RECOMMENDED PHLEBOTOMY GUIDELINES FOR PREVENTION AND THERAPY OF CAPTIVITY-INDUCED IRON-STORAGE DISEASE IN RHINOCEROSES, TAPIRS AND OTHER EXOTIC WILDLIFE

Donald E. Paglia, MD

UCLA Hematology Research Laboratory, Department of Pathology and Laboratory Medicine, UCLA School of Medicine, 10833 LeConte Avenue, Los Angeles, CA 90095 USA

Abstract

Captive conditions, most likely related to non-native diets, induce progressive iron accumulation in a wide range of exotic wildlife, and iron toxicity contributes to high morbidity and mortality in affected species. Preventive and therapeutic strategies focus on dietary manipulations to limit iron uptake and on physical removal of excess iron by pharmacologic chelation or phlebotomy. Until nutritional management becomes an effective alternative, repetitive phlebotomies provide the simplest and most practical method to prevent and reduce iron overburden and its adverse effects. Guidelines are presented for a phlebotomy protocol applicable to rhinoceroses, tapirs and other animals based on the broad clinical experience in humans affected by iron storage disorders.

Introduction

Over the past decade, convincing evidence has accumulated that African black *(Diceros bicornis)*[4,7-12,15] and Sumatran *(Dicerorhinus sumatrensis)*[7,8,10,11] rhinoceroses, as well as tapirs of all four species (*Tapirus* spp.),[13] develop pathologic overloads of iron under a variety of captive conditions. In rhinoceroses, blood and necropsy studies confirm that (a) iron accumulates progressively in multiple organs in proportion to time in captivity, (b) that iron stores in captive animals reach levels hundreds or thousands of times greater than found in the wild, and (c) that iron overload is frequently associated with histopathologic and biochemical evidence of severe cellular damage and dysfunction.

Although iron is an essential element, its presence in excess is invariably deleterious. Free iron actively catalyzes generation of highly toxic hydroxyl free radicals that in turn cause oxidative damage to cell and organelle membranes, to nucleoproteins, and to structural, functional and enzymatic proteins at multiple molecular sites.[1,3,6]

One of the principal clinical effects of excess iron is increased virulence of microorganisms and increased host susceptibility to infections of all types,[5,14,17,18] therefore iron overload may be largely responsible for the high incidence of diverse and exotic infectious diseases known to affect black and Sumatran rhinoceroses and tapirs in captivity. Additional evidence linking iron overload to other disorders has also been accumulating,[7,12] although direct cause-and-effect relationships have not been unequivocally established. Nonetheless, an enormous body of experience with iron storage disease in humans and other animals has demonstrated that iron overburdens of the magnitude observed in these species can contribute to and/or directly cause severe, life-threatening, multi-system disorders, some of which are clinically similar to dyscrasias affecting them in captivity.

Rationale for Repetitive Phlebotomy

To avoid the well known deleterious effects of chronic iron toxicity, several strategies have been proposed and tested over the past few years to prevent and/or reduce iron overburdens in affected rhinoceroses and other species. Decreasing the bioavailability of dietary iron has received the greatest attention, including formulation of low-iron feeds and addition of iron-binding components (such as tannins) to standard captive diets. These important approaches deserve continued investigation since they could substantially benefit both captive-born calves and wild-caught animals, both of which have normal iron stores initially. Unfortunately, even if 100% effective, interdiction of iron uptake would have no effect on the pathologic overloads already existing in most of the captive populations.

Since mammals cannot excrete iron physiologically, even when present in great excess, preexistent iron overburdens can only be corrected by physical removal of the metal.

One standard therapeutic approach is pharmacologic chelation with desferrioxamine (Desferal, Novartis Pharmaceuticals, NJ). Chelation is the preferred treatment to correct iron excess in avians, in many other small captive animals, and in children with chronic anemias who accumulate iron from multiple blood transfusions. We have found that desferrioxamine actively mobilizes storage iron for urinary excretion in rhinoceroses, but it remains prohibitively expensive to consider its routine use in large animals.

The second effective approach to prevention and/or correction of iron overload is repetitive phlebotomy, the standard of practice for humans with highly common genetic predispositions to overload iron. Since hemoglobin contains a fixed amount of iron (0.34% by weight), removal of a liter of blood with 15 g/dl hemoglobin content physically reduces an individual's iron load by 0.5 g. Thus, the iron removed by repetitive phlebotomy can be precisely calculated by measuring the volume and hemoglobin content of the blood removed

Guidelines for a Repetitive Phlebotomy Program

Historically, the seemingly draconian practice of blood-letting was one of the earliest known attempts to treat human ills and was also used in veterinary medicine as far back as ancient Egypt. Although its justification was often dubious, a sound scientific basis for this practice emerged in modern times to treat a number of iron-overload syndromes affecting humans[2] and other animals. In veterinary practice, therapeutic phlebotomy has been documented by numerous case reports in the literature, but seldom with consensus on optimal procedures for any given species. In humans, however, experience with this technique has been much more extensive due to the high frequency of iron-storage disorders, particularly hereditary hemochromatosis caused by mutations of the *HFE* gene, the most common genetic defect in the U.S. population.

Definitive guidelines for diagnosis and management of human hemochromatosis have been published by the Practice Guideline Development Task Force of the College of American Pathologists (CAP)[19] and by the U.S. Centers for Disease Control and Prevention (CDC).[16] The following recommendations for rhinoceroses and tapirs are based largely on extrapolation of these guidelines modified by consideration of the practical aspects of working with exotic wildlife in captive settings. It is expected that further modifications will be individually tailored to the special characteristics of each animal and to accommodate circumstances unique to each institution.

Candidate Populations, Selection Criteria and Goals

The browser rhinoceros species appear to be at higher risk than grazers for development of pathologic iron overloads in captivity, but under certain circumstances even the latter can accumulate iron excessively. We therefore recommend that the iron loads of all captive rhinoceros and tapir species be assessed at earliest opportunity and periodically thereafter. In humans, the CDC recommends transferrin saturation (serum iron concentration divided by total iron binding capacity) as a simple inexpensive screening test. CAP guidelines cite persistent elevations of transferrin saturation (>60%) and serum ferritin concentrations (>200-400 ng/ml for women and men, respectively) as thresholds for diagnosis of pathologic iron loads in hereditary hemochromatosis. Serum ferritin concentration provides the single most reliable non-invasive indicator of total body iron stores. (Ferritin assays require species-specific reagents, and these are available for rhinoceroses and tapirs, as well as other species, at the Laboratory of Comparative Hematology, Kansas State University College of Veterinary Medicine.)

In humans, the primary therapeutic indication for phlebotomy or iron-chelation therapy is the demonstration of significantly increased iron stores even in the absence of any clinical manifestations of iron-storage disease. On the basis of our experience, we would recommend threshold values of 65-70 % transferrin saturation and 500 ng/ml serum ferritin, above which therapeutic intervention in rhinoceroses or tapirs would be clinically justified. These thresholds represent, respectively, double and triple the mean values we have measured in black rhinoceroses free-ranging in the African wild (34 % transferrin saturation and 180 ng/ml ferritin). Animals that are already overtly anemic should be excluded as candidates for a phlebotomy program.

Within each candidate species, two subpopulations merit distinction: (1) those with transferrin saturation and ferritin values consistently above the reference ranges, and (2) captive-born calves and animals of any age that have been recently translocated from the wild into captivity, (all of which initially have iron analyte values within normal ranges). For the first group, the long-term goal of a phlebotomy program is therapeutic, to remove the pathogenic iron as rapidly as possible.[19] By contrast, periodic phlebotomy for the second group would be a preventive measure with the goal of maintaining normal iron stores despite captive conditions that otherwise would inexorably lead to progressive iron accumulation. (In rhinoceroses, body iron stores can increase as much as tenfold in as little as 3 yr in captive newborns.)

Procedures

The essential qualification for inclusion of animals in a repetitive phlebotomy program would be their ability to tolerate such procedures without undue stress or endangerment to the animals themselves or to the keeper and veterinary staffs. An entire session of presentations at the 2002 Annual AAZV Conference amply demonstrated the value and effectiveness of operant conditioning in animals ranging from small primates to large carnivores and megavertebrates. Training animals to allow routine care and clinical management without sedation or anesthesia is now widely practiced, and venipuncture is one of the most common procedures performed.

Ear and forefoot interdigital veins are most conveniently used with rhinoceroses, but these vessels may be prone to thrombosis under the trauma of frequent venipuncture. In addition, they are generally too small to obtain large volumes of blood quickly, so they would not be optimal for repetitive phlebotomies. The medial radial vein of the rhinoceros foreleg, however, provides the most accessible channel for rapid removal of sizeable volumes through large-bore needles (18 gauge or greater), and rhinoceroses at several different institutions have already been conditioned to tolerate this procedure.

CAP guidelines emphasize that the rate of phlebotomy in both frequency and volume must be established for patients individually, and it is axiomatic that each animal in a phlebotomy program will dictate its own tolerance limits. Humans are generally bled 450-500 ml once or twice per week. For a 1,000-kg black rhino, that would be equivalent to ~7-15 liters weekly, clearly not a feasible amount. Experience with rhinoceros donors for transfusions, however, suggests that 1.5-3 liters or more are reasonable and achievable target volumes for typical phlebotomy sessions. *It should be emphasized that any amount of blood removed from an iron-overloaded animal would be beneficial in that it contributes to negative iron balance and reduction of excess storage iron.*

In humans, phlebotomy programs are initiated slowly, (for example, once every 2 wk over the first 2 mo) since this stimulates erythroid marrow to proliferate toward maximal rates of production. Subsequently, the frequency can be progressively increased to once or twice weekly. Ultimately, the goal of a repetitive phlebotomy program is to mobilize storage iron by inducing a slight blood-loss anemia. This can be accomplished by reducing packed red-cell volumes (PCV) by as little as 5% below their normal baselines. To quote the CAP guidelines, "…if the hematocrit before any phlebotomies is 45%, maintaining it at 40% provides an adequate challenge to the marrow without provoking symptoms of anemia."[19] Lowering each animal's baseline PCV by ~5% would thus serve as a valid objective, and no additional advantage is gained by decreasing it further.

Progress in reducing iron stores can be monitored by measuring serum iron, transferrin saturation, and ferritin every 3-6 mo. These values, however, can be altered by concurrent infectious and inflammatory processes, making trends shown by periodic assays far more reliable than isolated values alone. Serum iron typically remains elevated until the storage pool nears depletion. As tissue iron stores are mobilized to replace hemoglobin lost by phlebotomy, ferritin concentrations in the plasma gradually decline. If repetitive phlebotomies eventually return the storage pool to normal, both the serum ferritin and red cell MCV would decline, reflecting the decreased availability of storage iron for enhanced erythropoiesis. In some species (such as rhinoceroses), reticulocytes mature before leaving the marrow, therefore erythropoietic responses are better assessed by changes in MCV than by reticulocyte counts.

Cost/Benefit Assessment and Secondary Benefits

Many captive black rhinoceroses already have extreme elevations of storage iron, dramatically reflected by serum ferritin concentrations in the thousands, tens of thousands, and greater (compared to a normal range of 100-200 ng/ml). Phlebotomy programs will require long-term commitments to contribute substantively to the welfare of these animals. Even if iron stores were eventually returned to normal, less frequent phlebotomies (perhaps one every 3-4 mo) would still be of value to prevent reaccumulation of the metal. These would not be necessary, however, if current dietary research studies result in measures that effectively reduce enteric iron uptake.

Any phlebotomy program would demand significant time commitments from personnel involved in animal training and performance of the procedure. Additionally, institutional administrators must be persuaded to commit staff time and other costly resources to institute remedial programs for problems that are not clinically obvious. (Rhinoceroses in particular seem to have evolved a capacity to remain outwardly stoic despite the presence of extensive internal damage or disease.) In humans, phlebotomy programs have been shown to be unequivocally cost-effective by avoiding the huge future expense of treating chronic and terminal multi-system disorders that are the inevitable consequences of prolonged iron overburden. Since chronic iron toxicity is as insidious as it is pernicious, it generally remains undetected until terminal failure of some critical organ system occurs. Nonetheless, progressive dysfunction of various organs contributes to deteriorating quality of life in affected animals as well as shortened life spans.

Operant conditioning of animals to tolerate venipuncture has at least two potentially important fringe benefits. It allows the veterinary staff easier access to obtain diagnostic blood samples for any disorder that might develop, and it provides a ready route for therapeutic parenteral administration without sedation, should that become necessary. Additionally, for a relatively small investment in a core central facility, blood removed by phlebotomy could be fractionated for long-term preservation and storage of red cells, leukocytes, platelets, and plasma as a resource to treat future hemolytic, hemorrhagic, infectious, or other disorders without jeopardizing additional rhinoceroses as donors of fresh blood. Potential sites and funding sources for a centralized national blood bank for rhinoceros blood components are currently being investigated.

LITERATURE CITED

1. Britton, R.S., A.S. Tavill, and B.R. Bacon. 1994. Mechanisms of iron toxicity. *In:* Brock, J.H., J.W. Halliday, M.J. Pippard, and L.W. Powell (eds.). Iron Metabolism in Health and Disease. W.B. Saunders Company, Philadelphia, Pennsylvania. Pp. 311-351.
2. Crosby, W.H. 1958. Treatment of haemochromatosis by energetic phlebotomy. One patient's response to the letting of 55 litres of blood in 11 months. Brit. J. Haematol. 4:82-88.
3. Halliwell, F., and J.M.C. Gutteridge. 1986. Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. Arch. Biochem. Biophys. 246:501-514.
4. Kock, N., C. Foggin, M.D. Kock, and R. Kock. 1992. Hemosiderosis in the black rhinoceros *(Diceros bicornis)*: A comparison of free-ranging and recently captured with translocated and captive animals. J. Zoo Wildl. Med. 23:230-234.
5. Kontoghiorghes, G.J., and E.D.Weinberg. 1995. Iron: Mammalian defense systems, mechanisms of disease, and chelation therapy approaches. Blood Rev. 9:33-45.
6. McCord, J.M. 1998. Iron, free radicals, and oxidative injury. Semin. Hematol. 35:5-12.
7. Paglia, D.E. 2000. Captivity acquired hemochromatosis resembling Bantu siderosis in browser, but not grazer, rhinoceroses. Blood 96:484a.
8. Paglia, D.E., and P. Dennis. 1999. Role of chronic iron overload in multiple disorders of captive black rhinoceroses *(Diceros bicornis)*. Proc. Am. Assoc. Zoo Vet. Pp. 163-171.
9. Paglia, D.E., and R.W. Radcliffe. 2000. Anthracycline cardiotoxicity in a black rhinoceros *(Diceros bicornis)*: Evidence for impaired antioxidant capacity compounded by iron overload. Vet. Pathol. 37:86-88.
10. Paglia, D.E., and E.D. Weinberg. 2001. Iron loading in birds and mammals - A notable, yet often unrecognized initiator of disease and death. Iron Disorders Institute Magazine i.d.Insight, 2nd quarter, p. 8.
11. Paglia, D.E., E.S. Dierenfeld, and I-H Tsu. 2001. Pathological iron overloads acquired in captivity by browsing (but not by naturally grazing) rhinoceroses. Abstracts of the Inter. Elephant and Rhino Res. Symp., Vienna, Austria. P. 46.
12. Paglia, D.E., D.E. Kenny, E.S. Dierenfeld, and I-H Tsu. 2001. Role of excessive maternal iron in the pathogenesis of congenital leukoencephalomalacia in captive black rhinoceroses *(Diceros bicornis)*. Am. J. Vet. Res. 62:343-349.
13. Paglia, D.E., C.L. Miller, S.H. Foerster, J.E. Wynne, I-H Tsu, and D.E. Kenny. 2000. Evidence for acquired iron overload in captive tapirs (*Tapirus* spp.) Proc. Am. Assoc. Zoo Vet., New Orleans, Louisiana. Pp. 124-126.
14. Payne, S.M., and R.A. Finkelstein. 1978. The critical role of iron in host-bacterial interactions. J. Clin. Invest. 61:1428-1440, 1978.
15. Smith, J.E., P.S. Chavey PS, and R.E. Miller. 1995. Iron metabolism in captive black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceroses. J. Zoo Wildl. Med. 26:525-531.
16. Thomas, S., and D. Snyder. 1998. Centers for Disease Control (CDC) Guidelines for Hereditary Hemochromatosis. American Hemochromatosis Society. CDC Website.
17. Weinberg, E.D. 1974. Iron and susceptibility to infectious disease. Science 184:952-956.
18. Weinberg, E.D. 1978. Iron and infection. Microbiol. Rev. 42:45-66, 1978.
19. Witte, D.L., W.H. Crosby, C.Q. Edwards, V.F. Fairbanks, and F.A. Mitros. 1996. Hereditary hemochromatosis. Clin. Chim. Acta 245:139-200.