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Comparison of twice-daily vs once-daily deferasirox dosing in a gerbil model of iron cardiomyopathy

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Abstract

Objective—Despite the availability of deferoxamine chelation therapy for more than 20 years, iron cardiomyopathy remains the leading cause of death in thalassemia major patients. Effective chelation of cardiac iron is difficult; cardiac iron stores respond more slowly to chelation therapy and require a constant gradient of labile iron species between serum and myocytes. We have previously demonstrated the efficacy of once-daily deferasirox in removing previously stored cardiac iron in the gerbil, but changes in cardiac iron were relatively modest compared with hepatic iron. We postulated that daily divided dosing, by sustaining a longer labile iron gradient from myocytes to serum, would produce better cardiac iron chelation than a comparable daily dose.

Methods—Twenty-four 8- to 10-week-old female gerbils underwent iron dextran—loading for 10 weeks, followed by a 1-week iron equilibration period. Animals were divided into three treatment groups of eight animals each and were treated with deferasirox 100 mg/kg/day as a single dose, deferasirox 100 mg/kg/day daily divided dose, or sham chelation for a total of 12 weeks. Following euthanasia, organs were harvested for quantitative iron and tissue histology.

Results—Hepatic and cardiac iron contents were not statistically different between the daily single-dose and daily divided-dose groups. However, the ratio of cardiac to hepatic iron content was lower in the divided-dose group (0.78% vs 1.11%, $p = 0.0007$).

Conclusion—Daily divided dosing of deferasirox changes the relative cardiac and liver iron chelation profile compared with daily single dosing, trading improvements in cardiac iron elimination for less-effective hepatic chelation.

Although routine deferoxamine usage has improved life expectancy in thalassemia major patients, iron cardiomyopathy remains the leading cause of death [1]. Consequently, assessment of chelator efficacy must address the ability to prevent cardiac iron accumulation and remove previously stored cardiac iron. Iron uptake and clearance mechanisms differ in the liver and heart, so it is not surprising that kinetics of iron transport do as well [2]. While deferoxamine improves cardiac function and clears cardiac iron when administered as a continuous infusion, its cardiac efficacy decreases substantially when administered intermittently, even if liver iron burdens appear to be adequately controlled [3,4]. These observations highlight the importance of chelator half-life with respect to mobilization and scavenging of cardiotoxic-free iron.

Deferasirox is a novel tridentate oral iron chelator that was recently approved for treatment of transfusional iron overload. In humans, deferasirox is administered on a once-daily regimen and has a half-life of 12 to 16 hours. Previous work in cell culture, gerbils, and humans suggests that daily deferasirox administration removes cardiac iron [5–7]. Intuitively, twice-daily deferasirox dosing would yield more homogenous suppression of non-transferrin-bound plasma iron species and lower peak drug levels. Our goal was to determine whether this regimen would also improve cardiac iron chelation compared with equivalent once-daily administration in the gerbil. We hypothesized that divided dosing would improve cardiac iron removal without compromising hepatic iron clearance.

Methods

All animal studies were done with approval of the Institutional Animal Care and Use Committee of Children's Hospital Los Angeles. Twenty-four 8- to 10-week-old female Mongolian gerbils (*Meriones unguiculatus*) were obtained from Charles River Laboratories and housed in the Children's Hospital of Los Angeles accredited animal care. All 24 gerbils underwent iron dextran-loading (200 mg/kg/week) for 10 weeks, followed by a 1-week iron equilibration period. Animals were divided into three treatment groups of eight animals each. Iron chelation was initiated with deferasirox at 100 mg/kg/day, either in single or divided dosing. Sham chelation was performed in eight iron-loaded animals. Chelation duration was 12 weeks. Following the chelation period, animals were euthanized by CO₂ overdose and organs harvested for quantitative iron and tissue histology. Liver and heart were weighed wet and portions sent to Mayo Medical Laboratories (Rochester, MN, USA) for quantitative iron measurements. The remainder of the organs was immersion-fixed in formalin, paraffin-embedded, and processed with hematoxylin and eosin, Masson's trichrome and Prussian-blue iron stains.

Results

Hepatic iron

Organ weight, wet-to-dry weight ratio, dry weight iron concentration, and organ iron content are presented in Table 1. Hepatic iron content was 43.1 ± 5.2 mg in the sham-chelated control animals. Daily single dose and daily divided dose deferasirox reduced hepatic iron content to 17.7 ± 4.8 mg and 21.1 ± 5.3 mg, respectively (both significant with respect to control). Differences in organ iron content differences reflected lower iron concentrations as well as decreased organ weight. Liver water content was significantly increased (13.4%) in the animals treated with once-daily deferasirox, but not in the twice-daily treated group (6.3%).

Cardiac iron

Twice-daily deferasirox lowered cardiac weights, iron concentration, and iron content relative to sham-chelated animals (Table 1). Similar trends were observed in the daily single-dose group, however, these values did not reach statistical significance. Wet-to-dry ratio was similar among the three groups, suggesting that chelation did not change water content.

While the decrease in cardiac iron content was nearly twice as large in the daily divided-dose-treated animals compared with daily single dosing (−28.2% vs −15.4%), this difference did not reach statistical significance.

Neither liver nor cardiac iron content, by themselves, was statistically different for the two treatment groups, however, the relative profile was noticeably different.

Figure 1A compares cardiac vs hepatic iron content between the three groups. Data for daily single-dose-treated animals and daily divided-dose treatment animals are shown. Within each

treatment group, cardiac and hepatic iron contents are strongly correlated, likely representing interanimal differences in drug bioavailability as well as pretreatment organ iron contents. The daily single-dose-treated animals (triangles) lie leftward and superior of the daily divided-dose-treated animals (open circles); visually, it is quite easy to place a linear discriminator to separate the treatment groups. The leftward shift of points of the daily single-dose group indicates stronger hepatic chelation, while the upward shift reflects inferior cardiac iron removal.

This relationship is most easily quantitated by comparing the ratio of cardiac to hepatic iron content (Fig. 1B). Sham-chelated animals had only $0.54\% \pm 0.12\%$ as much iron in their heart compared with their liver. Daily deferasirox therapy doubled this ratio to $1.11\% \pm 0.2\%$, reflecting the greater ease in removing hepatic iron relative to cardiac iron. In contrast, twice-daily deferasirox dosing demonstrated a ratio of $0.78\% \pm 0.09\%$ ($p = 0.0007$) indicating a more favorable cardiac iron profile.

Histology

Figure 2 compares 10 weeks of iron loading followed by 12 weeks of sham, single-dose and divided-dose chelation, respectively, in heart and liver. Cardiac iron distribution demonstrates both interstitial and myocyte staining as described previously [6]. After chelation treatment, Prussian-blue staining were visibly decreased in single-dose and divided-dose specimens, but the cardiac iron distribution was not fundamentally different between the two groups.

In the liver, sham-chelated animals demonstrate balanced iron distribution in hepatocytes, Kupffer cells, and large aggregates of phagocytic cells. Deferasirox-chelated animals demonstrate striking clearance of iron in the hepatocellular pool with relatively little clearance from the reticuloendothelial system. Histological appearance of single-dose and divided-dose-treated animals is similar.

Discussion

This study demonstrates that dosing interval is an important determinant of chelator efficacy in different organs. Divided dosing, by providing a more homogenous suppression of labile iron species, extracted more cardiac iron for any given reduction in hepatic iron. Intuitively, one would expect similar behavior in other organs that principally uptake labile iron, such as the pancreas and pituitary, but these organs were not examined in this study. The results parallel the improved cardiac efficacy of deferoxamine when administered as a continuous infusion compared with intermittent administration.

The deferasirox dose was chosen based on our earlier study evaluating the dose-response of deferasirox in the gerbil model. The highest dose, 100 mg/kg daily, was shown to significantly reduce hepatic iron content, while lower doses were not effective [8]. Gerbils have a 7.8-fold higher surface area to mass ratio, and corresponding metabolic rate, than humans. The gerbil deferasirox dose of 100 mg/kg translates to only two-thirds of the standard human dose (20 mg/kg) when normalized according to body surface area; this is appropriate given that there is not ongoing transfusional iron accumulation.

In addition to providing broader suppression of non-transferrin-bound iron species, divided dosing will yield lower peak drug levels. This could potentially reduce chelator toxicity, e.g., the increased hepatic water content observed in the divided-dose-treated animals. However, lower peak levels might also reduce hepatic iron removal through drug interactions with serum proteins. Deferasirox binds very highly to plasma proteins, in particular to albumin. In vitro studies demonstrate that protein-drug binding saturates within physiologically relevant ranges [9]. Higher peak levels of deferasirox observed in daily deferasirox administration may saturate

plasma–protein binding, leading to an increased amount of unbound chelators and improved overall iron balance.

Our findings are potentially interesting in the clinical management of thalassemia. Cardiac iron removal is rate-limiting in patients treated with deferoxamine (four- to five-fold slower than liver iron removal) and continuous drug delivery is necessary to achieve favorable cardiac kinetics. Preliminary data suggests that deferiasirox, like deferoxamine, removes cardiac iron but is particularly potent at hepatic iron elimination [6]. Currently, deferiasirox therapy is administered once daily, leading to high patient satisfaction and compliance [10–12]. However, the present data suggests that twice-daily deferiasirox administration might represent a favorable option in patients with heavy cardiac iron deposition or who do not achieve negative iron balance with the largest recommended single daily dose. The potential pharmacokinetic benefits would have to be weighed against the potential for decreased patient compliance with more frequent dosing. As a result, prospective clinical trials are necessary before any conclusions can be drawn regarding human dosing of deferiasirox.

Limitations

Physiologic response to iron chelation varies considerably among individuals, making it difficult to compare treatment effects with practical group sizes. In this study, intrinsic variability obscured the differential behavior of twice-daily and once-daily dosing when liver and cardiac iron were viewed in isolation. Using the numbers from Table 1, power analysis suggests that we would have needed at least 33 animals per group to observe these differences. Thus, we cannot claim that twice-daily dosing produces lower cardiac iron content. However, Figure 1 clearly demonstrates that twice-daily dosing produces a different pattern or profile of iron removal from heart and liver, one that favors cardiac iron elimination at the expense of poorer hepatic iron clearance.

Metabolite and drug clearances tend to be faster in rodents than in humans, although protein–drug interactions may lessen differences for deferiasirox. If deferiasirox half-life is significantly shorter in gerbils, differences in chelation efficacy between once-daily and divided dosing might be less in humans. Unfortunately, there are no data regarding the pharmacokinetics of deferiasirox clearance in gerbils because the high volume of serum needed for the drug assay preclude serial sampling.

There are no perfect animal models to mimic human iron overload. While the iron-dextran gerbil model has a long track record assessing chelation efficacy, hepatic and cardiac iron kinetics, and distribution patterns differ from humans.

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Figure 1.

(A) Cardiac vs hepatic iron content in mg/g. Data for daily single-dose (QD)-treated animals (triangles) and daily divided-dose (BID)-treated animals (open circles) are shown. Controls represented by gray squares. Leftward shift of points of QD group indicates stronger hepatic chelation relative to BID group, while BID downward shift shows stronger cardiac chelation (B). Graphical summary of data in (A) of organ (heart/liver) iron content ratios. Ratio of BID group is significantly lower ($p = 0.0007$) than QD. BID dosing of deferisirox appears to improve cardiac iron clearance, with a concomitant reduction of liver chelation.

Figure 2. Histological examination of heart and liver ($\times 200$ magnification). Specimens were stained with Perl's Prussian blue iron stain. Less iron relative to controls exists in the daily divided-dose (BID)-treated and daily single-dose (QD)-treated animal organs, although no fundamental change in distribution was observed.

Table 1

Chelation efficacy in liver and heart

	Sham-chelated (n = 8)	Deferasirox QD (n = 8)	Deferasirox BID (n = 8)
Liver			
Organ weight (g)	4.62 ± 0.51	3.93 ± 0.55 *	3.71 ± 0.43 *
Wet-to-dry ratio	3.80 ± 0.28	4.31 ± 0.39 *	4.04 ± 0.29
[Fe] dry weight (mg/g)	35.5 ± 5.0	19.5 ± 5.0 *	22.9 ± 4.7 *
Organ iron (mg)	43.1 ± 5.2	17.7 ± 4.8 *	21.1 ± 5.3 *
% content change	—	−58.9	−51.0
Heart			
Organ weight (mg)	386.6 ± 38.6	363 ± 35.9	337.5 ± 26.9 *
Wet-to-dry ratio	5.19 ± 0.47	5.03 ± 0.21	5.02 ± 0.15
[Fe] dry weight (mg/g)	3.04 ± 0.42	2.68 ± 0.68	2.42 ± 0.52 *
Organ iron (mg)	0.227 ± 0.038	0.192 ± 0.044	0.163 ± 0.042 *
% content change	—	−15.4	−28.19

Data demonstrate changes in liver and heart organ weights and in iron concentration and content postchelation therapy relative to control. There were no significant differences in these specific measures between the QD and BID groups for both heart and liver, while numerous significant differences existed in both relative to control.

Values represent mean ± standard deviation, except where indicated.

BID = daily divided dose; QD = daily single dose.

* $p < 0.05$ vs control.