an Iron Chelator, to
Improve Neurologic Outcome
Following Complete Cerebral Ischemia in Dogs

Jerry E. Fleischer, William L. Lanier, James H. Milde, and John D. Michenfelder

Eleven minutes of complete cerebral ischemia was produced in 17 dogs by temporary ligation of the
venae cavae and aorta. Immediately prior to the ischemic episode, 7 dogs received deferoxamine, an
iron chelator, 50 mg/kg i.v., and 10 dogs received an equivalent volume of saline placebo i.v. Five dogs
failed to meet preestablished protocol criteria and were excluded from data analysis. Neurologic
recovery was evaluated by an observer blind to the treatment groups in the remaining 12 dogs at 48
hours postischemia. The neurologic effects of complete cerebral ischemia were compared between
dogs treated with deferoxamine and those receiving placebo treatment. One of 6 deferoxamine-treated
dogs was normal and 5 were moderately to severely damaged. Similarly, 1 of 6 placebo-treated dogs
was normal and 5 were moderately to severely damaged. The authors conclude that deferoxamine
does not provide cerebral protection in this model of complete cerebral ischemia (Stroke
1987;18:124-127)

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Following the surgical preparation, control measurements of arterial blood gases, mean arterial pressure (MAP), heart rate (HR), temperature, and blood glucose were obtained. Seven dogs received deferoxamine 50 mg/kg i.v. diluted in saline solution to 6.4 ml, infused over 10–15 minutes titrated to maintain MAP> 60 mm Hg. In pilot studies, this drug dose and infusion rate were found to be well tolerated without changes in MAP or neurologic exam. One dog, having received deferoxamine 50 mg/kg during pilot studies was observed for 7 days and then entered into the deferoxamine-treatment group of the experimental protocol. Ten placebo-treated dogs received an i.v. infusion of saline solution of 6.4 ml. In all dogs, preischemic MAP was maintained > 60 mm Hg with i.v. infusions of normal saline and/or 25 μg boluses of epinephrine as needed. On completion of the deferoxamine or placebo infusions, arterial blood gases, MAP, HR, temperature, and blood glucose values were measured. The inspired gas was changed to 100% oxygen 5 minutes preischemia and to room air 20 minutes postischemia (provided Pao2 > 60 mm Hg). Complete cerebral ischemia of 11-minute duration was achieved by a method previously described. Briefly, umbilical tapes around the ascending aorta and venae cavae were simultaneously occluded, confining the cardiac output to the coronary and pulmonary circulation. After exactly 11 minutes, the tape around the aorta was released, the tapes around the venae cavae having been released 15–20 seconds earlier. This period of ischemia in pilot studies produced control animals that survived 48 hours postischemia, but were uniformly injured neurologically.

Immediate postischemic treatment consisted of sodium bicarbonate 20 mEq and normal saline 50 ml i.v. and transient hyperventilation. Epinephrine (25–50 μg boluses i.v.) was given as needed to obtain a MAP > 60 mm Hg within 1 minute postischemia. The thoracotomy was closed, and residual air was aspirated through a chest tube, which was later removed. Arterial blood gasses were measured at 5 and 20 minutes postischemia and as needed thereafter. Mechanical ventilation was continued until spontaneous ventilation maintained the Paco2 < 45 mm Hg. The dogs were then extubated and observed. Arterial blood gasses were measured at 15–30 minutes postextubation and dogs were then returned to their cages. At 24 hours postischemia, dogs received water p.o. ad lib and/or an i.v. infusion of dextrose 5% in lactated Ringer’s solution (200 ml) as determined by physical exam.

Forty-eight hours postischemia, the dogs were evaluated neurologically and assigned to one of four categories by an observer blinded to the treatment groups. Grade 1 (no damage) dogs ate and behaved normally with coordinated movements. Grade 2 (moderate damage) dogs could stand alone but were ataxic or exhibited partial to complete blindness. Grade 3 (severe damage) dogs could not stand alone or were comatose. Grade 4 (dead) dogs died within 48 hours postischemia. The surviving dogs were killed after the 48-hour evaluation, and at necropsy the thorax was examined to assess the presence of pulmonary injury that may have affected the neurologic outcome.

For statistical comparison of physiologic variables between deferoxamine-treated and placebo-treated dogs, Student’s test for unpaired data was employed. For comparison of control, postdeferoxamine, and time of extubation variables, Bonferroni’s correction of the paired t test was employed. Statistical comparison of neurologic outcome between deferoxamine-treated and placebo-treated dogs was made using the Fisher exact test. Data are presented as mean ± SEM.

Results

Five dogs were excluded from the data analysis prior to final neurologic evaluation by the blinded observer for failure to meet the preestablished protocol criteria. In the deferoxamine-treated group 1 dog was excluded for hypotension in the preischemic period. In the placebo-treated group, 3 dogs were excluded for hypoxemia postischemia (Paco2 < 60 mm Hg) and 1 dog was excluded when found to have a pneumothorax at 8 hours postischemia. Of the 12 remaining dogs, the functional neurologic status at 48 hours postischemia was similar in both groups (Table 1). One dog in each group was judged normal, 5 dogs in each group had moderate or severe deficits, and no dogs were dead at 48 hours. The mean arterial pressures and arterial blood gasses taken at control, preischemia, and at extubation are presented in Table 2. There were no statistically significant differences between groups except for a decrease in mean arterial pressure, pH, and buffer base following deferoxamine infusion. These differences were not apparent at extubation. There were no statistically significant differences between treatment groups in the time to isoelectric EEG following aortic occlusion, the time to extubation postischemia (Table 3), or preischemia glucose values. One dog in the deferoxamine-treatment group required boluses of normal saline and epinephrine totaling 180 ml and 150 μg, respectively, to maintain MAP > 60 mm Hg preischemia. In the immediate postischemic period 1 dog from the deferoxamine-treated group and 1 from the placebo-treated group required boluses of epinephrine, 50 and 300 μg, respectively, to maintain MAP > 60 mm Hg. The dog entered into the study protocol after serving in the pilot study did not differ from other dogs in the deferoxamine-treated group and was scored a Grade 2 at 48 hours postischemia.

Table 1. Grade of Neurologic Damage at 48 Hours Postischemia

<table>
<thead>
<tr>
<th>Postischemia</th>
<th>Grade of neurologic damage*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
</tr>
<tr>
<td>Deferoxamine</td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>6</td>
</tr>
<tr>
<td>Deferoxamine</td>
<td>6</td>
</tr>
</tbody>
</table>

*No statistically significant differences were observed between placebo and deferoxamine treated groups.
The role of free radicals in postischemic brain injury remains unclear. White et al.6 and Babbs7 have recently suggested that during ischemia, increased intracellular levels of ferrous iron (released from ferritin, mitochondrial cytochromes, and iron-containing enzymes) may chelate with accumulated ADP and, during reoxygenation, and iron-containing enzymes) may react with superoxide radicals, yielding reactive hydroxyl radicals from superoxide radicals, with subsequent lipid peroxidation and cellular injury. They further hypothesize that deferoxamine, a selective chelator of ferric iron, which has been shown to cross the blood–brain barrier,12 may prevent or ameliorate postischemic neurologic injury. Kompala et al.8 have reported the effects of deferoxamine, 50 mg/kg, administered preischemia on subsequent neurologic outcome following an 11-minute period of complete cerebral ischemia. We found that dogs receiving deferoxamine developed a transient decrease in MAP and a slight metabolic acidosis, most likely secondary to the histamine releasing properties of deferoxamine.17 More importantly, we found no difference in neurologic outcome at 48 hours postischemia between the deferoxamine-treated and the placebo-treated groups.

### Discussion

Using a well established canine model3,14-16 we have evaluated the effects of deferoxamine administered preischemia on subsequent neurologic outcome following an 11-minute period of complete cerebral ischemia. We found that dogs receiving deferoxamine showed no statistically significant difference in neurologic outcome when evaluated at 48 hours postischemia. It appears that using this model of complete cerebral ischemia, deferoxamine administered preischemia does not provide cerebral protection.

### References


### Table 2. Mean Arterial Pressure (MAP), Arterial Blood Gases, and Blood Glucose at Control, Preischemia (After Deferoxamine or Placebo), and at Extubation (Mean ± SEM)

<table>
<thead>
<tr>
<th>State</th>
<th>n</th>
<th>MAP   (mm Hg)</th>
<th>PaO₂ (mm Hg)</th>
<th>PaCO₂ (mm Hg)</th>
<th>pH</th>
<th>Buffer base (mEq/l)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>157 ± 8</td>
<td>140 ± 6*</td>
<td>39 ± 1</td>
<td>7.32 ± 0.02</td>
<td>41 ± 1</td>
<td>97 ± 9</td>
</tr>
<tr>
<td>Placebo</td>
<td>6</td>
<td>159 ± 6</td>
<td>145 ± 10*</td>
<td>40 ± 1</td>
<td>7.32 ± 0.02</td>
<td>41 ± 1</td>
<td>106 ± 12</td>
</tr>
<tr>
<td>Deferoxamine</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia</td>
<td></td>
<td>149 ± 3</td>
<td>408 ± 20†</td>
<td>37 ± 2</td>
<td>7.33 ± 0.02</td>
<td>41 ± 1</td>
<td>97 ± 11</td>
</tr>
<tr>
<td>Placebo</td>
<td>6</td>
<td>114 ± 16†</td>
<td>400 ± 15†</td>
<td>38 ± 1</td>
<td>7.28 ± 0.02§</td>
<td>38 ± 15,</td>
<td></td>
</tr>
<tr>
<td>Deferoxamine</td>
<td>6</td>
<td>&gt; 70</td>
<td>86 ± 3§</td>
<td>37 ± 2</td>
<td>7.38 ± 0.02</td>
<td>43 ± 1</td>
<td></td>
</tr>
<tr>
<td>Extubation</td>
<td></td>
<td>70</td>
<td>79 ± 4§</td>
<td>35 ± 3</td>
<td>7.36 ± 0.04</td>
<td>41 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

*FiO₂ = 0.3.
†FiO₂ = 1.0.
§p < 0.05 vs. control.
¶p < 0.025 vs. control.
\[p < 0.05 vs. placebo.

### Table 3. Time to Isoelectric EEG and Time to Extubation in Deferoxamine- and Placebo-Treated Dogs (Mean ± SEM)

<table>
<thead>
<tr>
<th>Time</th>
<th>Placebo (n = 6)</th>
<th>Deferoxamine (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEG flat (seconds)</td>
<td>52 ± 8</td>
<td>34 ± 4</td>
</tr>
<tr>
<td>Extubation (minutes)</td>
<td>89 ± 9</td>
<td>103 ± 9</td>
</tr>
</tbody>
</table>
15. Steen PA, Newberg LA, Milde JH, Michenfelder JD: Cerebral blood flow and neurologic outcome when nimodipine is given after complete cerebral ischemia in the dog. J Cereb Blood Flow Metab 1984;4:82-87

Key Words • cerebral ischemia • deferoxamine
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