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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N RECENT years much interest has focused on finding treatments that, when administered after a period of complete cerebral ischemia, will decrease the incidence and severity of postischemic neurologic 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 clinical 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 research. A number of phenomena have been observed during the recirculation or postischemic period, some or all of which may contribute to the ultimate neurologic injury. These include 1) transient postischemic reactive hyperemia followed by a prolonged period of low flow (the delayed postischemic hyperperfusion 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 occurring during reperfusion, FFA may lead to the formation of free radicals, with ensuing membrane destruction.4,5 It is this last observation that forms the basis of our current study.

White et al6 and Babbs7 have postulated that formation of free radicals proceeds via iron-catalyzed reactions and that presence of an iron chelator during reperfusion following complete cerebral ischemia may provide cerebral protection by blocking or slowing such reactions. Kompala et al8 found improved survival in rats treated with deferoxamine, an iron chelator, following 6 minutes of cardiac arrest. The current study was designed to determine if deferoxamine administered intravenously prior to an episode of complete 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% nitrous 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 Paco 2 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 procaine 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, biparietal 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 CO 2 was monitored with a mass spectrometer (Perkin-Elmer 1100 Medical Gas Analyzer). Blood glucose was determined by a membrane-bound enzyme technique (Yellowsprings Instruments Model 23A Glucose Analyzer). All dogs received penicillin 600,000 units im and streptomycin 500 mg i.m. preischemia.
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 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 t-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 (PaO₂ < 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>n 1 (None) 2 (Moderate) 3 (Severe) 4 (Dead)</td>
</tr>
<tr>
<td>Placebo</td>
<td>6 1 3 2 —</td>
</tr>
<tr>
<td>Deferoxamine</td>
<td>6 1 4 1 —</td>
</tr>
</tbody>
</table>

*No statistically significant differences were observed between placebo and deferoxamine treated groups.
They further hypothesize that deferoxamine, a selective chelate with accumulated ADP and, during reoxygenation and reperfusion, may catalyze formation of highly 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 develop a transient decrease in MAP and a slight metabolic acidosis, most likely secondary to the histamine releasing properties of deferoxamine. 

Using a well established canine model, 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 developed a transient decrease in MAP and a slight metabolic acidosis, most likely secondary to the histamine releasing properties of deferoxamine. More importantly, we found no difference in neurologic outcome at 48 hours postischemia between the deferoxamine-treated and the placebo-treated groups.

In summary: Dogs treated with either deferoxamine (an iron chelator) or saline prior to 11 minutes of complete cerebral ischemia 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.

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

<table>
<thead>
<tr>
<th>State</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></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</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>Deferoxamine</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>Preischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</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>Deferoxamine</td>
<td>114 ± 16†</td>
<td>400 ± 15†</td>
<td>38 ± 1</td>
<td>7.28 ± 0.02</td>
<td></td>
<td>38 ± 15</td>
</tr>
<tr>
<td>Extubation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</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>Deferoxamine</td>
<td>&gt; 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.
¶FiO2 = 0.21.

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>

Discussion

The role of free radicals in postischemic brain injury remains unclear. White et al and Babbs 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 reperfusion, may catalyze formation of highly 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, may prevent or ameliorate postischemic neurologic injury. Kompala et al have reported the effects of deferoxamine, 50 mg/kg, in rats when given following resuscitation from 6 minutes of induced cardiac arrest. Survival was similar in both deferoxamine-treated and untreated rats up to 48 hours postischemia. At the end of 10 days there was 64% survival in the deferoxamine-treated rats vs. 36% survival in the untreated rats (p < 0.05). Neurologic examination was normal in all rats at 10 days postischemia. Badyylak and Babbs in a similar protocol in rats using 7 minutes of induced cardiac arrest followed by concomitant treatment with deferoxamine 50 mg/kg, lidoflazine (a calcium entry blocker) 2 mg/kg, and CO2 7% inspired reported that 75% of treated and 25% of untreated rats were alive at 48 hours postischemia (p < 0.01) and that at 10 days postischemia 60% of treated and 25% of untreated rats were alive (p < 0.05). In addition, at 15 days postischemia no neurologic deficits were noted in any survivors.

References

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Key Words • cerebral ischemia • deferoxamine
Failure of deferoxamine, an iron chelator, to improve neurologic outcome following complete cerebral ischemia in dogs.
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Stroke. 1987;18:124-127
doi: 10.1161/01.STR.18.1.124

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