Deferoxamine Posttreatment Reduces Ischemic Brain Injury in Neonatal Rats

Charles Palmer, MB, ChB; Rebecca L. Roberts, BA; Christopher Bero, PharmD

**Background and Purpose** Iron catalyzes the formation of damaging reactive species during cerebral reperfusion. Brain iron concentration is highest at birth, so the brain of the asphyxiated newborn may be at increased risk of iron-dependent injury. We investigated whether the ferric iron chelator deferoxamine could reduce hypoxic-ischemic brain injury in neonatal rats. Because deferoxamine has concentration-dependent activities other than iron chelation, we measured brain deferoxamine levels and calculated deferoxamine pharmacokinetic parameters.

**Methods** We produced hypoxic-ischemic injury to the right cerebral hemisphere of 7-day-old rats by right common carotid artery ligation followed by 2.25 hours of hypoxia in 8% oxygen. At 5 minutes of recovery from hypoxia the rats received 100 mg/kg deferoxamine mesylate or saline subcutaneously. Rats (saline, n=33; deferoxamine, n=38) were killed at 42 hours of recovery to assess early acute edema by measurement of hemispheric water content. Other rats (saline, n=31; deferoxamine, n=32) were killed at 30 days of age for morphometric determination of right hemisphere atrophy. In still other rats, we measured deferoxamine levels in blood and brain after hypoxia-ischemia.

**Results** Deferoxamine significantly reduced right hemisphere injury as measured by early water content (P<.01) and later atrophy (P=.019). Deferoxamine brain levels peaked between 100 and 200 μmol/L at 40 to 60 minutes after injection and exceeded serum levels by ±70%.

**Conclusions** Deferoxamine administered after induction of cerebral hypoxia-ischemia reduces injury in 7-day-old rats. Deferoxamine concentrates in the brain at levels between 100 and 200 μmol/L. At the concentrations achieved, deferoxamine might protect the brain through mechanisms unrelated to its ability to chelate iron. (*Stroke. 1994;25:1039-1045.)*

**Key Words** cerebral ischemia • deferoxamine • neuroprotection • free radicals • pharmacokinetics

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Free radicals contribute to hypoxic-ischemic brain injury. Superoxide and hydrogen peroxide are the first reactive oxygen species generated when the ischemic brain is reperfused with oxygenated blood. They are poorly reactive in aqueous solutions and do not cause tissue damage on their own. However, in the presence of transition metals like iron, they are converted into more damaging species such as the hydroxyl radical and other reactive oxygen complexes that can stimulate peroxidation of brain lipid.

Recently, Beckman et al9,10 showed that superoxide reacts rapidly with nitric oxide to form the potentially damaging peroxynitrite anion. Because nitric oxide and superoxide are both produced during cerebral reperfusion, the potential for peroxynitrite formation is greatly enhanced. In addition, iron contributes to peroxynitrite toxicity by enhancing its cleavage into a powerful nitrating agent. Accordingly, iron may potentiate reperfusion injury by catalyzing the generation of more damaging free radicals from superoxide and nitric oxide.

Brain, with its high lipid content, is most susceptible to peroxidative damage, and the degree of peroxidation correlates directly with regional iron concentration. This bears special relevance to the newborn as brain iron concentration is highest at birth.12,13 When iron is injected directly into the brain in the form of ferric chloride, heme, or hemoglobin, it induces lipid peroxidation and inhibition of the membrane-bound enzyme Na-K adenosine triphosphatase. Accordingly, iron chelation may reduce reperfusion or hemorrhagic brain injury.

Deferoxamine is an iron chelator in clinical use that has a very high affinity for ferric iron. Gutteridge et al20 have shown that deferoxamine binds ferric iron tightly and inhibits hydroxyl radical formation. Deferoxamine can prevent the inhibition of Na-K adenosine triphosphatase caused by intracerebral injection of iron-rich hemoglobin.

In the preterm newborn, intracranial hemorrhage is a major cause of neurological handicap. Therefore, it is particularly pertinent that hemoglobin-derived iron can catalyze damaging redox reactions. As it is important to minimize ischemic and hemorrhagic brain injury to the neonatal brain, we used a model of hypoxic-ischemic brain injury in the immature rat to determine if deferoxamine could reduce reperfusion injury.

The first objective of this study was to determine if deferoxamine administered by subcutaneous injection 5 minutes after a hypoxic-ischemic insult to 7-day-old rats could reduce brain injury. The second objective was to measure deferoxamine brain concentrations and determine deferoxamine pharmacokinetic parameters following subcutaneous injection. Brain levels are important because in vitro studies indicate that deferoxamine has other potential neuroprotective actions. These mechanisms are dose dependent and unrelated to the ability...
of deferoxamine to chelate iron (reviewed in References 25 and 26). The study found that deferoxamine quickly accumulates in the brain and that deferoxamine posttreatment reduces hypoxic-ischemic brain injury in neonatal rats.

Materials and Methods

Seven-day-old Wistar rats (Charles River) rats of either sex (n = 158) were anesthetized with a mixture of halothane (4%) halothane for induction, 1% to 1.5% for maintenance) and 30% oxygen, with the balance nitrogen oxide. The right common carotid artery of each rat was permanently ligated with 4-0 surgical silk through a midline neck incision. The wound was sutured, and the animals were allowed to recover with their dams in room air for 3 hours. The duration of anesthesia was generally less than 5 minutes.

Three hours after ligation, four rats were placed in each of six 400-mL airtight jars and exposed to a continuous flow of 8% oxygen–92% nitrogen gas mixture as described previously.29 The jars were partially submerged in a circulating water bath maintained at 36.8°C to provide a stable thermal environment. Air temperature in the middle of the jars was maintained between 33°C and 34°C. After 2.25 hours of hypoxia, the jars were opened to room air, and the rats were returned to their dams. At 5 minutes of recovery in room air, rats were randomly selected to receive either 100 mg/kg SC deferoxamine mesylate (Sigma) or an equal volume of saline.

In the acute injury protocol, rats (n = 71) were killed at 42 hours of recovery for measurement of water content in both cerebral hemispheres. In a separate protocol, rats (n = 63) were killed at 30 days of age for determination of right hemispheric atrophy. This combination of carotid ligation and hypoxia is known to produce cerebral edema and tissue injury ranging from selective neuronal necrosis to infarction of the right hemisphere ipsilateral to ligation.23-24 All animal procedures were in accordance with institutional guidelines.

Acute injury in the rats killed at 42 hours of recovery was assessed by harvesting portions (150 to 200 mg) of the posterolateral section of each hemisphere and comparing percentage of water content of the right hemisphere. Water content was determined from the difference between the total weight and the dry weight after dessication at 70°C for 72 hours. We expressed the water content as a percentage of the total weight.

Long-term neuropathological injury was assessed by examining the brains of rats at 30 days of age (23 days of recovery). Treated (n = 45) versus control (n = 42) groups were identified by ear clipping. This procedure caused minimal bleeding. The rats were killed with pentobarbital (150 mg/kg IP), and the brains were carefully removed from the skull and immersion fixed in a mixture of formaldehyde, acetic acid, and methanol, 1:1:8 vol/vol. At 23 days of recovery, the injured right hemispheres appeared shrunken and atrophic; the more severely damaged had cavitory lesions.

Right hemisphere atrophy was estimated by measuring the diameter of each hemisphere and calculating the ratio of the two measurements. Specifically, a 2-mm coronal slice was cut at the level of the midmamillary body, and the diameter of each cerebral hemisphere was measured with a micrometer under a dissecting microscope from the midpoint of a straight line connecting the base of the interhemispheric fissure to the midmamillary body. This coronal level was chosen because it represented an easily identifiable region that was consistently damaged. The ratio of the hemispheres (right divided by left) was calculated and expressed as a percentage. We measured the range (mean±2 SD) of interhemisphere asymmetry in 15 normal 30-day-old rats that had not been subjected to carotid ligation or hypoxia. In previous studies we have shown that the interhemisphere ratio correlates well with microscopic injury.23 We have also found that the left hemisphere size remains normal even when the right hemisphere is severely damaged (C.P., R.L.R., unpublished data, 1993).

Drug-induced hypothermia can be protective after hypoxia-ischemia, so we measured the effect of deferoxamine on core body temperature. Seven-day-old rats (n = 12) were positioned in plastic syringe barrels housed within a neonatal incubator set at 33.5°C. This temperature was chosen because we found that it approximated the rat’s core temperature while nesting. The rats were fitted with a rectal temperature probe (511, Yellow Springs Instrument Co); after a 75-minute equilibration period, two temperature recordings were obtained over a 15-minute interval and averaged (preadrugs temperature). The rats were then injected with the experimental dose of saline (n = 6) or deferoxamine (n = 6), and after 75 minutes of equilibration, three temperature measurements were obtained over 30 minutes and averaged (postdrug temperature). The effect of the drug on body temperature was determined by the postdrug-to-predrug temperature difference.

We measured deferoxamine levels in blood and brain spectrophotometrically by combining it with excess iron to form ferroferric acid. Fresh brain samples were homogenized 2:1 wt/vol in 0.1 mol/L phosphate buffer (pH 7.4), then centrifuged at 14,000g for 15 minutes. The supernatant was deproteinized with 10% vol/vol of 30% perchloric acid and centrifuged. Blood samples were collected from severed neck veins, and serum was separated by centrifugation. The serum was deproteinized in 10% vol/vol of 30% perchloric acid and 100 μL supernatant mixed with 200 μL distilled water. We mixed 300 μL of the serum or brain samples with 1800 μL freshly made 1 mmol/L ferric-nitrate-nitriloacetic acid in 50 mmol/L sodium acetate buffer (pH 5.0). After 30 minutes of incubation at room temperature, we measured absorbance at 429 nm. Absorbances were corrected for serum or brain blanks where appropriate, and concentration was determined from a regression line derived from deferoxamine standards. To verify this assay for brain tissue, we added known amounts of deferoxamine (20 to 100 μmol/L) to rat brain homogenates. We measured 91.8±5.6% (mean±SD) of the deferoxamine added to the brain homogenate.

We measured deferoxamine levels in the blood and both cerebral hemispheres of 7-day-old rats that had been exposed to hypoxia-ischemia as described above. At 5 minutes of recovery from hypoxia, the rats were injected with 100 mg/kg SC deferoxamine mesylate and decapitated at intervals up to 210 minutes later. We collected free-flowing blood from the severed neck veins and then removed the brain from the skull. The left and right cerebral hemispheres were separated, and a portion from the posterolateral aspect of each hemisphere was sampled. We also measured deferoxamine levels in a separate group of normal 7-day-old rats (n = 6) for comparison with the posthypoxic-ischemic group.

Plasma and brain deferoxamine concentrations versus time were analyzed using model-independent analysis (see the Table). The terminal elimination rate constant (κt) was estimated from the slope of the best-fitting regression line of the terminal portion of the plasma and brain concentration versus time curve (R²=.89 for plasma and .94 for brain). Area under the curve (AUC) from time zero to the last sampling period (time t) was calculated using the linear trapezoidal rule. The remaining area was calculated as AUC(t-∞)=C∞/κt (where C∞ equals the concentration at the last sampling period). Total AUC(0–t) was determined by summation. Area under the first moment curve, clearance, apparent volume of distribution, distribution coefficient, and mean residence time were calculated according to standard techniques.7,29

Results

Left hemisphere (contralateral to carotid ligation) water content was 87.55±0.32% (mean±SD) for the 33
### Serum Deferoxamine Pharmacokinetics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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</thead>
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<tr>
<td>$\alpha$</td>
<td>0.00683 min$^{-1}$</td>
</tr>
<tr>
<td>AUC(0–∞)</td>
<td>17,052.5 μmol/L per min</td>
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<tr>
<td>AUMC(0–∞)</td>
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<td>MRT</td>
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<tr>
<td>Cl</td>
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<tr>
<td>Vz</td>
<td>21.2 mL</td>
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<tr>
<td>$\Delta'$</td>
<td>1.41 L/kg</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>109.7 min</td>
</tr>
</tbody>
</table>

$\alpha$ indicates terminal serum elimination rate; AUC(0–∞), area under the curve to infinity; AUMC(0–∞), area under the first moment curve to infinity; MRT, mean residence time (time it takes to eliminate 63.2% of drug from the body); Cl, clearance; Vz, volume of distribution; $\Delta'$, distribution coefficient; and $t_{1/2}$, serum half-life.

Saline-treated and 87.57±0.26% for the 38 deferoxamine-treated rats. These results are not different and fall within the normal range as previously determined. The left hemisphere water contents were combined to form a normal reference range of 86.75% to 88.25% (mean±2 SD) against which the distribution of water content in the edematous right hemisphere is illustrated in Fig. 1. The right hemisphere (ipsilateral to ligation) water content of the 33 saline-treated rats was distributed bimodally, 12 of the 33 (36%) had a water content <88.5%, and 15 of the 33 (45.5%) had a water content >91%. In contrast, in the deferoxamine-treated rats the water content was <88.5% in 25 of 38 (66%) and was >91% in only 4 of 38 (10.5%). Water content was significantly less in the deferoxamine-treated rats ($P<.01$, Mann-Whitney $U$ test).

The ratio of the right-to-left hemisphere diameter for 15 normal 30-day-old rats (not subjected to carotid ligation or hypoxia) was 98.03±2.44% (mean±SD). Therefore, the normal range for hemisphere asymmetry that would include roughly 95% of normal brains is 97.14% to 102.93% (mean±2 SD). Accordingly, a ratio <97.14% can be regarded as abnormal and indicative of right hemisphere atrophy. The normal range (mean±2 SD) is indicated by the striped area in Fig 2, which shows the distribution of right hemisphere atrophy for the 63 experimental animals.

The distribution of right hemisphere atrophy was bimodal. This was especially evident in the 31 saline-treated rats, of which 8 (25%) had a ratio <50%. The severely atrophic brains had cavitary lesions, the sequelae of cystic infarction. In contrast, all the deferoxamine-treated rats had a ratio >50%. Eight of the 32 (25%) deferoxamine-treated rats were normal compared with only 3 of 31 (10%) saline-treated. Right hemisphere atrophy (and cavitation) was less in the deferoxamine-treated rats ($P=.019$, Mann-Whitney $U$ test).

None of the 71 rats treated from either treatment group died in the 42-hour short-term recovery to determine brain water content. In contrast, deaths occurred during the long-term recovery study to measure brain atrophy. More specifically, of the 87 rats that were

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**Fig 1.** Bar graph showing right hemispheric water content at 42 hours of recovery after hypoxia-ischemia. The normal range (mean±2 SD) for cerebral water content is illustrated. Rats were treated at 5 minutes of recovery after hypoxic-ischemic insult with 100 mg/kg SC deferoxamine mesylate or saline. The deferoxamine-treated rats had lower water contents than the saline-treated rats. $P<.01$ (Mann-Whitney $U$ test).

**Fig 2.** Bar graph showing right hemispheric atrophy determined morphometrically by the right-to-left (R/L) hemisphere diameter ratio. The normal range (mean±2 SD) of hemisphere asymmetry is illustrated by the striped area. Rats were treated at 5 minutes after hypoxia-ischemia with 100 mg/kg SC deferoxamine mesylate or saline. The deferoxamine-treated rats were less damaged than the saline-treated rats. $P=.019$ (Mann-Whitney $U$ test).
Deferoxamine values peaked in the serum 40 minutes after subcutaneous injection at 106±29 μmol/L (mean±SD). Thereafter, the levels declined with a half-life of 109.7 minutes. Brain levels were consistently higher than serum levels at all time points measured from 2 to 210 minutes after injection. Brain levels peaked between 40 and 60 minutes after injection at 192±23 μmol/L in the left (contralateral) hemisphere and 170±70 μmol/L in the right (ipsilateral) hemisphere. The differences between hemispheres in the post–hypoxic-ischemic rats were not significant. Six normal 7-day-old rats (not subjected to hypoxia or ischemia) were injected with 100 mg/kg SC deferoxamine. Their brain deferoxamine levels were 117±30.8 μmol/L at 60 minutes after injection. These levels are significantly lower than those we found in either hemisphere of the rats after hypoxia-ischemia (P<.001, unpaired t test). Although not identical, the elimination rate constants for deferoxamine obtained from blood (0.0068 min⁻¹) and brain (0.0078 min⁻¹) were similar.

Discussion

This study showed that hypoxic-ischemic brain injury in 7-day-old rats can be reduced by deferoxamine administered 5 minutes after the hypoxic-ischemic insult. The study showed that deferoxamine (100 mg/kg SC) rapidly accumulates in the brain and reaches levels between 100 and 200 μmol/L within the first hour. Deferoxamine reduced brain water content measured at 42 hours of recovery from hypoxia-ischemia (Fig 1). It also reduced right hemisphere atrophy and cavitation at 23 days of recovery (Fig 2). Deferoxamine did not change the water content of the contralateral (left) hemisphere, indicating that it does not have a nonspecific dehydrating effect. Evident from Figs 1 and 2 is a bimodal distribution of injury, especially in the saline-treated rats. This bimodal distribution of damage is consistent with an earlier study in which we used the identical model of injury to show that allopurinol administered after hypoxia-ischemia reduces brain injury. During the 23 days of recovery in that study, only 1 saline-treated rat died of the 64 treated with allopurinol or saline. In contrast, during the present study 24 of 87 rats died. That deferoxamine did not influence mortality is suggested by the similar incidence in mortality in the control group. Mortality during the long-term recovery study may have been unrelated to the hypoxic-ischemic insult because none of the 71 rats in the short-term 42-hour recovery study died, and in our previously reported study mortality was uncommon.

It is possible that deferoxamine may protect against the more severe mode of injury more effectively than against the mild injury; we found in a preliminary study that deferoxamine offered no protection when the injury was less severe than achieved in this study. In the former study, rats were subjected to the same hypoxic-ischemic insult as described earlier. Twenty-two rats were treated with 100 mg/kg SC deferoxamine at 5 minutes of recovery from hypoxia, and 26 received saline. Although we aimed to obtain a more severe injury, we obtained very little atrophy in either treatment group. Specifically, 10 of 26 (39%) of the saline-treated rats were not damaged, and only 1 of 26 had a right-to-left hemisphere diameter ratio <50%. Consequently, in that study we did not show that deferoxamine reduced brain atrophy (P=.44). We attributed the mild degree of atrophy to temperature instability during hypoxia. When the study was repeated with a new circulating water bath that did not allow water temperature to fall below 36.8±0.2°C during hypoxia, more severe injury was attained, and we were able to demonstrate neuroprotection with deferoxamine.

In earlier animal models of cerebral ischemia, deferoxamine has shown mixed results. In a rat cardiac arrest model, deferoxamine administered during early resuscitation increased long-term survival. In a canine cardiac arrest model, deferoxamine administered immediately after resuscitation reduced lipid peroxidation and limited the levels of low-molecular-weight iron as compared with the untreated controls. In another canine model of brain ischemia, pretreatment with
Deferoxamine (50 mg/kg) did not improve neurological outcome.23 Patt et al34 reduced ischemic brain edema in gerbils by dietary brain iron depletion or by deferoxamine pretreatment but not with the iron-saturated form of deferoxamine (ferrioxamine).

For deferoxamine to be neuroprotective it must have access to its intracellular or extracellular targets. Yet there are reasons that deferoxamine does not penetrate brain cells easily and that it is taken up only by cells undergoing active pinocytosis.35,36 Brain levels are not usually reported, apart from a single study nearly 20 years ago.19 How deferoxamine penetrates the injured, immature blood-brain barrier is unknown.

The present study shows that deferoxamine enters the brain of the 7-day-old rat. This is confirmed by finding that brain deferoxamine levels consistently exceed the serum levels. The relatively large volume of distribution, 21.1 mL/Δ (1.4 L/kg) for the 7-day-old rats (average weight, 12.94 g), indicates that deferoxamine distributes from blood to the brain and perhaps other tissues. This volume of distribution in newborn rats is higher than the 50% of body weight in the neonate, but not the same for dogs.19 Although this study was performed on rats after hypoxia-ischemia, we have also shown that deferoxamine crosses the blood-brain barrier of the normal 7-day-old rat. The brain levels attained in normal rats were, however, only 65% of the levels achieved in rats after hypoxia-ischemia. Deferoxamine levels were the same in both hemispheres of the post-hypoxic-ischemic rats, although only the right (ipsilateral) hemisphere was permanently injured in this model. The left hemisphere underwent temporary biochemical alterations.39

The concentration of iron ions available in biological fluids to stimulate free radical reactions is considered to be very small even when acidosis or oxidant stress is severe. According to Halliwell,25 it is rarely greater than 5 μmol/L. Equimolar concentrations of deferoxamine can inhibit radical reactions, so deferoxamine brain levels greater than 10 μmol/L should have been sufficient to chelate adequately and thwart iron's availability for free radical reactions. We found that deferoxamine brain levels exceeded 10 μmol/L from as early as 2 minutes after subcutaneous injection. Using the calculated elimination rate, we extrapolated that it remained above 10 μmol/L for 6.8 hours. If 5 μmol/L is an adequate chelator concentration, then chelator levels were present in the rat brain for an additional 110 minutes, a total of 8 hours after subcutaneous injection. Because deferoxamine is an inhibitor of iron-dependent free radical reactions, it has been used to "probe" the role of iron in several animal models of reperfusion injury and trauma. Yet in vitro deferoxamine is not only an iron chelator; it has other concentration-dependent actions that need to be considered before attributing neuroprotection with deferoxamine to iron chelation alone. For instance, both deferoxamine and iron-saturated deferoxamine (ferrioxamine) scavenge superoxide37 and hydroxyl radicals.26 But, as the brain and serum levels in our study do not exceed the 0.5 to 1 mmol/L levels required to be an effective scavenger, this mechanism is unlikely to be responsible for its protective effect.

In a recent study both deferoxamine and ferrioxamine reduced cold-induced edema in mice.38 The dose was calculated to reach a concentration of 0.85 mmol/L assuming even distribution in total body water. However, our study showed that deferoxamine was not evenly distributed in total body water but concentrated in the brain at levels almost 70% higher than in the blood. This confirms the earlier report by Keberle,19 who found that deferoxamine brain levels exceeded serum levels in dogs 5 hours after administration. Therefore, assuming even distribution of deferoxamine in total body water will lead to underestimation of brain levels. This is important to investigators using deferoxamine in vivo who wish to maintain levels below the hydroxyl radical scavenging levels of 0.5 to 1 mmol/L. In addition, at levels above 0.5 mmol/L in vitro, deferoxamine may stimulate cyclooxygenase activity, causing an increase in prostacyclin production.39

At the 100 to 200 μmol/L brain levels in our study, deferoxamine could have inhibited peroxynitrite toxicity.39,40 It could have achieved this first by preventing metal-catalyzed nitration by peroxynitrite51 and second by direct interaction with peroxynitrite, preventing its cleavage into a hydroxyl-like radical and another potent oxidizing agent, nitrogen dioxide.31 Deferoxamine, even at low concentrations between 20 and 100 μmol/L, is a potent inhibitor of peroxynitrite-mediated oxidation. In contrast, when saturated with iron, deferoxamine loses this inhibitory activity.9 Beckman et al9,10 have argued that peroxynitrite may have greater toxicity than extracellularly generated hydroxyl radicals, partly because of its ability to cross cell membranes. It remains possible that the neuroprotective effect of deferoxamine in this study was not only through iron chelation but via peroxynitrite inhibition.

In this study we did not directly explore other potential mechanisms of deferoxamine, apart from showing that deferoxamine does not exert its protective effect by lowering rat body temperature. In this model, there is a close correlation between core temperature and brain temperature.41 It is important to show that deferoxamine does not reduce brain temperature, because reduction of a few degrees in temperature, even after hypoxia-ischemia, can be protective.42

We selected the deferoxamine dose for this study from previous animal studies that showed that deferoxamine could reduce ischemic and cold-induced brain injury.34,43 We did not perform dose-response studies, so it is possible that at higher doses deferoxamine might be more protective. On the other hand, higher doses can cause circulatory shock and auditory and retinal toxicity44 and a nitroxide radical is formed from the interaction of deferoxamine with superoxide and hydroxyl radicals.45,46 Some of the toxic effects of deferoxamine can be reduced by using deferoxamine chelated to high-molecular-weight compounds like dextran and hydroxethyl starch.7 These compounds have demonstrated protection in animal models of head injury and ischemia.47,48

It is possible that the protection we achieved with deferoxamine is unique to the immature brain as iron uptake by the immature brain is rapid compared with the adult,13 and we have found that the blood vessels of the normal rat under 7 days of age stain more prominently for iron compared with older rats.29 In addition, the newborn rat, like the preterm human infant, has low levels of superoxide dismutase.50 Furthermore, recent studies have shown that excitotoxic injury to immature
neurons and oligodendrocytes is mediated in part by loss of cellular antioxidants and free radical injury.\textsuperscript{31,52} In summary, this study showed that 100 mg/kg SC deferoxamine rapidly concentrates in the brain of the 7-day-old rat to levels between 100 and 200 μmol/L. Deferoxamine reduces brain injury even when administered 5 minutes after induction of hypoxia-ischemia. We have not, however, substantiated the role played by iron in reperfusion injury because at the brain deferoxamine levels achieved in this study, nonchelator actions may contribute to neuroprotection. The protective mechanisms of posttreatment deferoxamine will need further study.

Acknowledgments

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The involvement of oxygen free radicals in the pathogenesis of ischemic brain injury is supported by several lines of experimental evidence that have been succinctly summarized in the introduction of the preceding article by Palmer and coworkers, who found that deferoxamine, an iron chelator, is effective in a posttreatment regimen to reduce acute and chronic pathological consequences of ischemic-hypoxic insult in newborn rats. This study is based on a well-established model, with the outcome measures validated by the authors in previous studies.\(^1\) In addition to the authors’ expertise in pharmacotherapies in this model, the large sample sizes and a study of the pharmacokinetics of deferoxamine make this article stand out among a number of recent publications addressing a similar goal. An interesting observation in this study is the rapid accumulation of deferoxamine in the injured brain to reach high levels that are likely to be relevant to its therapeutic effects. The blood-brain barrier in newborn rats is not well developed and may be more permeable to therapeutic agents like deferoxamine. A future study correlating blood-brain barrier function and brain penetration of this therapeutic agent will be helpful in understanding why deferoxamine is preferentially distributed in the immature brain after ischemic-hypoxic insult. An alternative explanation for greater brain accumulation of deferoxamine may have additional neuroprotective action other than iron chelation. A dose-response study including measurement of brain deferoxamine content will be useful in delineating the mechanism(s) of action of deferoxamine.

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