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Deferasirox and deferiprone remove cardiac iron in the iron-overloaded gerbil

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Abstract

Introduction—Deferasirox effectively controls liver iron concentration; however, little is known regarding its ability to remove stored cardiac iron. Deferiprone seems to have increased cardiac efficacy compared with traditional deferoxamine therapy. Therefore, the relative efficacy of deferasirox and deferiprone were compared in removing cardiac iron from iron-loaded gerbils.

Methods—Twenty-nine 8- to 10-week-old female gerbils underwent 10 weekly iron dextran injections of 200 mg/kg/week. Prechelation iron levels were assessed in 5 animals, and the remainder received deferasirox 100 mg/kg/D po QD (n = 8), deferiprone 375 mg/kg/D po divided TID (n = 8), or sham chelation (n = 8), 5 days/week for 12 weeks.

Results—Deferasirox reduced cardiac iron content 20.5%. No changes occurred in cardiac weight, myocyte hypertrophy, fibrosis, or weight-to-dry weight ratio. Deferasirox treatment reduced liver iron content 51%. Deferiprone produced comparable reductions in cardiac iron content (18.6% reduction). Deferiprone-treated hearts had greater mass (16.5% increase) and increased myocyte hypertrophy. Deferiprone decreased liver iron content 24.9% but was associated with an increase in liver weight and water content.

Conclusion—Deferasirox and deferiprone were equally effective in removing stored cardiac iron in a gerbil animal model, but deferasirox removed more hepatic iron for a given cardiac iron burden.

Transfusional iron overload is a major cause of morbidity and mortality in thalassemia, sickle-cell disease, and other chronic anemias. Regular transfusions deliver between 0.3 and 0.5 mg of iron per kg per day or nearly 10 g per year in a 70-kg man.¹ Although iron is toxic to many organ systems, cardiac deposition remains the leading cause of death.² Subcutaneous deferoxamine chelation prevents cardiac dysfunction, but the regimen is onerous, requiring subcutaneous infusions 8–12 h per day, 5–7 days per week.³ Unfortunately, the discomfort and inconvenience of long, subcutaneous infusions discourages many patients from optimal therapy. Noncompliance is lethal; patients who take less than 225 doses/year have a 50% mortality by 30 years of age.⁴

The oral chelator deferasirox offers inherent advantages with respect to chelation compliance.⁵ Deferasirox can be administered as a single morning dose because of its long elimination half-life (11–19 h).^{1,6} When administered at 20 mg/kg/day, deferasirox produces comparable iron balance to deferoxamine therapy administered at 40 mg/kg/day, 5 days weekly.^{7,8}

Although deferasirox seems to control total iron burden, little data exist regarding cardiac chelation efficacy. Deferasirox's long half-life should suppress labile iron species, or NTBI, over an entire day.⁹ As the heart selectively takes up labile iron species, deferasirox may offer greater protection against cardiac iron uptake than intermittent deferoxamine therapy.¹⁰ In myocyte cultures, deferasirox readily enters myocytes, binds iron, and prevents redox-cycling; however, the ability for deferasirox to mobilize and remove stored cardiac iron has not been well characterized in either humans or animals.^{11,12}

Therefore, the purpose of this study was to determine the efficacy of deferasirox to extract cardiac iron in a gerbil model. As deferiprone removes cardiac iron effectively in humans, the cardiac chelation efficacy of deferasirox was compared with comparably dosed deferiprone. The gerbil emulates many of the functional abnormalities observed in human iron cardiomyopathy.^{13–20} This model has also been used to study chelator efficacy.^{14,15,19–22} This study differs in that iron loading and chelation were performed sequentially, rather than concurrently, to assess stored iron mobilization rather than prophylaxis of iron accumulation.

METHODS

Animals and iron loading

All animal studies were conducted with approval of the IACUC of Children's Hospital Los Angeles. Overall, twenty-nine 8- to 10-week-old female Mongolian gerbils (*Meriones unguiculatus*) were obtained from Charles River Laboratories and housed in the CHLA-accredited animal care facility. All animals received 10 weekly subcutaneous injections of iron dextran (Sigma Chemical Co., St. Louis, Mo) at a dose of 200 mg/kg. After the last injection, a 13-day iron equilibration period was allowed before starting chelation therapy.

Chelation

Overall, 5 animals were sacrificed before initiation of chelation therapy to characterize initial iron levels. The remaining 24 iron-loaded gerbils were divided into the 3 groups of 8 animals each: sham-chelated gerbils, deferasirox, and deferiprone-treated animals. All animals received chelation for 12 weeks.

To avoid the stress of chronic, repeated gavage feeding, deferiprone and deferasirox were homogeneously mixed in plain peanut butter for oral feeding via a 1-mL syringe; all chelators were provided by Novartis Pharma, AG (Basel, Switzerland).

Deferasirox was given at a single daily dose of 100 mg/kg and deferiprone at a dose of 375 mg/kg/day divided into 3 equal doses. Chelator doses were based on a previous dose-finding study²¹; these doses represent 67% of human values when normalized for body surface area. Oral chelator administration gave the animal approximately 0.15 mL of peanut butter per day, providing <1/1000 of the binding capacity of the administered chelator in administered iron.

MRI

Pilot data suggested strong hepatic efficacy using deferasirox,²¹ so liver R2 was measured in 4 animals from the deferasirox group at 8 weeks to monitor for overchelation. The MRI techniques have previously been described.²³

Electrocardiography and exercise tests

Electrocardiography and exercise tests were performed at baseline, immediately before chelation, and at the end of the study. Limb lead electrocardiography was performed using a standard electrocardiograph (Mac Vu; Marquette/GE Medical Systems, Milwaukee, Wis). Animals were sedated with a single intraperitoneal injection of ketamine (100 mg/kg) plus

xylosine (10 mg/kg) and positioned supine for electrode placement. PR, QRS, QTc, and RR intervals were averaged over 5 consecutive heartbeats.

Maximum running time was assessed on a rodent treadmill (Exer 6M; Columbus Instruments, Columbus, Ohio) equipped with an electrified grid. Gerbils were acclimated for 10 min at treadmill speeds of 10 m/min several hours before the exercise tests. Animals were run at systematically increasing treadmill speeds, beginning at 10 m/min and increasing at a rate of 2.5 m per min every 3 min. Gerbils were run to exhaustion, with exhaustion determined as spending more than 10 consecutive seconds on the stimulator grid, or staying on it for more than half of the time.²⁰ To ensure maximum effort, examinations were repeated 2 days apart, with the longer exercise time used for analysis.

Histology and organ iron assessment

Euthanization was performed with 5% CO₂ according to institutional guidelines. After sacrifice, the hearts and livers were removed, weighed, and sent for quantitative iron determination (Mayo Medical Laboratories, Rochester, Minn). Tissue dry weight and dry weight iron concentrations were recorded as well. Liver and heart were immersion-fixed in 10% formalin, paraffin-embedded, and stained with Prussian blue, Masson's trichrome, and H&E.

All histologic sections were reviewed in a blinded fashion by an experienced pathologist. Cardiac iron deposition, muscular hypertrophy, and fibrosis were scored with respect to location and intensity using a relative scale from 0 to 4. Hepatic iron staining was assessed separately in the sinusoidal cells and hepatocytes. The number, size, and staining intensity of lobular aggregates of reticuloendothelial cells were also scored on a 0 to 4 scale. The pathologist also assigned a total iron score reflecting visually weighted contributions of each of these iron pools.

Portions of each heart were processed for electron microscopy using standard techniques. Imaging was performed on a Philips CM 12 transmission electron microscope in the Childrens Hospital Los Angeles Pathology Department.

Statistics

Iron concentration, iron content, organ weight, and wet-to-dry weight ratio were analyzed using one-way ANOVA over the 3 treatment arms (sham-chelated, deferasirox, and deferiprone-treated animals). The mean of each treatment group was compared with the mean value from the sham-chelated animals using Dunnett's test, which corrects for multiple comparisons. A one-sided test was used for iron concentrations and iron content, based on pilot data demonstrating chelator efficacy²¹; a two-sided analysis was used for organ weight and wet-to-dry ratio. Comparison of these variables between 10-week controls and sham-chelated animals was performed by an unpaired *t*-test. Electrocardiographic intervals and running times were processed in an identical manner. Linear regression was also used to assess the relationship between electrocardiographic intervals and organ iron concentration. Histology scores were assessed using Wilcoxon signed-rank analysis because of the limited number of grades used in the scoring. Bonferroni correction was applied for multiple comparisons.

RESULTS

All animals tolerated the iron loading and chelation without any apparent ill effects. After 8 weeks of chelation, the estimated iron concentration by MRI was 4.4-mg/g wet weight, so chelation was continued.²³ One animal from the deferasirox group died from an anesthetic complication. It was healthy before sedation.

Chelation efficacy is summarized in Table I. Cardiac and liver iron concentrations and contents after sham chelation were significantly lower than observed in the 10-week control animals, representing spontaneous (not chelator-mediated) iron redistribution and elimination ($P < 0.001$). All subsequent chelator comparisons are reported with respect to the sham-chelated animals, not the 10-week control animals.

Both chelators lowered wet and dry weight cardiac iron concentrations. Deferiprone therapy produced the lowest iron concentrations but was associated with a 16.5% increase in cardiac mass. Figure 1 demonstrates a scattergram of wet-weight cardiac iron concentration versus heart weight. Clear separation exists between the treatment groups. Heart weight and heart iron concentration are also inversely related in the sham and unchelated animals. This observation justifies the use of iron content, rather than concentration, as a metric for chelator efficacy. Cardiac iron content was decreased 20.5% by deferasirox ($P = 0.05$) and 18.6% by deferiprone ($P = 0.06$), respectively. The increased cardiac weight observed with deferiprone did not reflect increased hydration as wet-to-dry weight ratios were similar to sham controls.

Both chelators were also effective in the liver. Hepatic iron content fell 51% with deferasirox and 24.9% with deferiprone. Interestingly, deferasirox- and deferiprone-treated animals exhibited similar wet-weight iron concentrations; however, organ weight and water content (wet-to-dry weight ratio) were increased in the deferiprone group. The interaction between liver weight and iron concentration is summarized in Fig 2 and is even more striking than for the heart. Larger organs were again associated with lower wet-weight iron concentrations; for sham-chelated animals, the trend was relatively strong ($r = 0.74$). The unchelated animals sacrificed at 10 weeks demonstrated a parallel relationship having similar slope. Thus, organ growth seems to modulate iron concentration in the absence of chelation, producing paradoxical statistical independence of liver iron content and organ weight ($r = 0.30$, $P = 0.07$). Effective chelation represents parallel shifts of this relationship, corresponding to changes in organ iron content.

The response of heart and liver iron to chelation was correlated. Figure 3 demonstrates a scattergram comparison of heart and liver iron content based on treatment group. Correlation coefficient was 0.81 ($P < 0.0001$). Deferasirox data are shifted leftward relative to deferiprone results, indicating relatively stronger liver chelation for any degree of cardiac iron loading.

Average histology scores are summarized in Table II. Mean iron scores were better correlated ($r = 0.70$) with wet-weight iron concentration than cardiac iron content or dry-weight concentration (not shown). Mean iron scores decreased with chelation but only reached statistical significance for the deferiprone group. Myocyte hypertrophy was noted in the deferiprone-treated animals, concordant with the observed increase in cardiac mass. Decreases in cardiac fibrosis scores with chelation did not reach significance.

Mean liver histology scores are demonstrated in the bottom of Table II. Mean and hepatocyte iron scores paralleled quantitative iron values, but descriptors of reticuloendothelial burden did not. Correlation between mean iron score and wet-weight iron concentration had an r -value of 0.86 when compared across all groups. Kupffer cell iron staining was higher in the deferasirox-treated animals than the animals that underwent sham chelation; sinusoidal iron staining was comparable with that observed in the 10-week control animals (Fig 4). In contrast, deferiprone therapy produced balanced chelation, with significant reductions in cytoplasmic iron and phagocyte aggregates and no increase in Kupffer cell burden.

Cardiac iron staining was regional. In the right and left ventricular free walls, the staining was heaviest in the endocardium and myocardium. The interventricular septum demonstrated 50% greater staining on the right ventricular portion (left ventricular epicardium). With chelation,

the right ventricle cleared most readily, followed by the endocardial and myocardial components of the left ventricle and interventricular septum.

On a cellular level, cardiac iron redistribution was readily apparent on both light and electron microscopy. Figure 5 compares cardiac iron loading after 10 weeks of iron dextran injections versus 10 weeks of iron loading followed by 12 weeks of sham chelation. At 10 weeks, iron staining is exclusively endomyosial, residing in interstitially distributed endothelial cells. After sham chelation, Prussian-blue staining is visibly decreased, concordant with the net decrease in cardiac iron measured biochemically. Nonetheless, detectable *myocyte* iron staining is noted on both light and electron microscopy, suggesting a slow iron redistribution process. Iron chelation therapy with both chelators attenuated the redistribution of stainable iron. Chelation therapy produced no other discernable microstructural changes on either light microscopy or electron microscopy.

EKG assessment demonstrated subtle changes in the PR, QRS, and QTc intervals with iron loading and chelation. As all animals were handled identically for the first 11 weeks, baseline and iron-loaded/prechelation data points were pooled among the groups. Iron loading *shortened* the QTc interval 7.4% ($P = 0.003$) and broadened the QRS duration 10.6%, although the latter did not reach statistical significance ($P = 0.06$). Chelation with deferasirox antagonized the changes in QTc interval and shortened QRS duration, relative to sham-chelated animals. Deferasirox and deferiprone also significantly prolonged the PR interval relative to sham controls; however, values were similar to both mean baseline and prechelation values. PR, QRS, and QTc intervals were weakly correlated to heart and liver iron concentration, with correlation coefficients ranging from 0.33 to 0.60, ($P < 0.04$ for all). The strength and direction of these changes were concordant with therapy, suggesting that drug effects were primarily being modulated through iron chelation rather than through nonspecific mechanisms.

Despite the high liver and cardiac iron levels achieved in this protocol, animals remained asymptomatic and did not exhibit any functional limitations. As all animals were treated identically up until chelation, data from baseline and pretreatment were pooled. Running times after iron loading were 15% higher than baseline ($P = 0.02$), which likely reflects a training or maturity effect, although cardiac function has previously been shown to *improve* in the gerbil for mild cardiac siderosis.²⁰ ANOVA demonstrated no significant difference among the treatment groups after chelation. No statistical correlation was observed between running time and either liver or cardiac iron.

DISCUSSION

Although liver iron seems to be a good surrogate for total body iron,^{24,25} it is an incomplete marker of extrahepatic organ iron burden or toxicity. Patients may have significant cardiac deposition despite reassuring liver iron and ferritin levels. Different chelators seem to have different accessibility to hepatic and extrahepatic iron stores. For example, deferoxamine works more rapidly and efficiently in removing liver iron than cardiac iron.²⁶ In contrast, deferiprone seems to remove iron from the heart effectively^{27,28} despite being relatively inefficient in controlling hepatic iron content.^{27,29} Given the clinical consequences of cardiac iron deposition, it is clear that any *new* chelator should be assessed for both cardiac efficacy and liver efficacy.

The primary finding of this study is that deferasirox and deferiprone were equally effective at removing stored cardiac iron in the gerbil at a rate between 1.6% and 1.7% per week. Both deferasirox and deferiprone prevented redistribution of iron from endomyosial deposits to myocytes, and both antagonized subtle electrocardiographic changes associated with iron. Iron loading was insufficient to cause significant functional abnormalities. Deferiprone was

associated with cardiac hypertrophy and increased cardiac mass; however, the etiology is uncertain. Chronic anemia is known to produce compensatory hypertrophy.^{30,31} Hemoglobin levels were not measured in this study, but high-dose deferiprone therapy has previously been associated with marrow suppression in rat models.^{32–34} A direct hyperplastic effect of deferiprone cannot be excluded; however, it has not previously been described in animal or human studies.

Cardiac and liver iron levels were highly correlated; however, deferasirox had lower liver iron contents for comparable cardiac iron burdens. Deferasirox was particularly efficient at hepatocyte clearance, reflecting its predominantly biliary elimination.¹² Deferiprone was half as effective at clearing total liver iron, but it lowered both reticuloendothelial stores and hepatocyte stores. The hepatomegally and increased hepatic water content observed in the deferiprone-treated animals has not been previously been described. Nonspecific organ atrophy was observed in rats given comparable doses over 1 to 3 months.^{33,34} The animals did not exhibit any physical signs of liver dysfunction and liver enzymes were not performed, so the clinical significance of the hepatomegally is undetermined.

Although significant electrocardiographic and exercise abnormalities have been described in the gerbil model, the functional abnormalities in this study were subclinical. PR, QRS, and QTc intervals were weakly correlated with liver and cardiac iron, but changes were subtle. The QRS broadening observed in this study is consistent with observations using optical and direct electrophysiologic measurements in gerbil.^{17,18} This conduction delay is thought to occur through reduced sodium currents and enhanced fast sodium channel inactivation.

The *shortening* of PR and QTc intervals with iron overload, although superficially paradoxical, is consistent with the bimodal functional effects of iron previously described in this model.²⁰ Mild iron loading produces a positive inotropic effect with improved contractility and performance. Although the mechanism is unknown, oxidants are known to stimulate calcium release from the sarcoplasmic reticulum.^{35,36} Acutely, increased intracellular calcium will behave in a similar manner as increased catecholamine stimulation, leading to improved myocyte contractility, faster atrioventricular conduction (shorter PR interval), and faster repolarization (shorter QTc interval). At higher concentrations, ferrous iron can also decrease sarcoplasmic calcium release by antagonizing the ryanodine receptors,³⁷ creating a potential mechanism for chronic heart failure.³⁸ Therefore, the subtle EKG findings observed in this study may represent early changes in the large pathologic spectrum of iron cardiomyopathy.

The absence of detectable differences in exercise performance also suggests that myocyte iron loading produced in this study was relatively mild. Previous studies in this model demonstrate exercise impairment between 20 and 47 weeks of iron dextran loading.²⁰ As the total duration of this study was 23 weeks, significant differences were not necessarily expected. However, treadmill testing did serve as an important negative control for drug-induced exercise impairment.

The efficacy of deferasirox to remove cardiac iron has not previously been assessed *in vivo*. Studies in myocyte cultures demonstrate that deferasirox rapidly enters myocytes and binds labile intracellular iron species, leading to decreased free radical production. Deferasirox and deferiprone both entered myocytes more readily than deferoxamine. Although these studies are encouraging, cell culture systems imperfectly model *in vivo* effects such as the interactions between drug and serum proteins. The current experiments suggest that deferasirox has comparable cardiac activity with deferiprone in an intact rodent model and superior hepatic chelation ability. Unfortunately, human studies of deferasirox cardiac efficacy are currently lacking, although prospective trials have been initiated.

Limitations

Rodent models are imperfect surrogates for chelator efficacy in humans. Differences in iron storage and accessibility as well as drug half-life limit extrapolation to human disease. The iron-dextran-loaded gerbil is an established model but exhibits some notable deviations from human disease. Cardiac iron deposition first occurs interstitially, with subsequent myocyte redistribution. Although interstitial iron deposition is nearly universal in thalassemia patients,³⁹ unlike for hemochromatosis patients,⁴⁰ it is less prominent than found in rodent models. Second, cardiac and liver iron levels were tightly correlated in this study in both treated animals and untreated animals, which suggests less asymmetry in organ loading and clearance rates of iron compared with humans.^{26,41} This finding could also reflect the more strenuous iron loading and chelation regimens used in experimental models when compared with patients.

This study was designed to assess chelation efficacy, not toxicity. As a result, no assessment of hepatic, renal, or bone marrow function was collected, limiting the authors' ability to interpret the clinical significance of some histologic findings.

SUMMARY

This article compares the efficacy of deferasirox and deferiprone in removing previously stored iron in a gerbil model of iron overload. Deferasirox and deferiprone both reduced cardiac iron content approximately 20% over 3 months. Cardiac and liver iron elimination were correlated, but deferasirox was nearly twice as potent in the liver for any given cardiac iron level. PR, QRS, and QTc intervals were weakly correlated with hepatic and liver iron concentrations. Exercise performance was not significantly different among the groups.

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Abbreviations

ANOVA	analysis of variance
EKG	electrocardiogram
IACUC	Institutional Animal Care and Use Committee
LIC	liver iron concentration
MRI	magnetic resonance imaging
NTBI	non-transferrin bound iron
PR	PR interval
QRS	QRS interval
QTc	corrected QT interval
RR	interbeat interval

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Fig 1. Scattergram depicting cardiac iron concentration in milligram/gram wet weight versus cardiac weight in grams. Solid dots represent values from the sham-chelated animals; mean and standard deviation for this group are indicated by the x and y error bars. Values for 10-week controls (+ signs), deferasirox chelation (triangles), and deferiprone chelation (squares) are shown.

Fig 2. Scattergram depicting LIC in milligram/gram wet weight versus liver weight in grams. Symbols are identical to the previous figure. The dotted line represents linear regression between LIC and liver weight for the sham-chelated animals (r -value 0.74).

Fig 3.

Scattergram demonstrating the interdependence between heart and liver iron content. Solid dots represent values from the sham-chelated animals; mean and standard deviation for this group are indicated by the x and y error bars. Values for 10-week controls (+ signs), deferasirox chelation (triangles), and deferiprone chelation (squares) are shown. When all groups are pooled, a strong linear relationship exists ($r = 0.81$), which is indicated by the solid line. However, points from deferasirox-treated animals consistently lie leftward of this relationship, whereas points from deferiprone-treated animals lie rightward, indicating that deferasirox has stronger hepatic chelation for a given degree of cardiac iron removal.

Fig 4.

Representative Prussian-blue images depicting hepatic iron staining after 12 weeks of sham chelation (**A**), deferasirox therapy (**B**), and deferiprone therapy (**C**). Comma-shaped cells represent individual Kupffer cells. Large, intensely stained lobular masses of reticuloendothelial cells are observed in all images. Hepatocyte staining manifests as blue “background” staining at this magnification but is discernable as discrete intracellular collections at high power. Deferasirox (**B**) produced striking hepatocyte clearing, but increased Kupffer cell staining is observed. Deferiprone (**C**) has modest hepatocyte clearing and less intense lobular mass staining. Kupffer cell staining is comparable with sham-chelated animals.

Fig 5.

(A) Prussian blue (left) and electron micrograph (right) of gerbil left ventricular myocardium after 10 weeks of iron loading. Staining is confined to endothelial cells located in the interstitium. Myocytes appear remarkably clear by both light and electron microscopy. (B) Similar pictures from a sham-chelated animal (10 weeks of iron loading and 12 weeks of sham chelation). Staining remains predominantly interstitial, but evidence exists of multiple, small iron deposits between mitochondria within the myocytes.

Table I

Liver and heart weights, water content, and iron concentration/content

	Heart			
	10-week Control (N = 5)	Sham Chelated (N = 8)	Deferasirox (N = 8)	Deferiprone (N = 8)
Organ weight (mg)	340.8 ± 29.7	333.4 ± 40.3	332.4 ± 27.2	388.4 ± 35.5
Wet-to-dry ratio	4.74 ± 0.11	4.79 ± 0.24	5.04 ± 0.34	5.03 ± 0.13
[Fe] dry wt (mg/g)	4.33 ± 0.63	3.10 ± 0.32	2.63 ± 0.40	2.28 ± 0.37
[Fe] wet wt (mg/g)	0.913 ± 0.133	0.648 ± 0.075	0.524 ± 0.081	0.454 ± 0.071
Organ iron (mg)	0.309 ± 0.028	0.215 ± 0.028	0.171 ± 0.029	0.175 ± 0.024
% content change	+43.7%	—	−20.5%	−18.6%

	Liver			
	10-week Control (N = 8)	Sham Chelated (N = 8)	Deferasirox (N = 8)	Deferiprone (N = 8)
Organ weight (g)	4.57 ± 0.55	3.89 ± 0.61	3.45 ± 0.50	5.11 ± 0.55
Wet-to-dry ratio	4.33 ± 0.17	3.85 ± 0.28	4.20 ± 0.26	5.14 ± 0.88
[Fe] dry wt (mg/g)	44.9 ± 5.7	36.2 ± 5.60	20.7 ± 3.4	26.9 ± 6.3
[Fe] wet wt (mg/g)	10.38 ± 1.23	9.42 ± 1.38	4.97 ± 0.96	5.29 ± 1.24
Organ iron (mg)	47.0 ± 3.7	36.1 ± 3.6	17.7 ± 4.5	27.1 ± 6.8
% content change	+30.1%	—	−51.0%	−24.9%

Notes: Data represent mean ± standard deviation. Bold text reflects values that have $P < 0.05$ with respect to the sham-chelated group.

Table II

Summary of cardiac and liver histology scores

Heart	10-Week Control (N = 5)	Sham Chelated (N = 8)	Deferasirox (N = 8)	Deferiprone (N = 8)
Mean Iron Score (9 sites)	1.9 ± 0.5	1.7 ± 0.7	1.3 ± 0.6	1.1 ± 0.5
Myocyte Hypertrophy (3 sites)	1.7 ± 0.5	1.5 ± 0.4	1.8 ± 0.1	2.2 ± 0.4
Interstitial Fibrosis (4 sites)	1.1 ± 0.5	0.7 ± 0.1	0.4 ± 0.2	0.3 ± 0.1
Liver	10-week Control	Sham Chelated	Deferasirox	Deferiprone
Mean Iron Score	3.8 ± 0.5	3.6 ± 0.5	1.0 ± 0.0	1.6 ± 0.7
Cytoplasmic Iron	3.4 ± 0.6	3.3 ± 0.5	0.6 ± 0.5	1.6 ± 0.7
Kupffer Cell iron	3.0 ± 1.0	1.5 ± 0.5	3.3 ± 0.7	1.8 ± 0.7
Lobular Masses	3.8 ± 0.5	3.5 ± 0.8	3.5 ± 0.8	2.3 ± 0.9

Notes: Data represent mean ± standard deviation. Bolded scores represent significance value $P < 0.05$ with respect to sham-chelated animals.