Effect of oral administration of excessive iron in adult ponies

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Objective—To evaluate the potential of excess dietary iron to cause hepatic lesions similar to those described in horses with suspected iron toxicosis or hemochromatosis.

Design—Prospective study.

Animals—6 adult male ponies.

Procedure—4 ponies received 50 mg of iron/kg (22.7 mg/lb) of body weight each day by oral administration of ferrous sulfate, which contained 20% elemental iron; 2 ponies received only the carrier (applesauce). Complete blood counts, serum biochemical analyses, and hepatic tissue biopsies were performed, and serum iron concentrations were measured. Blood and tissue samples were obtained at days 0 and 2, and at the end of weeks 1, 3, 6, and 8 after administration of iron was initiated. Treatment was discontinued after 8 weeks, and hepatic iron concentrations were measured at 28 weeks.

Results—Hepatic iron concentrations, serum iron concentrations, percentage saturation of transferrin, and serum ferritin concentrations were increased, compared with baseline and control concentrations, by week 8. Adverse clinical signs or histologic lesions in the liver were not detected in any ponies. At 28 weeks, hepatic iron concentrations had decreased.

Conclusions and Clinical Relevance—Histologic lesions were not seen in the hepatic biopsy specimens obtained from the ponies treated with ferrous sulfate. It was concluded that it would be unlikely for iron toxicosis to develop in adult ponies or horses during a period of < 8 weeks when food or water contained increased amounts of iron. It is suspected that previous reports of hepatopathies in animals with hemosiderosis in accumulation may represent a primary hepatopathy with secondary hemosiderosis accumulation, especially if the only source of iron is via oral consumption. (J Am Vet Med Assoc 2001;218:400-404)

Iron is known to be a protoplasmic toxin, which, if it accumulates in tissues at sufficient concentrations, results in iron-induced lipid peroxidation and organelle dysfunction, such as mitochondrial death mediated by free radical production. Excess iron intake is one of the more common causes of toxicosis in children. Iron fumarate caused hepatic cirrhosis and death in neonatal foals when it was administered in a paste shortly after birth. Overdoses of injectable iron have been known to cause toxicosis in horses and pigs. In horses with mild iron toxicosis, histologic lesions consist of moderate and irregular periportal fibrosis, proliferation of small bile ducts, and accumulation of hemosiderin in Kupffer cells and the epithelium of large bile ducts. Foals with more severe cases of iron toxicosis have massive periportal necrosis of hepatocytes, bile ductule proliferation, and fibrous connective tissue proliferation. Mixed inflammatory cell infiltration and cholestasis are observed in some instances.

Recently, there have been reports of adult horses dying because of hepatopathies, associated increased hepatic iron concentrations, and suspected iron toxicosis often resulting in unexpected death in Oregon, Texas, Washington, and California. In Oregon, histologic lesions in horses were confined to the liver with periportal hepatic fibrosis, bile duct proliferation, and hemosiderosis; hepatic iron content was 2- to 4-fold above the reference value. Increased serum biochemical variables in these horses included: γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST) activities, and serum iron concentrations. Hemosiderosis and hemochromatosis also have been reported in horses that had hepatic fibrosis, biliary hyperplasia, and hepatic iron concentrations up to 20 times the reference value. Evidence of excess iron in the diet of these horses could not be verified. These cases were different from human familial hemochromatosis, where there is inappropriate absorption of iron, and the hereditary form seen in Salers cattle, because the iron binding capacity was not saturated and the 3 horses in that report were of different breeds.

The National Research Council nutrient requirements of horses does not list a maximum tolerable concentration for iron in the diet, and ponies have been fed 500 to 1,000 µg of ferric citrate/g of feed with no effect. The daily requirement of iron for horses is cited as only 40 µg of iron/g of feed. The iron content of typical diets for horses varies considerably: Most forages contain between 100 and 400 µg of iron/g of feed on a dry matter basis. Grains contain less iron (barley, 88 to 97 µg of iron/g of feed; corn, 35 µg of iron/g of feed; oats, 40 µg of iron/g of feed). Molasses contains 507 µg of iron/g of feed on a dry matter basis. A typical hay and grain diet would provide approximately 2 mg of iron/kg (0.9 mg/lb) of body weight per day.

The purpose of the study reported here was to determine whether excess dietary iron would cause hepatic lesions similar to those described in horses with suspected iron toxicosis or hemochromatosis.
Materials and Methods

Horses—Six healthy sexually intact male ponies of mixed breeding and weighing between 69 and 179 kg were used in the study. Ponies were given 1 dose of an anthelmintic (ivermectin, \( \text{200 \, mg/kg [91 \, \mu g/lb]} \)) 3 to 10 weeks prior to the beginning of the study, but were not treated with any drug or chemical after that other than the iron supplement. Ponies were housed at the animal isolation facilities at Oregon State University under standards approved by the Animal Use and Care Committee.

Experimental design—Four ponies were used in the treated group, and were given ferrous sulfate, 20\% elemental iron, orally in applesauce once daily on the back of the tongue. Two ponies (controls) were given only the applesauce carrier. Each treated pony received 50 mg of iron in the form of ferrous sulfate per kg (22.7 mg/lb) each day. This dose was selected because, in a pilot study, an adult horse had no harmful clinicopathologic effects after being given 40 mg/kg of iron per day, and it was reported in a previous study that ponies receiving 500 to 1,000 \( \mu g \) of ferric citrate/g of feed had no adverse effects; however, serum biochemical or histologic changes were not mentioned.\(^{14}\) In our study, elemental iron was estimated to be 2,500 \( \mu g/g \) of feed; this was calculated by estimating that the ponies would consume 2\% of their body weight, because it has been reported that horses will voluntarily consume between 2 and 2.5\% of their body weight in dry matter each day.\(^{14}\) All ponies were fed the same grass hay, which consisted of a mixture of Timothy and Orchard grass (iron content, 108 \( \mu g/g \)) with water and salt available ad libitum. The value of 108 \( \mu g/g \) was used in calculating the total iron dosage. Feed was not weighed because the forage contained such a small percentage of total iron intake. Because the control ponies were therefore consuming approximately 2 \( \mu g/kg \) (0.9 \( \mu g/lb \)) of iron, the treated ponies received approximately 25 times that amount of iron each day.

All ponies were monitored daily during the treatment period, and any abnormal physical findings were recorded. A thorough physical examination was performed at the time blood samples were obtained. Blood samples were obtained at the same time each day, prior to the feeding of iron (baseline, day 0), at day 2, and the end of weeks 1, 3, 6, and 8 after treatment with iron was initiated. Complete blood counts and serum biochemical analyses, including GGT, AST, alkaline phosphatase (ALP), and sorbitol dehydrogenase (SDH) activities, as well as total bilirubin, protein, albumin, bile acids, and BUN concentrations, were determined. A serum iron profile\(^{15,f}\) (serum iron, total iron binding capacity, and serum ferritin) was performed, and the percentage saturation of transferrin was calculated. Samples of hepatic tissue were obtained by performing percutaneous biopsy, using a 14-gauge notch cutting biopsy needle, at time 0 and weeks 1, 3, 6, 8, and 28. Biopsy specimens were obtained from a site on the right side beneath the 14th intercostal space at a line drawn between the point of the shoulder and the tuber coxae. Two specimens, approximately 1 mm in diameter by 15 mm long, were removed at each time. Tissue samples were fixed in neutral-buffered 10\% formalin, sectioned, and stained with H&E and Perls iron stain. Frozen hepatic tissue samples were analyzed for iron content by use of inductively coupled plasma (ICP) analysis,\(^a\) and reported as \( \mu g \) of iron/g of liver (dry weight). Fecal samples were collected from the stall at the time of blood collection (approx 24 hours after the previous dose of iron had been administered). Fecal samples were analyzed for occult blood by use of a benzidine test pad\(^d\) and for iron content by use of ICP analysis.\(^b\) Fecal parasite egg counts were performed using the McMaster’s method prior to feeding iron, and during week 7.

Results

Evidence of disease was not detected during repeated clinical examinations, with the exception of 1 of the treated ponies that became anemic by week 3 (PCV, 14.7\%; hemoglobin concentration, 4.9 g/dl), and the anemia did not resolve with administration of iron. During week 7, a necropsy was performed on this pony and numerous strongyles were found in the gastrointestinal tract. Strongyle eggs in the feces had increased from 275 eggs/g of feces prior to treatment with iron to 6,600 eggs/g of feces at week 7. No other lesions that may have resulted in anemia were found and it was concluded that the anemia was caused by parasitism. Data from this pony was included in our results through week 6, but not at week 8, which included only the 3 remaining ponies that were being treated with iron. Strongyle egg counts (25 to 375 eggs/g of feces) in the remaining ponies at week 7 were within limits commonly seen in field conditions in Oregon.

There was no histologic evidence of liver damage in any of the ponies fed 50 mg of iron/kg for 8 weeks. However, there appeared to be a slight increase in the amount of hemosiderin deposited in Kupffer cells, compared with livers of control ponies, which contained no stainable iron (Fig 1).

Because the standard deviation was so large, the number of ponies was small, 1 of the ponies receiving iron was lost, and a normal distribution of the data could not be assumed, it was not possible to determine statistical differences in our results. Serum concentrations of total bile acids increased during week 8, but did not increase above reference range values for our laboratory and it was believed this was not physiologically significant. There was no apparent increase in any hepatic enzyme activity after dosing with 30 mg of iron/kg for 8 weeks.

Packed cell volume, erythrocyte counts, and hemoglobin concentration did not change in the ponies being fed excess iron (except in the pony that developed the high parasite load and anemia). Although continued feeding of excess iron did not improve the anemic condition of this pony, the hepatic iron concentration continued to increase. Hepatic iron contents were similar in control and treated ponies before the initiation of the study, but during the study, hepatic iron content (mean ± SD) in the treated ponies decreased to 3,435 ± 1,614 \( \mu g \) of iron/g of liver, compared with iron contents in control ponies, which remained at 716 ± 182 \( \mu g \) of iron/g of liver. Twenty weeks after cessation of excess iron administration in the 3 remaining ponies, hepatic iron concentration decreased to 1,494.7 ± 684.9 \( \mu g \) of iron/g of liver.

Serum iron concentration increased above the laboratory reference range values by the end of week 1, and continued to increase through week 6; however, this increase was not significant. Percentage saturation of transferrin increased during the administration of
The anemic pony had 100% saturation at week 6. Serum ferritin concentrations increased by week 3, and continued to increase until week 6.

We were unable to identify feces from individual ponies at each time they were sampled; therefore, it was difficult to interpret the data. However, the fecal iron content of the samples we could identify ranged from 274 to 3,210 µg/g of feces on a dry matter basis in control ponies and at time 0 from the treated ponies, when all ponies were receiving approximately 108 µg of iron/g of hay in their diet. The iron content of feces identified from ponies receiving approximately 2,500 µg of iron/g of feed ranged from 582 µg of iron/g of feces early in the feeding trial to as high as 7,850 µg of iron/g of feces after 6 weeks of treatment. Fecal occult blood was not detected (except from the anemic pony at weeks 0 and 1, and in all ponies that we could identify the feces from weeks 6 and 8; this included 3 treated ponies and both control ponies).

**Discussion**

Our experiment failed to induce any pathologic changes in the liver following 8 weeks of excess oral administration of iron. None of the changes previously seen in proven instances of iron toxicosis or suspected cases of toxicosis were observed in any of the ponies receiving 50 mg of elemental iron/kg. The amount of hemosiderin observed histologically in hepatic tissue biopsy specimens increased as the measured hepatic iron concentration increased, but the increase was negligible, compared with previous reports. The serum biochemical variables that would indicate hepatic insult were not increased, although there was an increase in serum and hepatic iron concentrations. By week 8, serum iron, serum ferritin, percentage transferrin saturation, and hepatic iron concentrations increased, which is consistent with increased iron intake.

Iron homeostasis is primarily controlled by rate of intestinal absorption and loss of intestinal epithelial cells, because there is not an effective mechanism for excretion. Iron is excreted in bile; however, 40% may be absorbed before reaching feces. With increased consumption of iron, decreased amounts will be absorbed, and in true iron deficiencies, the absorption rate may be up to 15 times that of clinically normal animals. The intestinal mucosa regulates iron with 2 phases of absorption: a rapid phase in the duodenum and a slow phase in the ileum, with a lesser amount of absorption in the stomach and colon. Typically, approximately 5 to 10% of the iron content in food is absorbed, with the ferrous form being more highly absorbed, compared with the ferric form. The low pH of the gastric content provides a slurry that facilitates the brush border conversion of ferric iron to ferrous iron. Heme iron is taken up within enterocytes by a poorly understood process with 2 outcomes: it will be stored as ferritin within the enterocyte, which will later be lost as the cell is sloughed, or it will be transferred across the basolateral membrane of the enterocyte to the plasma, bound to transferrin, and transferred to the bone marrow for incorporation into erythrocytes. Iron is stored as ferritin and hemosiderin within the bone marrow, with additional iron accumulation in the liver and spleen.

Even with our limited fecal sample size, results of this study do not disagree with the concept that intestinal absorption of iron is decreased after iron accumulates in the body, and is somewhat protective. In the samples we obtained, fecal iron content increased after ponies received excess iron; after receiving > 60 times the National Research Council's recommended minimum amount of iron, the ponies still did not become ill.

The body of a healthy 500-kg (1,100-lb) horse contains approximately 33 g of iron. Most (67%) of this is found in hemoglobin or myoglobin. Because iron is primarily stored as either hemosiderin or ferritin, < 1% of the total iron is in the serum or plasma in horses. Species-specific serum ferritin concentrations are a better estimate of the total amount of stored iron, and in 1 study, serum ferritin concentrations correlated well with non-heme iron in the liver and spleen.
Serum iron is bound to transferrin for transport; however, transferrin is not easily measured immunologically. Therefore, total iron binding capacity is determined along with the percentage of transferrin saturation with iron. Because serum iron comprises a small proportion of total body iron, and there is such a wide range of values (reference range, 50 to 198 μg/dl) in healthy horses, serum iron concentrations correlate poorly with total iron stores. Also, serum iron concentrations in horses vary with breed. Other factors that commonly cause serum iron concentration to increase include hemolysis, administration of corticosteroids, hepatic disease with release of iron, and possibly iron toxicosis, especially in young animals or animals given parenteral injections of iron supplements in addition to improperly cleaned glassware causing laboratory error.

In this study, ferrous iron was used because it is more highly absorbed, compared with ferric iron, and therefore, has a higher potential to cause toxicosis when administered orally. Iron that is injected is more toxic than iron administered orally, which is attributable to oversaturation of transferrin in the plasma, thereby leading to increased free iron. Free iron catalyzes the Haber-Weiss reaction that produces hydroxyl radicals, and also reacts with oxygen to produce perfrerulion compounds that have an oxidizing potential equal to or greater than that of hydroxyl radicals. Oxygen radicals can interact with cell membranes and result in lipid peroxidation. Racehorses given IV injections of iron dextran may become ill or die unexpectedly. By injecting the iron, the homeostatic mechanism of the intestinal mucosa is bypassed, allowing for higher concentrations of free iron to accumulate in the body. Iron toxicosis has been studied in rodents and rabbits, and death has been reported with administration of iron intravenously and orally. Iron toxicosis has been attributed to metabolic acidosis, cardiovascular collapse, and gastrointestinal tract damage with resulting sepsis and endotoxemia. In studies in which guinea pigs were used, iron accumulates within lysosomes and the oxygen radical damage increases the fragility of hepatic and myocardial lysosomes because of iron-catalyzed membrane peroxidation, with resultant lysosomal damage and cell death, with hemosiderin accumulates in the liver, spleen, heart, pancreas, and other tissues. In other studies in horses with associated hepatic hemosiderin accumulation, myocardial hemosiderin accumulation was not detected. In iron toxicosis, lysosomal damage in cardiac muscle would release hydrolytic enzymes and the potential cardiac damage could result in death.

Acute cases of iron toxicosis also result in gastrointestinal mucosal necrosis and hemorrhage, with the damaged mucosa allowing for increased absorption of iron. In our study, the fecal occult blood test results were positive only in the parasitized pony early in the study, and in the other ponies sampled at week 6, which may have been attributable to intestinal ulceration caused by the excess iron (however, control ponies also had positive results). It is difficult to interpret this data because of the insensitivity of the test. The benzidine reagent we used tested for peroxidase activity of hemoglobin; therefore, if hemoglobin is metabolized by intestinal microorganisms, it will not react with the test reagent. In a previous study, approximately 100 ml of whole blood had to be placed in the stomach of adult horses by nasogastric tube before it was detectable on the fecal benzidine test. Although inaccurate, the benzidine test was the most practical test that we had to indicate intestinal bleeding. There are also many other substances that have peroxidase activity, such as myoglobin and meat by-products, that may cause a false-positive reaction with this test.

There are reports of adult horses being poisoned by oral administration of iron; however, the amounts of iron used were much less than the amounts given to the ponies in our report. In 1 report, a horse received only 0.6 mg of iron/kg (0.27 mg/lb) daily in the form of ferrous sulfate, compared with the 50 mg/kg given daily to our ponies for 8 weeks. We were unable to induce the hematologic and histologic findings detected in the horse of the previous report; it may have had preexisting liver disease, resulting in excess concentrations of iron, similar to another report of hepatic toxicosis in horses. An interesting feature of specific field cases of hepatopathy associated with increased hepatic iron concentrations was the severe histologic hepatic periportal fibrosis with considerable hemosiderin accumulation in horses that appeared healthy and in good body condition. In Oregon and California, no source of excessive iron intake was determined after analysis of feed, water, and mineral supplements and no other toxicants or causes were determined. Unexpected death and hepatopathies have been reported in equine serum hepatitis (Thilier's disease), which is an ill-defined syndrome that may or may not be preceded by the administration of biologics. A number of hepatopathies may exist that can impair biliary excretion of iron and result in hepatic hemosiderin accumulation. Diffuse pathologic involvement, such as portal fibrosis, may explain why some horses with hepatopathies accumulate excessive amounts of iron and others do not.

Although this study had a number of shortcomings (small number of ponies, loss of 1 pony, and inability to identify the source of some of the fecal samples), we concluded that it would be difficult to induce hepatic toxicosis in an adult pony consuming iron free choice in the feed or water, at least over a period of 8 weeks. The prior instances of hepatopathies associated with high hepatic iron concentrations that have been reported may have been caused by an unknown hepatotoxic insult that is gone by the time histologic lesions and hemosiderosis are observed.

References


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