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Authors

Hodges, Yvonne K

Weinberger, Howard D

Stephens, Janet

et al.

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Desferri-Exochelin, A Lipid-Soluble, Hexadentate Iron Chelator, Effectively Removes Tissue Iron

Yvonne K Hodges[1], Howard D. Weinberger[1], Janet Stephens[2], Marcus A. Horwitz[3], Lawrence D. Horwitz[1].

[1]Cardiology, Colorado Health Sciences Center, 4200 E 9th Ave, Denver, CO, 80262,
[2]Pathology, Exempla St. Joseph Hospital, 1835 Franklin Street, Denver, CO 80218.
[3]Medicine, University of California – Los Angeles, 10833 Le Conte Avenue, Los Angeles, CA, 90095

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Author for Correspondence and Proofs:

Lawrence D. Horwitz MD

University of Colorado Health Sciences Center

Cardiology B130

4200 E. Ninth Avenue

Denver CO 80262

Phone: 303-315-4398

FAX: 303:315-5082

email: lawrence.horwitz@uchsc.edu

ABSTRACT

Chronic iron-overload is damaging to the heart, liver and other organs. Better iron chelators are needed to treat this serious medical condition. We studied the uptake and distribution of the lipid-soluble, hexadentate iron chelator desferri-Exochelin 772SM (D-Exo) and evaluated its efficacy in removing iron from tissue in rodent models. After an intravenous bolus of tritiated D-Exo to rats, counts rapidly disappeared from the blood and rapidly appeared in 15 organs studied, usually peaking within 15 minutes. There was considerable uptake in the heart and liver, two organs especially susceptible to damage from clinical iron overload. To assess actual decreases in cardiac and hepatic iron in response to D-Exo, we studied mice loaded with 42 mg of iron dextran (2100 mg/kg). Untreated, iron-loaded mice sacrificed 9 weeks later had a 4-fold increase in cardiac iron and a 20-fold increase in hepatic iron compared with controls that were not iron-loaded. In iron-loaded mice treated with 7 mg of D-Exo i.p. 4 days/wk for 8 wks (total 224 mg), tissue iron, measured by atomic absorption, was reduced by 20% in the liver and 25% in the heart ($P<0.01$ for each organ). During the first 8 hr after a D-Exo dose, iron was excreted in the urine. Mice treated with D-Exo gained weight normally and showed no evidence of toxicity. In conclusion, in this iron-overload mouse model, D-Exo administered i.v. or i.p. rapidly diffuses into multiple organs, including the heart and liver, and effectively removes iron without apparent toxicity.

ABBREVIATIONS: D-Exo, desferri-Exochelin; DPM, disintegrations per minute

INTRODUCTION

Chronic iron overload, due to hemochromatosis or frequent transfusions for anemias, results in adverse outcomes including cardiac and hepatic dysfunction (1). Although iron can be removed by phlebotomy in hemochromatosis, this is not feasible in thalassemia or other anemias. The current treatment for these conditions is administration of an iron chelator, deferoxamine (2). Deferoxamine removes excess iron from the body and improves clinical outcomes *(*1,3). Because of its hexadentate structure, it prevents iron-mediated production of toxic free radicals by the Fenton reaction (4). However, deferoxamine, a lipid-insoluble compound, does not readily enter cells and is rapidly excreted (2,5,6). In addition, deferoxamine has serious side effects that require limitation in the tolerable daily dose (1,2, 5,7). Therefore, to achieve clinically useful effects, it is generally necessary for deferoxamine to be administered parenterally as daily 12-24 h infusions (8). This regimen frequently results in poor patient compliance.

The development of more easily administered and less toxic iron chelators suitable for clinical applications is highly desirable, due to the limitations of deferoxamine (2, 5, 8). One characteristic that enhances organ uptake of an iron chelator is lipid-solubility, which facilitates entry across lipid components of cell membranes. The siderophores secreted by *Mycobacterium tuberculosis* are a family of lipid-soluble, hexadentate iron chelators, termed “exochelins” (9-11). These siderophores have evolved over thousands of years to be nontoxic. They readily enter cells and prevent oxidative reactions (10). In this study, we administered a synthesized exochelin to rodents by intravenous and intraperitoneal routes and measured their ability to remove excess iron from the heart and liver.

METHODS

Exochelins

A large family of secreted siderophores has been isolated from cultures of *M. tuberculosis* and characterized (9-11). These siderophores have a common core structure and are both lipid and water soluble. The relative degree of lipid solubility is determined by the composition of side chains that terminate in ester moieties (9). A highly lipid soluble exochelin, desferri-Exochelin 772SM (D-Exo), has been synthesized. The number refers to the molecular weight of the iron-bound compound and the letters to the side chain structure, which contains serine (S) and terminates in a methyl ester (M). The structure of iron-bound Exochelin 772SM is shown in Figure 1. The molecular weight of D-Exo is 719, while the designation 772 refers to the weight of the iron-bound compound.

Radiolabeled Exochelin

Tritium-labeled D-Exo (5 Ci/mmol) was a gift from Keystone Biomedical, Inc. The procedure for labeling the compound involved synthesis of dibromoexochelin (3, 5 position on the aromatic ring) followed by a tritium-bromide exchange.

Protocols

All experiments conformed to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no.85-23 revised 1996). Institutional Animal Care and Use Committees of the University of California at Los Angeles or the University of Colorado Health Sciences Center approved the experiments.

1. Blood Disappearance and Organ Uptake of Tritiated D-Exo in Rats after Intravenous Administration

Fifteen male Sprague-Dawley rats, weighing approximately 200-gram in weight, were anaesthetized with ketamine (25mg/kg) and xylacine (1.3 mg/kg). Each rat received 100 μCi tritium-labeled D-Exo intravenously in 250 μl of 10% normal saline administered as a single bolus into one of the tail veins.

The rats were divided into four groups destined to be sacrificed at 15 min (n=4), 1 hr (n=4), 4 hr (n=3) and 24 h (n=4) after injection. After the animals were sacrificed, the heart, lung, thymus, liver, spleen, stomach, small intestine, large intestine, kidney, bladder, testes, and brain as well as samples of the skull bone, skeletal muscle, and abdominal fat tissue were collected, and a urine sample withdrawn from the bladder. For blood pharmacokinetic analysis, blood was withdrawn from the opposite tail vein at 2, 5, 10, and 15 min and 1, 4 and 24 h after injection.

For blood measurements, two 10 μl aliquots of blood from each time point were added to 1 ml of TS-2 tissue solubilizer (Research Products International Corp., Mount Prospect, IL) and incubated overnight at 37^{o}C. For tissue measurements, two samples of each tissue were individually weighed, suspended in 1 ml of TS-2, and incubated overnight at 37^{o}C until the tissue was dissolved. For urine measurements, two 25 μl aliquots of urine were each added to 1 ml of TS-2 and incubated overnight at 37^{o}C. Ten ml of 4a20 liquid scintillation fluid (Research Products International Corp.) was added to each sample and DPM quantitated in duplicate aliquots using a beta scintillation counter. DPM/ml of blood

or urine, DPM/g of tissue, and DPM/organ were calculated and averaged for each sample.

2. Effect of Multiple Intraperitoneal Injections of D-Exo on Organ Iron Levels in Mice

Experiments were performed on 5 week old male B6D2F1 NIH hybrid mice (Harlan Sprague-Dawley Indianapolis, IN). Twenty-two mice [weighing 20.87 g ± 0.28 (SE)] received ferric hydroxide dextran complex (Iron Dextran; 98 mg/ml; Sigma-Aldrich Co., St. Louis, MO) given by intraperitoneal injections (i.p.) in three divided doses over 5 days. The total dose of iron was 42 mg/mouse (2100 mg/kg). An additional 9 mice did not receive iron dextran.

Five groups of animals were studied. Three days after iron-loading, eight of the iron-loaded mice received 7 mg D-Exo i.p., 4 days/week for 8 weeks (Iron; D-Exo group). The total dose of D-Exo was 224 mg over the 8 week treatment period. The vehicle was a mixture of equal parts ethanol and polyethylene glycol diluted in 0.09% NaCl (17.5:17.5:65). Eight iron-loaded mice received only vehicle without D-Exo (Iron; Vehicle group), and 6 iron-loaded mice received neither D-Exo nor vehicle (Iron; No treatment group). Four of the iron-free mice received neither D-Exo nor vehicle (No iron, No treatment group) and five of the iron-free mice received vehicle (No iron, Vehicle group). Initially the mice received Harlan Teklad standard rodent chow (Catalog # 8640) and tap water. The diet and water were changed after the last injection of iron and before the first treatment to an iron-deficient rodent chow (Harlan Teklad, cat # TD80396, containing 2 to 5 mg iron/kg) and iron-free “nanopure” water.

Before sacrifice, the mice were anaesthetized with pentobarbital (60 mg/kg i.p.). To prevent blood coagulation, heparin (5 units/gm i.p.) was administered and blood, hearts, livers, and spleens were removed for measurement of tissue iron levels or histological analyses.

Urine Iron Analysis

Following a modification of the method of Kurien and Scofield, urine was collected from individual mice for 8 h from a clean plastic animal cage without bedding (12). The urine was stored frozen at -20^{o}C and then analyzed later for iron concentration. Urinary iron was quantified by a colorimetric, microtiter plate ferrozine-based assay modified from that of Fish (13). Plastic or acid washed containers were used to minimize contaminating iron. Iron in 0.2 ml urine samples was chelated by 0.15 ml of iron-chelating reagent [2 mol/L ascorbic acid, 5 mol/L ammonium acetate, 6.5 nmol/L ferrozine, 13.1 mmol/L neocuprine (Sigma)]. Absorbance was measured at 562 nm and compared with ferrous ethylene diammonium sulfate standards.

Echocardiographic Analyses

Prior to administration of iron and again at the end of the treatment period, all mice had echocardiographic assessments of left ventricular size and function. Mice were restrained by hand or with light sedation using 2,2,2 tribromoethanol dissolved in tert-amyl alcohol (Avertin; Sigma-Aldrich Co., St. Louis MO; 200 mg/kg). Doses of Avertin were minimized to assure heart rates above 400 beats/min. The mice were placed in the prone position on an acoustic gel pad. A GE Vingmed Vivid Five echocardiography machine (GE Medical, Milwaukee WI) with a pediatric 10.0 MHz wide-band annular array transducer

with frequencies up to 10 Hz was used. Two-dimensional and M-mode images were obtained in the short-axis orientation. The two-dimensional images were measured at 396 frames/s. Echocardiographic measurements of chamber size and wall thickness in systole and diastole were made “off-line” from stored digital data. Measurements from 3 cardiac cycles were averaged. Left ventricular systolic function was assessed as percent fractional shortening (difference between diastolic and systolic internal diameter divided by internal diameter in diastole) (14, 15). The investigator (H.W.) who performed and analyzed the echocardiographic measurements was blinded regarding the treatment status of each mouse.

Tissue Iron Quantitation

Individual liver and heart samples were dried, ashed in a muffle furnace for 24 h at 450°C, digested with concentrated nitric acid, and re-ashed. The ash was dissolved in diluted HCl and total iron content was measured with a flame atomic absorption spectrophotometer (model 2380; Perkin-Elmer Corporation, Norwalk, CT). Calibration standards were verified with each set of analyses. The detection limit for iron at 248.3 nm was 0.1μ/ml.

Histology

Tissues were fixed in 10% formalin, embedded with paraffin, sectioned, mounted on slides, and stained with hematoxylin and eosin, or trichrome stain for fibrosis or Prussian blue stain for iron. The pathologist (J.S.) who analyzed the slides was blinded as to the treatment status of each mouse.

Statistics

Statistical analyses were done by ANOVA or linear regression. $P<0.05$ was considered to be significant.

RESULTS

1. Studies of Tritiated D-Exo in Rats

Blood Concentrations after Single Dose Intravenous Administration

Disappearance curves of the radioactive counts obtained in two rats are shown in Figure 2. There was a rapid early decline in counts. The curves were well described by a single compartment model and the individual values for $t_{1/2}$ were 8 and 42 mins.

Organ Concentrations after Single Dose Intravenous Administration

Organ counts as mean values from groups of mice sacrificed at time intervals ranging from 15 min to 24 h are shown in Figure 3. All organs exhibited peak counts at either 15 min or 1 h. Subsequently, there was a gradual decrease in counts from the peak level with detectable counts still present at 24 h. Most organs had similar disappearance curves. Four and 24 h after injection, counts in the heart were 35% and 8% of the peak level and counts in the liver were 20% and 9% of the peak level, respectively. The $t_{1/2}$ was 2 h in the heart and 0.9 h in the liver. Relatively high counts in the small intestine were noted, a finding compatible with biliary excretion of iron-chelator complexes. Disappearance was extremely slow in the brain and testes.

Urine Concentrations after Single Dose Intravenous Administration

As shown in Figure 4, substantial counts were detected in the urine within the first four hours after i.v. injection. Counts/ml of urine fell afterward, a finding consistent with rapid clearance via the kidneys.

2. Effects of Daily Administration of D-Exo in Iron Overloaded Mice.

Body Weight

All of the mice remained active during the 64 days of the study and appeared to be healthy. Within each group, the mice progressively gained weight and there were no significant differences among the three groups of iron-loaded mice. The two groups that did not receive iron had greater weight gains than the three groups that were iron-loaded.

Tissue Iron

Results of organ iron concentrations for the heart and liver are shown in Figures 5A and 5B respectively. Iron-loading markedly increased iron concentrations in both organs. Due to the relatively small amount of tissue available, there was considerably more variation within each treatment group in the heart compared with the liver. In both the heart and liver, there were no significant statistical differences between the iron-loaded group that received vehicle and the iron-loaded group that did not receive vehicle. Therefore, the analysis of the D-Exo treatment was done against the combined values of the vehicle and non-vehicle iron-loaded groups. Treatment with D-Exo resulted in a 25% decrease in iron concentration in the heart and a 20% decrease in iron concentration in the liver ($P < 0.01$ for each). In the heart, there was a significant reduction when the D-Exo group was compared with the untreated, iron-loaded group but the apparent reduction was not significant when compared with the vehicle-treated, iron-loaded group alone. In the liver, D-Exo reduced iron concentrations significantly compared with either the untreated or vehicle treated groups.

Echocardiography

Results of echocardiograph measurements of left ventricular end-systolic and end-diastolic dimension and fractional shortening during systole are shown in Table 1. There were no changes in left ventricular dimensions or systolic function due to iron-loading or treatment in any of the groups studied.

Histology

In sections from two mice that were neither iron-loaded nor treated (No Iron; No Treatment group), hematoxylin and eosin (H&E) stains of the hearts, livers and spleens were normal and trichrome stains showed no evidence of fibrosis. By Prussian blue staining, neither mouse had iron visible in the heart, one mouse had trace amounts of iron in the liver, and both mice had trace amounts of iron in the spleen. There were similar findings in the one mouse studied that was treated with Vehicle only (No Iron; Vehicle group).

In two mice exposed to iron dextran and not treated with D-Exo, H&E staining of the heart, liver and spleen revealed evidence of interstitial iron but normal architecture, and trichrome staining showed no evidence of fibrosis in any of the three organs. Prussian blue stains of the heart demonstrated iron in interstitial cells primarily, with only occasional myocytes staining positively. In the liver, Prussian blue staining was dense in Kupffer cells and stippled in hepatocytes. In the spleen, the Prussian blue stain was strongly positive.

In one mouse exposed to 42 mg of iron dextran and treated with D-Exo, the histological findings were similar to those in the iron-loaded mice that were not treated with D-Exo. In heart, liver, and spleen, the H&E stain had evidence of interstitial iron but normal cellular architecture, and the trichrome stain was negative for fibrosis. In the heart, Prussian blue staining demonstrated iron in interstitial cells but only an occasional myocyte stained positively. In the liver, there was dense Prussian blue staining in Kupffer cells and stippled staining in hepatocytes. In the spleen, the Prussian blue staining was strongly positive. Representative sections are shown in Figure 6.

Urinary Iron Excretion

Measurements were carried out on Day 35 of the treatment period. Iron was not detected in the urine in iron-loaded mice that were not treated with D-Exo (data not shown). Three of the iron-loaded mice in the D-Exo treatment group were injected with 7 mg of D-Exo and placed into individual clean cages. Over an 8 h period following the injection, the fluid from each urination was collected and placed in a 1.5 ml microfuge tube. The tubes were frozen at -20^{o}C and subsequently analyzed for iron concentration.

Results are shown in Figure 7. Each mouse urinated three times between 50 and 250 μl per sample following injection with D-Exo. The mouse in panel B did not excrete detectable iron into the urine at the third voiding. Iron was not detected in the urine 24 h after iron chelation in any of the three mice. Therefore, there was rapid excretion of iron after injection of D-Exo that was easily detectable the first eight hours but was no longer detectable by 24 hours.

DISCUSSION

Patients with iron overload due to thalassemia major or other anemias requiring frequent transfusions will usually die by age 30 from heart failure or other complications if the excess iron is not removed (1). The only treatment previously available for patients with iron overload from multiple transfusions has been deferoxamine, a lipid-insoluble, hexadentate iron chelator that has limited capacity to enter cells and is rapidly metabolized. To be effective, it must be given in 12 or 24 hour subcutaneous or intravenous infusions on a daily basis (2,8). Deferoxamine prevents iron-dependent oxidative processes (4,10), removes cardiac and liver iron in animals (3,16), and prolongs survival in iron-overloaded animals (3) and thalassemia patients (1). However, the requirement for prolonged infusions is expensive and patient compliance is often poor.

Two chelators suitable for oral administration have been tested clinically in patients with thalassemia. Deferiprone is a bidentate molecule that has had limited ability to remove iron; severe toxicity has prevented it from being approved by the U. S. Food and Drug Administration (FDA) (17,18). In November 2005 the FDA approved deferasirox, a tridentate molecule, for clinical use. Given orally deferasirox effectively removed iron. However, it was associated with a number of side effects (19). These included nausea, abdominal pain, abnormal blood tests of liver and kidney function, hearing and visual disturbances, and rashes. Long term efficacy is unknown.

D-Exo has several properties that make it potentially attractive for the treatment of iron overload. It binds to iron with very high affinity, enabling it to slowly remove iron from

transferrin, lactoferrin and ferritin, but does not remove iron from hemoglobin or vital iron-containing enzymes (20). As the siderophore for *Mycobacterium tuberculosis*, it has evolved into a molecule capable of coexisting harmlessly with a human host. Neither in this study nor in earlier studies have we encountered any serious toxicity due to this agent. Because it is lipid soluble, D-Exo rapidly enters cells (10). In contrast, lipid-insoluble, water-soluble deferoxamine cannot readily enter cells and probably exerts its effects primarily in the extracellular fluid (7). While having the advantage of lipid solubility, D-Exo is sufficiently water soluble to allow easy handling and administration (9).

In addition to a capacity to create negative iron balance, optimally effective chelators should also neutralize the deleterious effects of iron during the removal process. A number of groups have presented evidence that iron overload is associated with oxidant injury (21-23). Desferri-exochelins and deferoxamine are hexadentate molecules that bind to all six of the active sites on the iron molecule, efficiently blocking hydroxyl radical formation, an important mechanism of iron-induced tissue injury (4, 5, 10). We have previously reported that D-Exo prevents oxidative injury in animal models of ischemia and reperfusion, a setting in which there is high activity of reactive oxygen species (10). Bidentate or tridentate molecules can block generation of this highly toxic free radical only if sufficient concentrations are present for two or three molecules to bind to each ferric ion – such concentrations could be difficult to achieve *in vivo* (24). Therefore, a potential detriment of bidentate or tridentate iron chelators is that their iron-chelator complexes could be capable of catalyzing reactions with oxygen species that generate more reactive free radicals capable of causing tissue injury (2, 5, 24).

In this study, we demonstrated that the hexadentate, lipid-soluble chelator D-Exo efficiently accesses multiple organs. After an i.v. bolus dose of tritium-labeled D-Exo in rats, blood counts peaked rapidly followed by disappearance with a $t_{1/2}$ of considerably less than an hour. Simultaneously, there was rapid uptake of counts in all 15 organs studied. Beginning within 60 minutes after injection of tritiated D-Exo, counts gradually decreased in all organs but were still substantial 24 h later. Uptake was substantial in the heart and liver, two organs highly susceptible to damage from clinical iron overload. Assuming that radioactivity remained associated with intact D-Exo, it appears this highly lipid-soluble chelator rapidly enters organs. Radioactive labeling does not allow distinction between iron-free and iron-bound exochelin. Since urinary counts were easily detectable within the first hour, there was rapid clearance by this route. In addition, the relatively high levels of radioactivity in the small intestine probably reflect considerable biliary excretion.

To assess actual decreases in cardiac and hepatic iron in response to D-Exo, we utilized a mouse model in which iron-loading was achieved by injection of iron dextran. When untreated, iron-loaded mice were sacrificed on day 64, eight weeks after iron-loading, there was a 4-fold increase in cardiac iron and a 20-fold increase in hepatic iron compared with the control group. This degree of iron overload over this relatively brief period of time did not result in cardiac decompensation or histologic evidence of cardiac or liver damage. Substantial amounts of histologically detectable iron were present in cardiac interstitial cells but only a few cardiac myocytes contained sufficient iron to stain positively with Prussian blue. No myocyte disruption, hypertrophy or fibrosis was detected.

Within two target organs, heart and liver, known to be vulnerable to damage in clinical iron overload syndromes; there were substantial reductions in iron content. In the liver, the reduction was 20% and in the heart the reduction was 25%. Since no echocardiographic abnormalities were noted in the untreated iron-loaded mice, we could not ascertain whether the D-Exo would have been efficacious in preventing iron-mediated cardiac injury. We did establish that iron was effectively removed from the heart and liver. We did not observe any D-Exo toxicity. Mice treated with D-Exo appeared to be healthy and gained weight normally.

Ideally, an iron chelator should be easy to administer in effective dosages, have a high affinity for iron, be able to remove iron from critical organs and from the body, have low toxicity, and be capable of preventing deleterious oxidative processes that could lead to organ injury. Deferoxamine, the treatment currently available, exhibits toxicity at high doses and requires prolonged daily infusions to reduce iron accumulation effectively. In clinical settings, compliance with a daily regimen of deferoxamine is poor (2). The diffusible, oral chelators deferiprone and deferasirox reduce total body iron loads, although their long term efficacy is unknown. Both have significant side effects and, because they lack the hexadentate structure of D-Exo and deferoxamine, their iron-bound complexes could theoretically participate in oxidative reactions (2, 5, 24). D-Exo, a lipid-soluble, hexadentate, high-affinity iron chelator, given as intraperitoneal boluses in mice three times a week, effectively removed excess iron from the heart and liver, organs that are susceptible to injury from long-term exposure to iron-overload conditions. The lack of evidence of toxicity of D-Exo to date is also attractive. While the characteristics of

this agent are potentially advantageous, whether it will prove to be clinically useful is unknown. Further studies will be required at increased doses to ascertain whether larger amounts of iron can be removed without serious toxicity and whether oral administration is effective.

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Figure Legends

Figure 1. Structure of iron exochelins.

The structure of Exochelin 772SM is shown in the iron-bound form. The molecular weight is 772 in the iron-bound form and 719 in the D-Exo form.

Figure 2. Blood radioactivity disappearance curves for two rats treated with a bolus of i.v. D-Exo.

Each rat was injected intravenously with tritium-labeled D-Exo, after which blood was obtained at the time-points indicated. DPM/ml of blood was determined as described in Methods. Each point represents the average of the counts for two 10 μl-aliquots of blood.

Figure 3. Organ radioactivity disappearance curves for rats treated with a bolus of i.v. D-Exo.

Rats were injected with tritium-labeled D-Exo, euthanized 15 min, 1h, 4h, or 24h later, and 15 tissues were assayed for radioactivity as described in Methods. Each point represents the mean ± SE of the log DPM/g counts for 3 (4h time point) or 4 (15 min, 1h, and 24 h time points) animals.

Figure 4. Urine radioactivity in rats treated with a bolus of i.v. D-Exo.

Counts in urine samples collected postmortem from the bladder in rats euthanized at the time intervals shown following administration of a bolus of D-Exo. Values are mean ± S.E. in data from three rats at each time point.

Figure 5. Myocardial and hepatic iron levels in iron overload mice.

Myocardial (Top Panel) and Hepatic (Bottom Panel) iron concentrations. Open bar: No Iron; Vehicle group; hatched bar: Iron; D-Exo group; closed bar: Iron; Vehicle group; crosshatched bar: Iron, No treatment group.

Figure 6. Histology in myocardium of iron-loaded, D-Exo-treated mice

H & E (A), Prussian Blue (B), and Trichrome (C) stains of myocardium from one iron-loaded mouse treated with D-Exo. The H & E stain demonstrates normal myocyte architecture. The Prussian Blue stain reveals moderate amounts of iron that is mostly interstitial but also present in myocytes. The Trichrome stain shows no evidence of fibrosis.

Figure 7. Urinary Iron excretion after treatment with i.p. D-Exo.

Panels A-C depict the iron in the urine of three individual iron-loaded mice. Each mouse urinated three times between 50 and 250 μl in the 8 h period after injection with 7 mg D-Exo. Urine collection and iron concentration determination are described in Methods. The mouse in panel B did not excrete detectable iron in the urine at the third voiding.

Figure(s)

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Figure 1

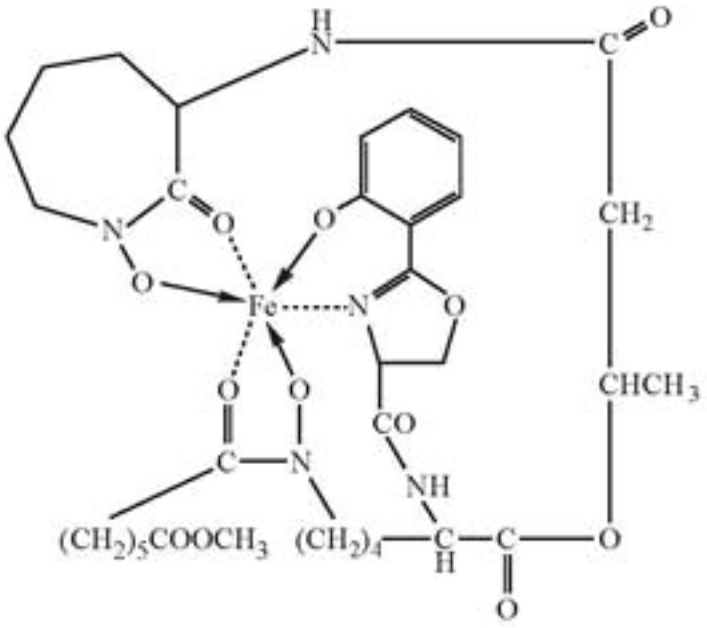

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Figure 2

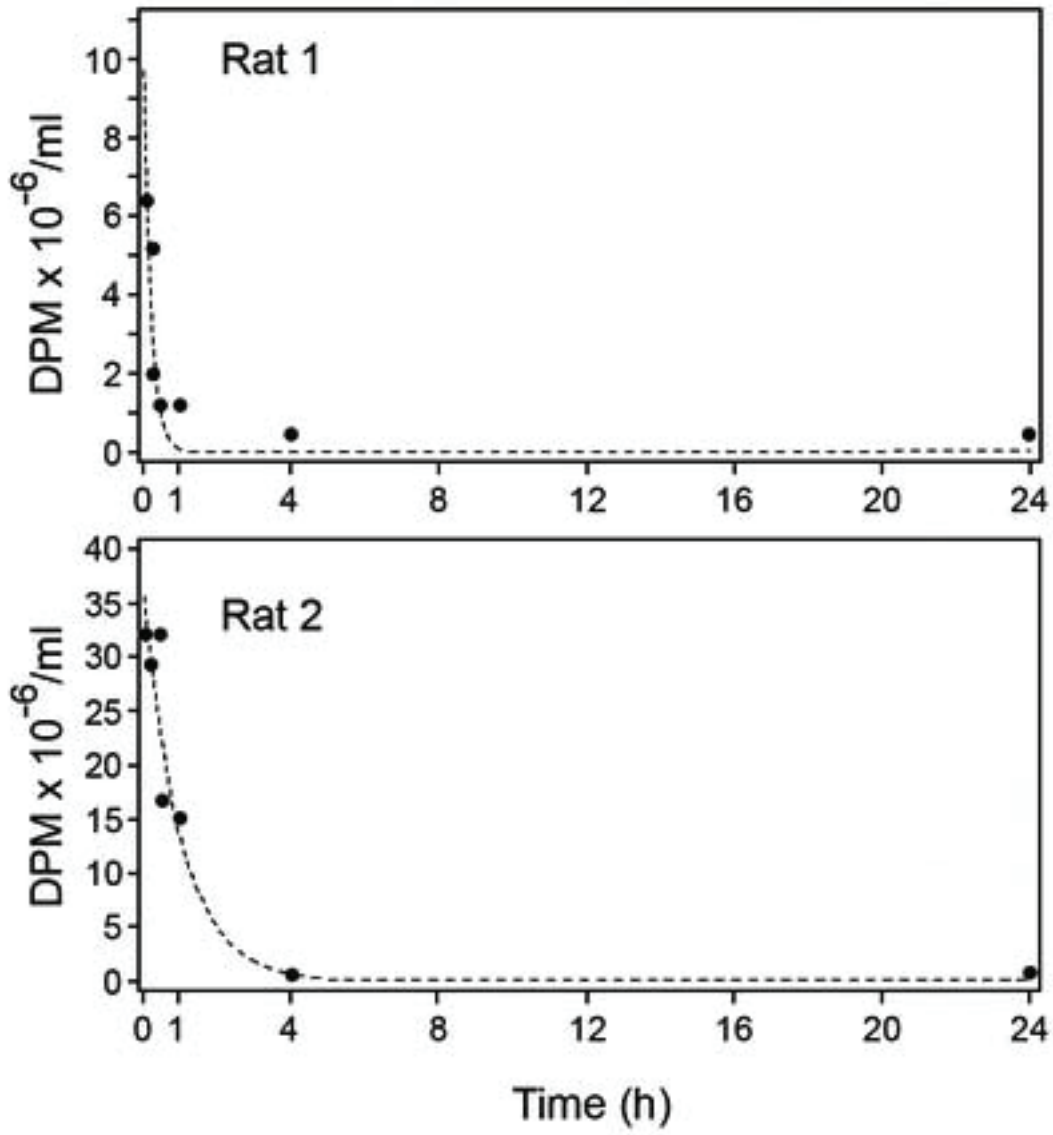

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Figure 3

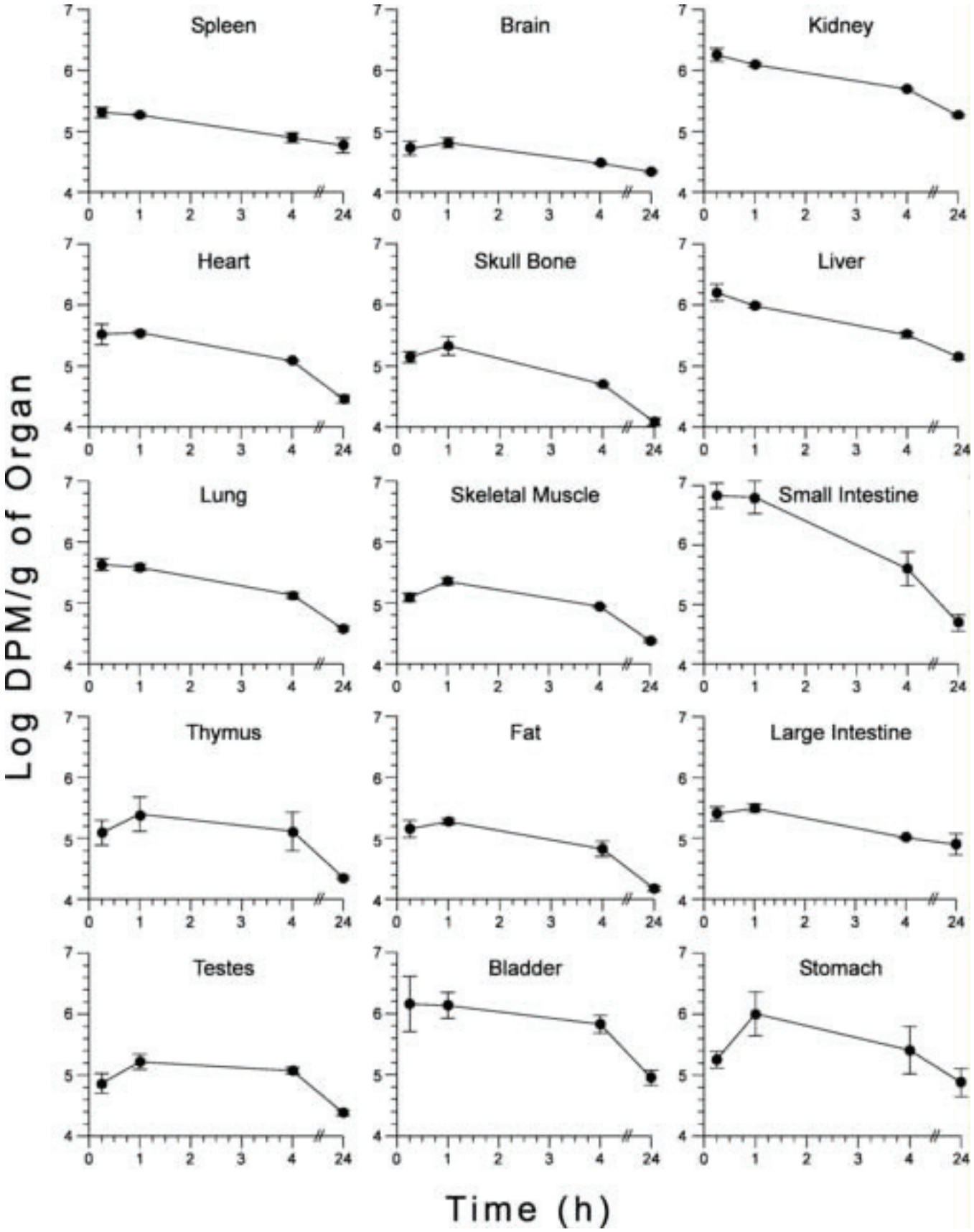

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Figure 4

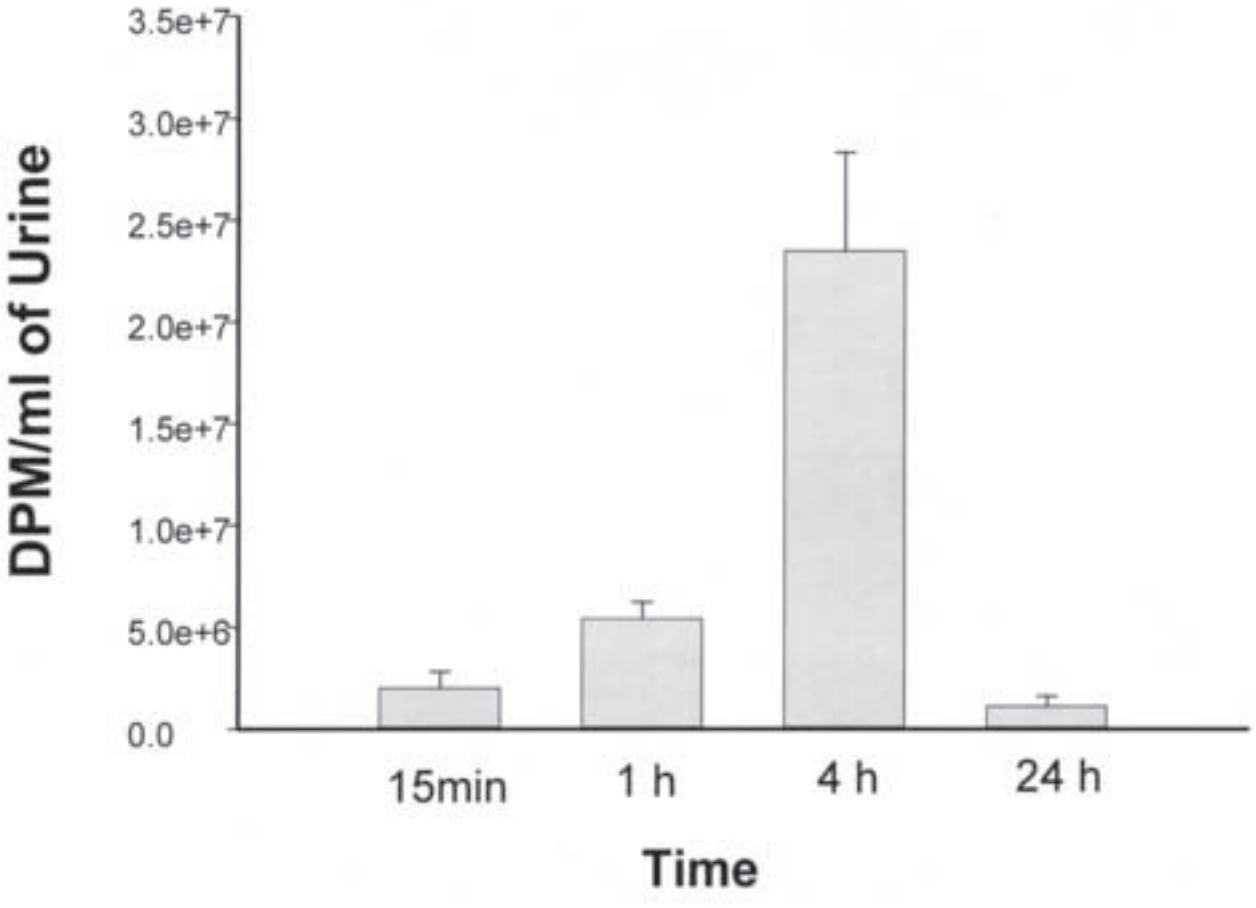

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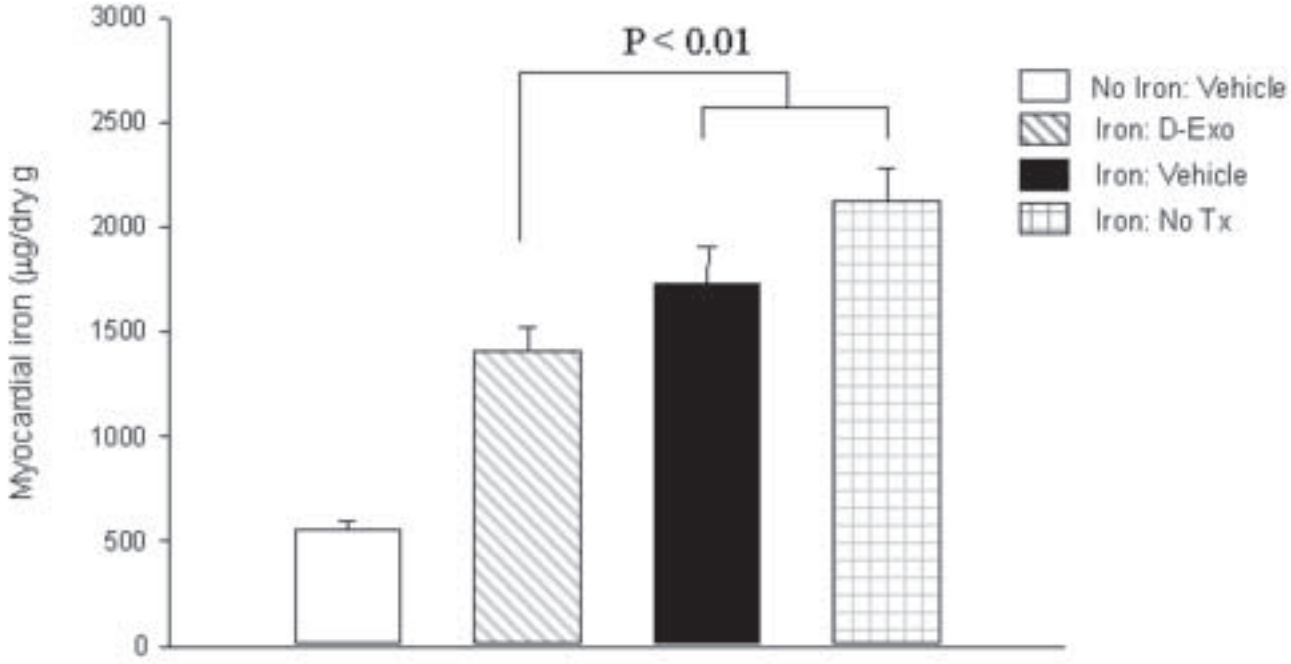
Figure 5



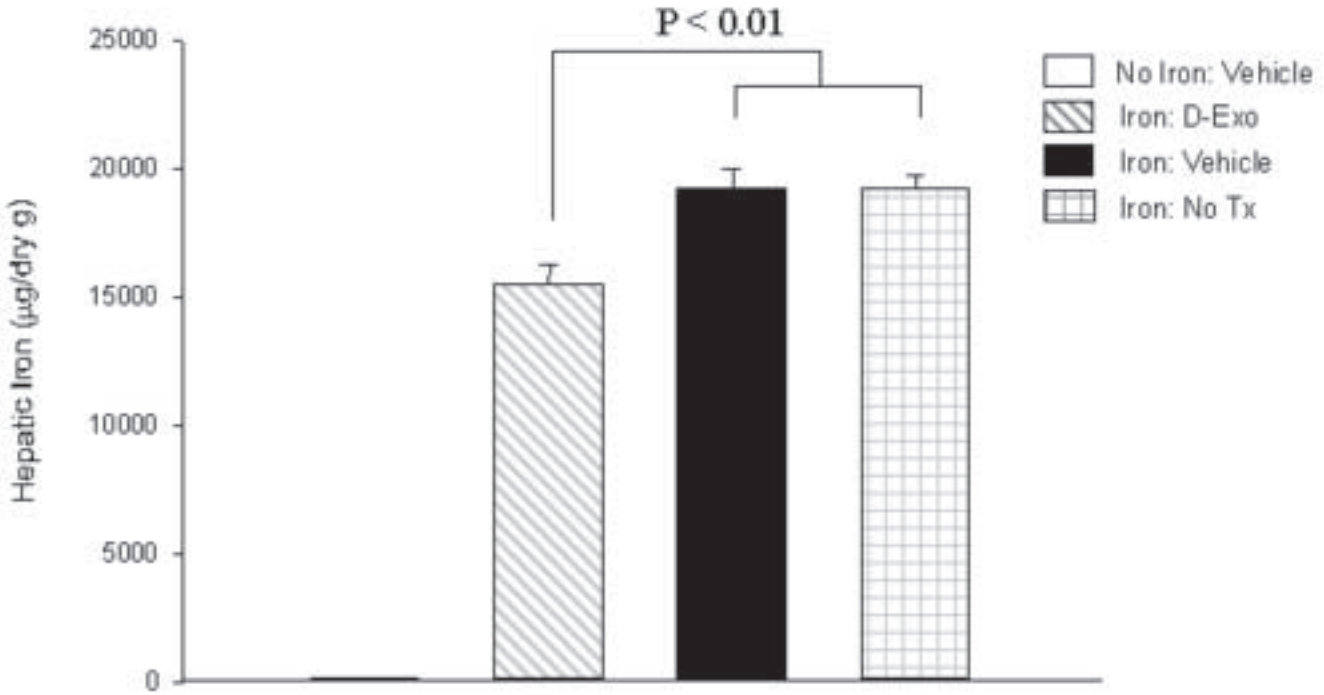


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Figure 6

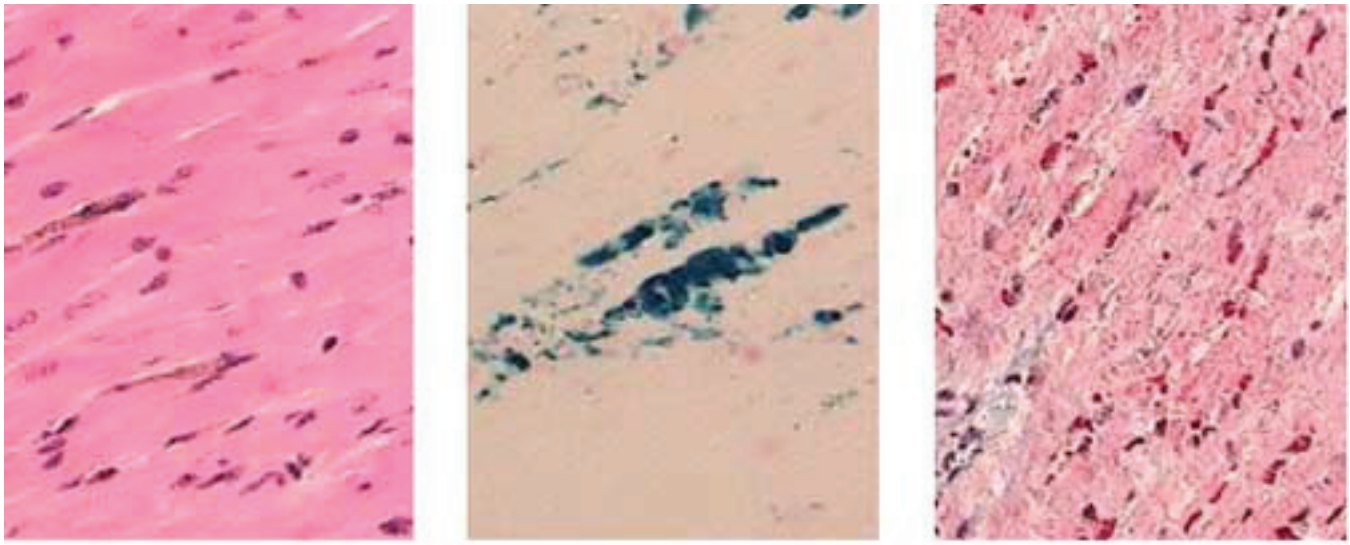

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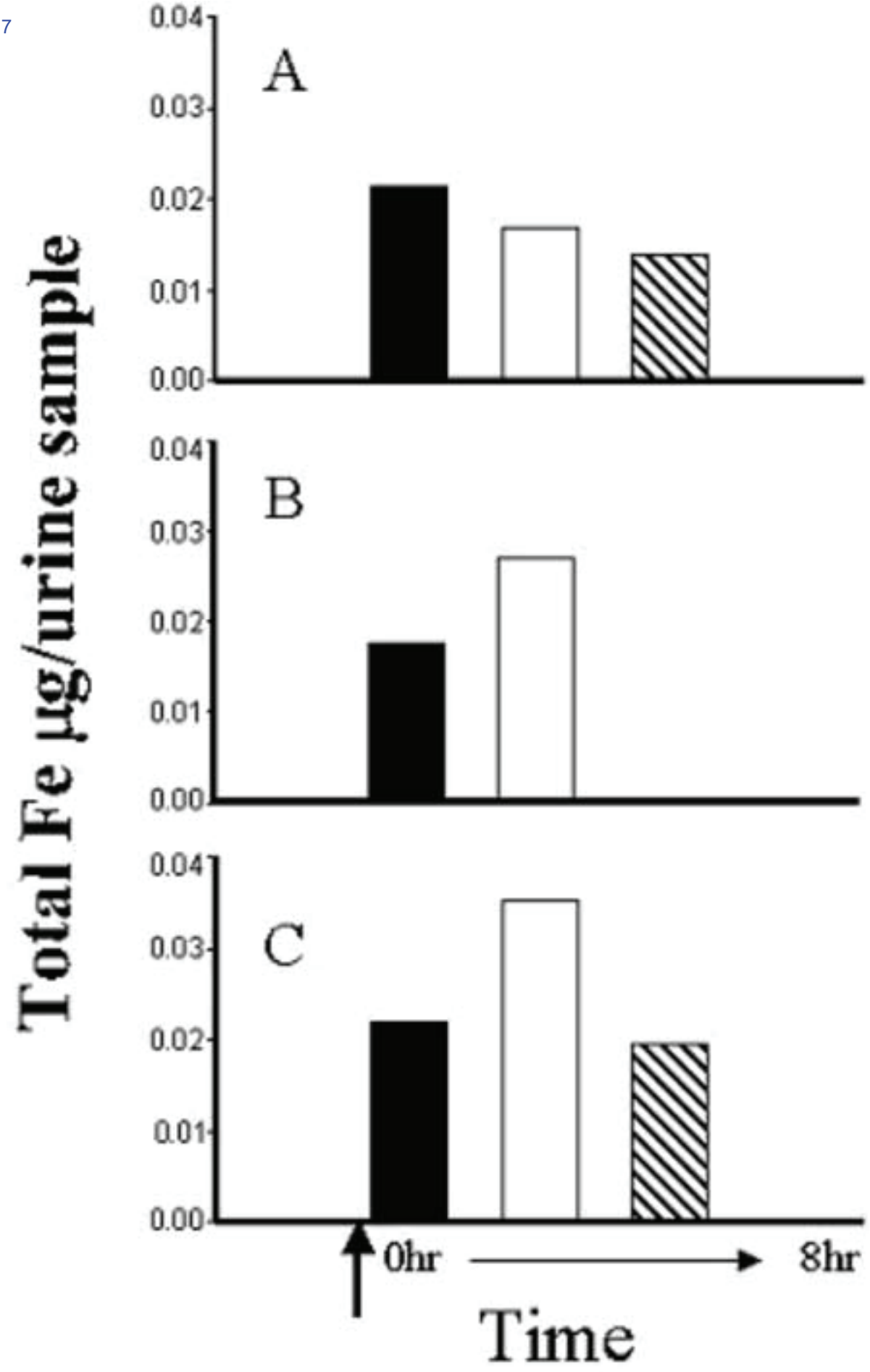
Figure 7



Table(s)

	DAY 0			DAY 64		
	LVIDd	**LVIDs**	**%FS**	**LVIDd**	**LVIDs**	**%FS**
No Iron;No Treatment n=4	3.91 ± 0.07	1.44 ±0.06	63 ± 1	3.56 ± 0.07	1.72 ± 0.05	52 ± 1
No Iron; Vehicle n=5	3.73 ± 0.1	1.42 ± 0.04	62 ± 2	3.88 ±0.07	1.68 ± 0.07	57 ± 2
Iron; No Treatment n=6	3.70 ± 0.05	1.44 ± 0.04	61 ± 1	3.69 ± 0.02	1.54 ± 0.04	58 ± 1
Iron; Vehicle n=8	3.66 ± 0.04	1.47 ± 0.05	60 ±1	3.84 ± 0.04	1.68 ±0.05	56 ± 1
Iron; D-Exo n=8	3.77 ± 0.06	1.48 ± 0.06	61 ± 1	3.68 ± 0.1	1.79 ±0.05	51 ± 1

LVIDd= left ventricular end diastolic dimension, LVIDs= left ventricular end systolic dimension, %FS = fraction shortening Values are mean ± S.E.