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Iron Does Not Cause Arrhythmias in the Guinea Pig Model of Transfusional Iron Overload

Lana Kaiser,[1,*] John Davis,[1] Jon Patterson,[4] Ryan F Boyd,[1] N Bari Olivier,[4] George Bohart,[3] and Kenneth A Schwartz[1]

Cardiac events, including heart failure and arrhythmias, are the leading cause of death in patients with β thalassemia. Although cardiac arrhythmias in humans are believed to result from iron overload, excluding confounding factors in the human population is difficult. The goal of the current study was to determine whether cardiac arrhythmias occurred in the guinea pig model of secondary iron overload. Electrocardiograms were recorded by using surgically implanted telemetry devices in guinea pigs loaded intraperitoneally with iron dextran (test animals) or dextran alone (controls). Loading occurred over approximately 6 wk. Electrocardiograms were recorded for 1 wk prior to loading, throughout loading, and for approximately 4 wk after loading was complete. Cardiac and liver iron concentrations were significantly increased in the iron-loaded animals compared with controls and were in the range of those reported for humans with thalassemia. Arrhythmias were rare in both iron-loaded and control guinea pigs. No life-threatening arrhythmias were detected in either group. These data suggest that iron alone may be insufficient to cause cardiac arrhythmias in the iron-loaded guinea pig model and that arrhythmias detected in human patients with iron overload may be the result of a complex interplay of factors.

Abbreviation: ECG, electrocardiogram

Iron absorption changes in response to dietary intake, total body iron, and requirements for production of red blood cells. Normally, iron absorption is highly regulated by altering dietary iron absorption from the small intestine.[3] This tight regulation is critical because humans have no mechanism to excrete excess iron; consequently, absorbed iron stays in the body. Iron overload occurs when the control of intestinal absorption of iron is either altered or circumvented.

Transfusional iron overload results from repeated transfusion of red blood cells, as seen in persons with intractable anemia caused by thalassemia, sickle cell disease, bone marrow failure, or aggressive treatment of cancer.[4,22,31] Although transfusions are now routine and life-saving, repeated transfusions bypass iron regulatory mechanisms and result in iron overload. Each unit of blood contains approximately 250 mg of iron,[28] and with no mechanism for iron excretion, iron overload can occur relatively rapidly. Clinically, problems with iron overload develop after the patient has received about 100 units of red blood cells,[28] or approximately 25 g of iron. Initially iron is stored in the reticuloendothelial cells.[3] However, once these storage sites become saturated, iron is deposited directly into the parenchyma of liver, heart, and endocrine and other organs, resulting in multiorgan dysfunction.[3,4,21,22,31]

The thalassemias, one of the major hemoglobinopathies, are a group of inherited defects in the rate of synthesis of one or more or the globulin chains. This results in imbalance of chain production, ineffective erythropoiesis, hemolysis, and variable degree of anemia.[4,31] Although genetically and clinically diverse, the thalassemias are considered the most common genetic disorder worldwide, and carriers of the disorders are estimated to comprise 3% of the world's population.[4,31] The hallmark of β thalassemia, first described by Cooley, is the development of anemia and other symptoms within the first year of life.[4,31] However, symptoms of classic β thalassemia anemia (stunted growth, abnormal bone growth, hepatosplenomegaly, and features of the hypermetabolic state associated with anemia) are seen only in patients who are not transfused appropriately. If pediatric patients receive adequate red cell transfusions, growth and development are normal, and few complications are evident during the first decade of life. However, the effects of iron overload from repeated transfusions become apparent around the end of the first decade. Therefore, although repeated transfusions have prolonged the lifespans of patients with β thalassemia, transfusion-associated iron overload is now a leading complication of the disease.[4,31] The primary determinant of morbidity and mortality in patients with transfusion-dependent β thalassemia is iron's effect on the heart. Despite the fact that iron chelators have been available for more than 30 y, 50% of patients with β thalassemia die before their 35th birthday, the majority from cardiac complications.[4,31]

The clinical course of patients with transfusion-dependent thalassemia results from a combination of the disease itself and the consequences of repeated red cell transfusion.[4,31] Although the goal of treatment is to decrease the complications associated with thalassemia, the outcome of repeated transfusion is iron overload. The excess iron results in cardiac, hepatic, endocrine and skeletal disorders, and cardiac complications are the major cause of death in individuals with β thalassemia.[4,12,14,18,22,31] Although the human clinical literature alludes to a link between iron overload and cardiac arrhythmias, these potentially lethal events are

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Department of Hematology and Oncology, [1]College of Human Medicine, [2]Department of Small Animal Clinical Sciences, [3]Department of Large Animal Clinical Sciences, [4]Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI.
[*]Corresponding author. Email: kaiser@msu.edu

difficult to study in a clinical setting. Whereas early detection and treatment of arrhythmias should decrease morbidity and mortality, electrocardiographic changes are infrequent and unpredictable.[25] In addition, separating the role of iron from other confounding influences, including heart failure, anemia, diabetes, pharmacologic agents, acid–base–electrolyte disturbances, hypertension, smoking and other lifestyle choices, is impossible. Because the survival of patients with transfusional iron overload is determined primarily by the degree of iron-induced cardiac toxicity, it is important to characterize the types and frequencies of arrhythmias and correlate them with tissue iron concentrations. This goal can best be achieved in an animal model of transfusional iron overload.

There are several rodent models of iron overload, including models of both primary (hereditary hemochromatosis) and secondary (transfusional) iron overload. In the early 1990s, Carthew and colleagues[7-9] noticed that gerbils with endotoxin-induced hemorrhage developed hepatic fibrosis and iron accumulation, and they suggested that the gerbil could be a useful model of iron overload. At about the same time, the guinea pig model of transfusional iron overload was developed and characterized by Adams.[1,27,28] Using this model, we previously reported delayed conduction in Purkinge fiber–papillary muscle preparations from iron-loaded guinea pigs when compared with controls.[28] Delayed conduction occurred in the absence of cardiac contractile abnormalities and at a relatively low total dose of iron. The unexplained sudden death of 25% of guinea pigs iron-loaded to 1.5 g/kg suggested that death was related to cardiac arrhythmias. These data, coupled with reports that iron-loaded gerbils show delayed conduction and develop cardiac arrhythmias,[7,16,20,23,32,33] suggested to us that the iron-loaded guinea pig could provide a useful model of iron-induced cardiac arrhythmias. Our goal was to evaluate the development of cardiac arrhythmias in the guinea pig model of transfusional iron overload, using a dose of iron that is known to cause delayed conduction without effecting contractility in vivo.[28]

Materials and Methods

Animals. The experiments reported here were approved by the All-University Committee on Animal Use and Care at Michigan State University (East Lansing, MI). Female Hartley guinea pigs (*Cavia porcellus*, strain Mdh:SR[A]; weight, approximately 600 g; Bioport, Lansing, MI) were housed in the University Laboratory Animal Resources Facility. Guinea pigs tested negative for simian virus 5, reovirus, Sendai virus, lymphocytic choriomeningitis virus, and pneumonia virus of mice. Guinea pigs were housed individually in standard rat 'shoebox' cages (floor space, 143 in.^{2}; height, 7 in.), bedded on paper chips (Shepherd Specialty Papers, Watertown, TN), fed free-choice guinea pig chow (Guinea Pig Diet 7006, Harlan Teklad, Madison, WI), and provided with tap water in dishes. The room temperature was maintained at approximately 21 °C with a 12:12-h light:dark cycle.

Guinea pigs were allocated into 4 groups: iron-loaded, monitored (n = 10); iron-loaded, not monitored (n = 3); control, monitored (n = 5); and control, not monitored (n = 3). Monitored animals were surgically implanted with radiotelemetry devices for assessment of arrhythmias. In addition, tissue iron was measured from monitored animals. Because the entire heart was used to measure iron, histopathology of heart and liver was evaluated by using tissues from nonmonitored animals.

Chronic instrumentation with telemetry devices. Guinea pigs were anesthetized with isoflurane, and surgery was done in a designated surgical suite. All animals received the analgesic butorphanol (0.2 mg/kg) subcutaneously intraoperatively. For ambulatory long-term electrocardiogram (ECG) analysis in the conscious state, telemetry devices (model TA11 CTA-F40; Data Sciences International, St Paul, MN) were implanted by using a modification of the method of Gehrmann.[15] Under aseptic conditions, a midline incision was made on the back along the spine. An implantable wireless radiofrequency transmitter was inserted into a subcutaneous tissue pocket, and the leads were tunneled subcutaneously after infusion of lidocaine. One lead was tunneled to an area to the left of the scapula toward the apex of the heart and the second to the right. Both leads were anchored in place with a permanent suture. Incisions were closed with 4-0 Vicryl (Ethicon, Johnson & Johnson, Cincinnati, OH). Guinea pigs tolerated this procedure well and were eating, drinking, and engaging in usual behavior in less than 4 h after surgery. The use of intraoperative butorphanol and lidocaine appeared to manage postoperative pain successfully. After implantation of the transmitter, guinea pigs were housed in individual cages placed over a telemetry receiver (RPC-1, Data Sciences International) and were allowed 2 wk for recovery before initiation of the iron-loading protocol.

Iron loading. Both monitored and non-monitored animals were assigned randomly to iron-loaded or control groups. Iron-loaded animals were injected with iron dextran (Vedco, St Joseph, MO) intraperitoneally, and controls received dextran (Sigma Chemical, St Louis, MO). To decrease the chance of infection, iron dextran was purchased in 100-ml multiuse bottles and divided into single-use aliquots under sterile conditions. Each animal received the appropriate amount of iron in a single-use 1-ml syringe with 25-gauge needle. All syringes were prefilled and labeled prior to entry into the animal room.

The goal was to achieve a final iron load of approximately 2 g/kg. Because we previously identified delayed conduction in vitro at less than 25% this iron load,[28] we hypothesized that we would be able to detect arrhythmias at this load and then follow their development and progression. Guinea pigs were injected intraperitoneally after the skin was prepared with alcohol. Injections were made twice weekly with iron (50 or 100 mg/ml) at a dosage of either 200 or 400 mg/kg weekly. Initially iron was diluted to 50 mg/ml to facilitate accurate measurement. As the animals grew, the dose increased; consequently, to decrease the volume of the injection, the undiluted iron dextran (100 mg/kg) was used. In the first series of experiments, the dosage rate was 200 mg/kg weekly; because we determined that iron was excreted in the urine and bile of the guinea pig (data not shown), the dosage rate then was increased to 400 mg/kg weekly. The animals were weighed weekly, and the amount of iron injected was based on the previous week's weight. Mean duration of iron loading in monitored guinea pigs was 44 d (range, 37 to 51 d) and mean duration of dextran control loading in monitored guinea pigs was 42 d (range, 36 to 51 d).

ECG data acquisition and analysis. ECG traces were recorded during the 2-wk period after surgery, during the entire loading schedule, and for approximately 74 d after loading began. There was no significant difference between groups in the number of days to load, number of days monitored, or number of days monitored after loading was complete (data not shown). Recording

Table 1. Heart weight, liver weight, and cardiac and hepatic iron concentrations in iron- loaded (n = 8) and control (n = 5) guinea pigs

	Heart weight (mg)	Liver weight (mg)	Cardiac iron (mg/g dry wt)	Hepatic iron (mg/g dry wt)
Iron-loaded	1.8 ± 0.17	39.0 ± 2.5[a]	3.2 ± 0.42[b]	27.8 ± 2.6[c]
Control	2.3 ± 0.16	30.6 ± 1.0	0.26 ± 0.02	0.95 ± 0.15

[a]$P < 0.01$ compared with weight of liver from control animals.
[b]$P < 0.0001$ compared with concentration from control animals.
[c]$P < 0.00001$ compared with concentration from control animals.

was done at predetermined frequencies, intervals, and durations (5 to 10 s of recording every 10 to 15 min). Traces were visually reviewed daily. The decision to record at predetermined intervals, rather than continuously, was based several factors: (1) no ECG analysis software has been used and validated on guinea pig data; (2) preliminary experiments suggested that this protocol was adequate to identify arrhythmias; and (3) 10-s random recordings previously identified arrhythmias in iron-loaded gerbils.[23] The protocol called for increasing the frequency and duration of recording when abnormalities were noted. If the observer noted what appeared to be an abnormality, the frequency and duration of recording was increased (for example, 20 s every 5 min). Abnormalities were not evaluated by a veterinary cardiologist until the completion of all experiments.

Electrocardiogram analysis. ***Arrhythmia analysis.*** The primary outcome measure was the development of arrhythmias. Total number of arrhythmias, total number of pathologic arrhythmias, and the number of arrhythmias per minute were compared among groups. The endpoint of these experiments was the development of significant arrhythmias, not death. Significant pathologic arrhythmias were defined as brady- or tachyarrhythmias of sufficient duration to interfere with normal tissue perfusion, including sinus irregularity with bradycardia, atrial fibrillation or flutter, second and third degree atrioventricular block, junctional rhythms, runs of ventricular tachycardia, and ventricular fibrillation. Sinus arrhythmias, premature atrial contractions, and bundle branch blocks were not considered significant, but their presence was noted.

All traces were reviewed for arrhythmias. Any trace that appeared abnormal (either iron-loaded or control) was printed and placed sequentially in a notebook; the total number of abnormalities for each animal was noted. Traces were evaluated blindly by a veterinary cardiologist (NBO) and the type of arrhythmia noted on a checklist. Numbers and types of arrhythmias for each animal were recorded, and total number of arrhythmias, total number of pathologic arrhythmias, and number of arrhythmias per minute were compared among groups.

Heart rate and interval analysis. Three traces per week per animal were used for the measurement of heart rate, P duration, PR interval, QRS duration, and QT interval. Traces from midnight on Monday, Wednesday, and Friday of each week were used for analysis. Five traces were printed per page, and the clearest trace used for heart rate and interval analysis. If a trace was not suitable for interval analysis, traces on either side of the designated time point were used. All measurements were made manually by using a caliper. The mean value for each animal for each week was used to compare heart rate and intervals between weeks and among groups.

Necropsy. At the end of the study, all animals were euthanized by pentobarbital overdose and underwent gross necropsy. The heart and sections of liver from monitored animals were collected for iron determination. Sections of heart and liver from nonmonitored guinea pigs were collected for histopathologic examination. Tissues and organs from monitored animals that showed gross evidence of infection were collected for culture and histopathologic examination.

Tissue iron measurements. Cardiac and hepatic iron concentrations were determined by inductively coupled plasma emission spectroscopy as previously described.[2,28] Briefly, the entire heart and a section of liver were dried and acid-digested. This assay can be used to generate multielement profiles, including iron, and was done in the Toxicology Laboratory of the Diagnostic Center for Population and Animal Health, Michigan State University.

Histopathology. Sections of heart and liver from nonmonitored iron-loaded and control guinea pigs were processed routinely for histopathologic examination and stained with hematoxylin and eosin, Masson trichrome, and prussian blue stains. Sections were evaluated blindly by a veterinary pathologist (JP).

Statistics. Data are expressed as mean ± standard error of the mean (SEM). Comparisons of body weight, heart rate, and ECG intervals were made among groups (iron and control) at each time point and for each group over time by using multifactorial analysis of variance. One-time comparisons among groups (tissue iron, tissue weights) were made using unpaired *t* tests. A *P* value of less than 0.05 was the criterion of statistical significance. Data were analyzed after being entered into a statistical software package (SPSS, Chicago, IL).

Results

Guinea pigs. Duration of loading was not significantly different among groups (iron, 44.3 ± 2.7 d [range = 37 to 51 d]; control, 41.8 ± 3.4 d [range = 36 to 51 d]). Iron-loaded guinea pigs achieved a total calculated iron dose of 1.8 ± 0.1 mg/kg. Cardiac and hepatic iron concentrations in iron-loaded guinea pigs were significantly greater than those of controls (Table 1). Iron-loaded guinea pigs developed darkly pigmented skin but maintained normal behavior and gained weight throughout the experiment. Weight of guinea pigs was not different among groups prior to loading (iron, 671 ± 29 g; control = 663 ± 29 g), during the loading protocol, or at euthanasia (iron, 710 ± 28 g; control, 774 ± 38 g). With the exception of 1 monitored guinea pig found dead from an intestinal torsion 27 d after loading was complete, there was no mortality. One iron-loaded guinea pig was euthanized 15 d into the loading protocol because the transmitter had worked its way through the skin.

Arrhythmia analysis. ***Heart rate and intervals.*** As expected with growth, heart rate decreased significantly over time in both groups. Heart rate was greater in control animals at 4 of the 12 time points measured (Figure 1). Analysis of intervals revealed no specific pattern: P duration was greater in iron-loaded animals at 2 of the 12 time points; the PR interval was greater in controls at 3 time points; the QRS interval was different between the 2 groups

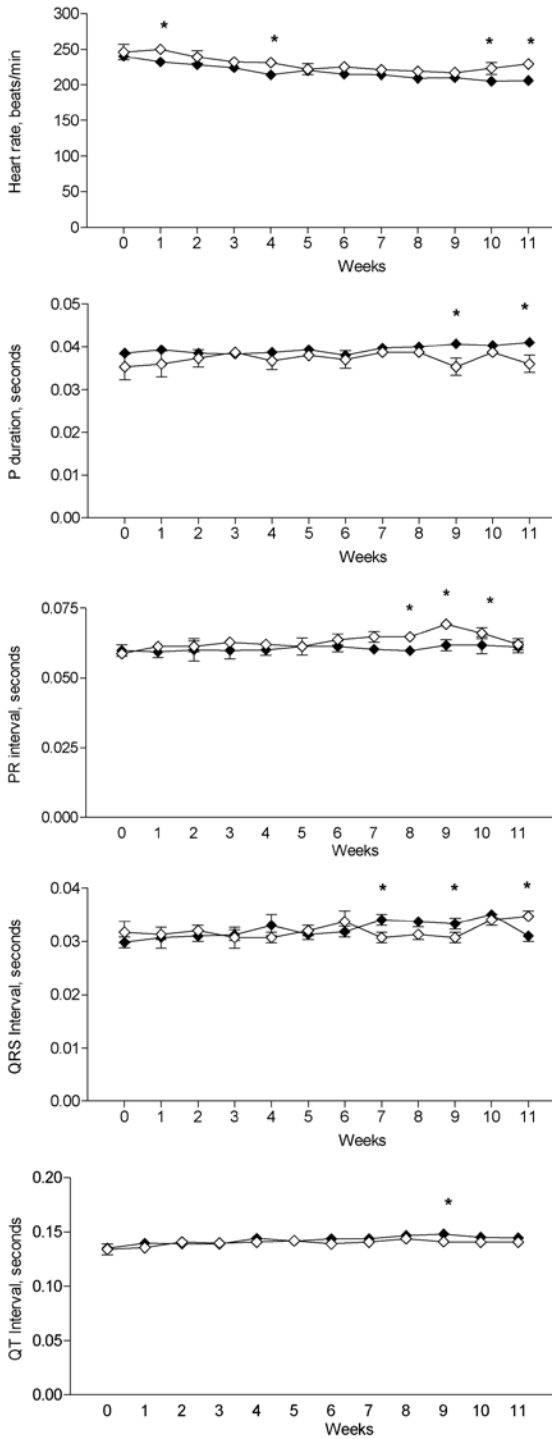

Figure 1. Heart rate, P duration, PR interval, QRS interval, and QT (listed in order from top to bottom) from electrocardiograms of iron-loaded (solid diamonds) and control (open diamonds) guinea pigs over time. Data are expressed as mean ± standard error of the mean. Zero represents before iron loading. Electrocardiogram traces were recorded before loading (week 0), during the entire loading schedule (iron, 44.3 ± 2.7 d [range, 37 to 51 d] versus control, 41.8 ± 3.4 d [range = 36 to 51 d]), and for approximately 74 d after loading began.*, $P < 0.05$ between values for iron-loaded and control animals at that time point. No trends or patterns were noted in any parameter measured. For iron-loading: n = 10 for weeks 0 through 3; n = 9 for weeks 4 through 5; n = 8 for weeks 7 through 9), and n = 7 for week 10. For control: n = 5 for weeks 0 through 7 and n = 3 for weeks 8 through 10.

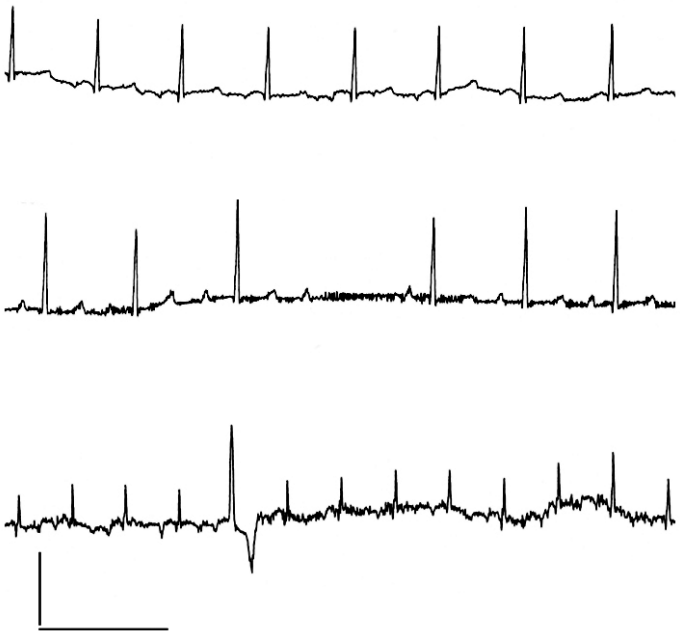

Figure 2. Representative electrocardiogram traces from guinea pigs showing a normal trace (top panel), second-degree block (middle panel), and premature ventricular contraction (bottom panel). The horizontal line represents 0.5 s; the vertical line indicates 0.5 mV.

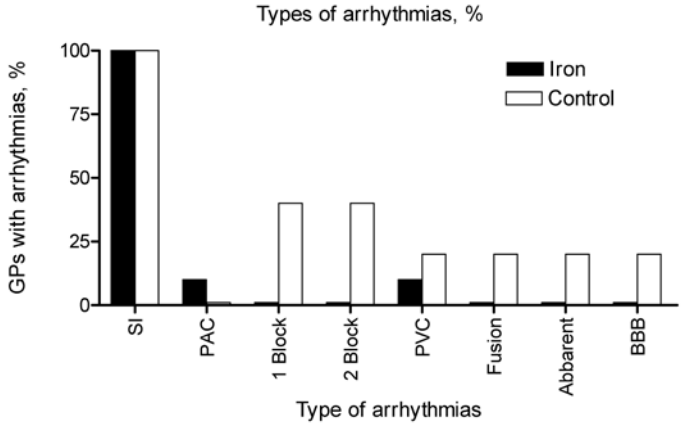

Figure 3. Types of arrhythmias noted in iron-loaded and control guinea pigs. SA, sinus arrhythmia; SI + brady, sinus irregularity with bradycardia; PAC, premature atrial contraction; 1 BLOCK, first-degree atrioventricular block; 2 BLOCK, second-degree atrioventricular block; PVC, premature ventricular contraction; Aberrant, apparent beats; BBB, bundle branch block. Control guinea pigs showed a greater variety of arrhythmias than did iron-loaded animals.

at 3 time points; and the QT interval was greater in iron-loaded animals at 1 point (Figure 1).

Arrhythmias. Arrhythmias were rare in both iron-loaded and control guinea pigs (Figures 2 and 3). There was no statistical difference among groups in total number of arrhythmias, total number of pathologic arrhythmias, number of minutes observed, or number of arrhythmias per minute (Table 2). The most common arrhythmia noted was sinus arrhythmia, which was seen in all guinea pigs and not considered pathologic. Types of arrhythmias noted are shown in Figure 3. The most common pathologic arrhythmia seen was sinus irregularity with bradycardia, which occurred in 6 iron-loaded and 2 control guinea pigs. Four iron-

Table 2. Summary of arrhythmia data from iron-loaded (n = 10) and control (n = 5) guinea pigs

	Total no. of arrhythmias	No. of arrhythmias/min	Total no. of pathologic arrhythmias	No. of pathologic arrhythmias/min
Iron-loaded	9.4 ± 4.5 (0 to 47)	0.08 ± .02 (0 to 0.26)	2.3 ± 0.9 (0 to 8)	0.05 ± 0.03 (0 to 0.03)
Control	18.8 ± 11.1 (4 to 63)	0.092 ± 0.02 (0 to 0.12)	4 ± 1.4 (0 to 8)	0.04 ± 0.1 (0 to 0.1)

Data are given as mean ± standard error of the mean (range). Observation periods did not differ significantly between groups (iron-loaded, 145.2 ± 49.8 min [15 to 538 min]; control, 181.7 ± 89.2 min [83 to 538 min]). Two iron-loaded guinea pigs did not complete the entire protocol (see text). There were no significant differences detected ($P > 0.05$).

loaded and 1 control guinea pig had no pathologic arrhythmias recorded. Three iron-loaded and 1 control animal had a single type of pathologic arrhythmia; 5 iron-loaded guinea pigs demonstrated 2 different types of pathologic arrhythmias; and 3 control animals had 3 different types of pathologic arrhythmias. Atrial fibrillation, ventricular tachycardia, and ventricular fibrillation were not noted in any guinea pig. One iron-loaded guinea pig was noted to have biphasic P waves throughout the experiment, and 1 control guinea pig was noted to have tall P waves—when compared with P waves from other guinea pigs, throughout the experiment.

In iron-loaded guinea pigs, the total number of arrhythmias observed throughout the experimental protocol ranged from 0 to 47 per animal, whereas the number of pathologic arrhythmias ranged from 0 to 8. In control guinea pigs, the total number of arrhythmias observed throughout the experimental protocol noted ranged from 4 to 63 per animal, whereas the number of pathologic arrhythmias ranged from 0 to 8. Neither the number of minutes recorded nor the mean number of arrhythmias (total or pathologic) per 1-min interval differed significantly among groups ($P > 0.05$; Table 2).

Necropsy. When compared with controls, all iron-loaded animals had dark red-brown staining of the skin, muscles, and internal organs. Other than pigmentation changes, the only other gross abnormality was hepatomegaly. The liver was significantly ($P < 0.01$) larger in iron-loaded animals compared with controls, but there was no difference in heart weights between groups (Table 1). There was no gross evidence of infection in any guinea pig. One iron-loaded guinea pig was found dead 27 d after the last iron dose. Cause of death was related to a strangulated loop of ileum that had become entrapped through a tear in the mesentery.

Histopathology. In sections of heart from all 3 nonmonitored iron-loaded animals, moderate numbers of hemosiderin-containing macrophages were scattered throughout the fibrous tissue stroma of the epicardium, myocardium, and endocardium, including valvular endocardium. The degree and distribution of iron pigment were similar in all 3 animals and as described previously.[27] Sections of liver from all 3 iron-loaded animals showed numerous small to large, predominantly randomly scattered aggregates of macrophages containing hemosiderin as previously described.[27] The aggregates were in sinusoids and portal triads. The capsular surface of liver sections was mildly to moderately irregular in contour. The irregularity of the capsule was not associated with fibrosis but apparently was due to accumulation of hemosiderin-laden macrophages in the capsule and the large cellular aggregates in the adjacent hepatic parenchyma. In general, however, fibrosis was relatively mild compared with the degree of iron accumulation. Sections of heart and liver from control animals were microscopically normal.

Discussion

Cardiac arrhythmias are not a prominent feature in conscious, freely moving iron-loaded guinea pigs monitored by telemetry. Although total cardiac and hepatic iron concentrations were similar to those reported in humans with transfusional iron overload[5,21,24] and other animal models,[7,16,19] our iron-loaded guinea pigs had only rare arrhythmias and showed no evidence of clinically significant arrhythmias. In contrast to other reports, our animals were not sick, did not demonstrate increased mortality, and showed no evidence of infection. These differences could mean that the guinea pig is not be the best model to study iron induced arrhythmias; that arrhythmias reported by other investigators were related to infection or acute iron toxicity, not chronic iron overload; that the iron loading, tissue iron levels, or distribution were insufficient to cause arrhythmias; or that iron alone is insufficient to cause arrhythmias.

Although the guinea pig model of transfusional iron overload shares many characteristics of transfusional iron overload in humans, like other rodent models, it is an imperfect reproduction of the human disorder. Unlike humans, rodents excrete iron, and rodents may store or buffer excess iron differently. Spontaneous losses of iron in rodents may make the rate of loading, not the total dose, a critical factor in model development. Although tissue iron levels in our guinea pigs were similar to those reported for humans, distribution of cardiac iron appeared dissimilar, with cardiac iron in human hearts located primarily in the myocytes, whereas in guinea pigs, at the doses reported here, cardiac iron was located predominately in the interstitium and macrophages.

In the guinea pigs monitored with implantable radiotransmitters, which allowed for frequent recording of conscious, unsedated, and freely moving animals, iron doses and a dosing schedule that resulted in delayed conduction but not significant cardiac arrhythmias. One explanation could be that iron was not present in myocytes. However, with the same dose and loading schedule, isolated guinea pig Purkinje fiber-papillary muscle preparations showed delayed and blocked conduction in iron-loaded animals when compared with controls,[28] suggesting that iron was present in appropriate quantity and location to alter electrical conduction. Iron is sufficient to induce tissue damage in guinea pigs is demonstrated by the altered stability of both hepatic and myocardial lysosomal membranes.[1]

Transient arrhythmias have been reported to occur in the 'time-compressed' gerbil model of iron overload, where the dose of iron was quadrupled "to accelerate ECG changes."[23] With the cited protocol, gerbils received "massive" amounts of iron (16 g/kg) within a short period of time and showed increased mortality and morbidity. Gerbils subjected to this protocol were described as having "failed to groom, were less active, and had coarse fur and stooped posture, and they breathed more rapidly

and shallowly."[33] These gerbils showed significant morbidity and mortality, and ECGs were evaluated for 10 s while the animals were anesthetized with ketamine.[23] Although the time-compressed gerbil model may closely mimic the human condition with regard to cellular distribution of iron, the overall state of ill health of the animals plus the accelerated loading schedule do not mimic the human disease, raising questions as to the role of iron in the reported arrhythmias.

Our iron-loaded guinea pigs had elevated cardiac and hepatic iron levels. Although meaningful values for cardiac iron of patients with iron overload are difficult to obtain,[5,17,21,24] the threshold for the development of heart failure and arrhythmias is reported to be 2 mg/g dry weight.[5,19] Cardiac and hepatic iron concentrations in a person with β thalassemia who underwent heart–liver transplant were reported to be 5.8 and 28.1 mg/g dry weight, respectively.[24] Prior to transplantation, the patient experienced heart failure, ventricular tachycardia (successfully treated with amiodarone), insulin-dependent diabetes, hypoalbuminemia, and cirrhosis. This patient emphasizes the difficulty in attributing cardiac arrhythmias solely to iron, because heart failure, acid–base and electrolyte abnormalities, hypoxia, pharmacologic agents, hypertension, smoking and other lifestyle choices, complications of diabetes, and anemia could each play a role in the conduction abnormalities noted.

In the animal models of iron overload, cardiac iron ranges from 2.9 to 12.9 mg/g dry weight and hepatic iron from 20 to 181 mg/g dry weight.[17] The concentrations of iron in our guinea pigs, as well as those reported in 3 gerbil studies,[7,16,19] are in the range of reported human values.[5,17,21,24] Tissue iron concentrations in gerbils subjected to a high-dose protocol[23,32,33] are higher than reports from the human literature.

Abnormal cardiac rhythm in humans has been described, but often discounted, in association with iron overload.[4-6,10-13,18,21,26,29,30] Despite a greater than 50% increase in arrhythmias documented by ECG in patients with clinical disease from iron overload,[6] "cardiac arrhythmias were noted but not considered to be related to hemochromatosis, since it is difficult to establish abnormalities were due solely to iron overload."[6] In a multicenter study of cardiac involvement of patients with β thalassemia intermedia who had received repeated transfusions, more than 40% had arrhythmias documented by ECG, whereas only 5% were reported to be in heart failure.[2] By use of Holter monitors, major arrhythmias were observed in 7 of 11 β thalassemia patients who were not chelated adequately, but none occurred in 17 patients with adequate chelation.[18] Decreased heart rate variability and increased ventricular late potentials, associated with ventricular tachycardia, has been reported to occur in patients with β thalassemia who had no clinical evidence of cardiac dysfunction.[14] Life-threatening arrhythmias in high-risk β thalassemia patients were abolished by continuous infusion of deferoxamine.[11] In this study, 6 of 17 patients had significant cardiac arrhythmias, and 4 of these patients had no evidence of congestive heart failure or other cardiac disease.[11] Atrial fibrillation was the most common arrhythmia, but ventricular and supraventricular tachycardia were reported also. Taken together, these data suggest that iron may be necessary but insufficient by itself to cause cardiac arrhythmia in iron-overload conditions.

It is impressive that arrhythmias have been documented as frequently as they have in patients with iron overload. It is also interesting that Obejero-Paz and colleagues[23] reported significant arrhythmias in their iron-loaded gerbil model by recording the electrocardiogram for only 10 s. Unlike other conditions associated with iron overload, arrhythmias are often transient, and leave no evidence of their existence unless fortuitously captured on an ECG. Furthermore, arrhythmias may not cause symptoms sufficient for patients to seek medical attention, and thus may not be discovered or may result in sudden unexplained death.

In our present study, iron did not cause cardiac arrhythmias. Although cardiac and hepatic iron concentrations were similar to those of humans with thalassemia, our iron-loaded guinea pigs did not demonstrate significant pathological arrhythmias. In fact, control animals had more arrhythmias, and more arrhythmias of greater clinical significance, than did iron-loaded animals. Iron alone, in the guinea pig at the dose and dosing schedule we used, does not cause cardiac arrhythmias. Whether iron alone, outside the context of other comorbid conditions, can cause arrhythmias in patients with iron overload remains to be elucidated.

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