IS IMPAIRMENT OF OXIDANT NEUTRALIZATION THE COMMON DENOMINATOR AMONG DIVERSE DISEASES OF BLACK RHINOCEROSES?

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Abstract

Several disparate diseases of high morbidity and mortality have had a major impact on the captive breeding program for African black rhinoceroses *(Diceros bicornis)*. These include a mucocutaneous ulcerative disorder, congenital leukoencephalomalacia, susceptibility to fungal pneumonia, and acute episodic hemolytic anemia. The latter appears to be related to some of the unusual metabolic features of rhinoceros erythrocytes, which differ radically from most other mammals, particularly in regard to ATP generation and antioxidant metabolism. Strategies based on these red cell characteristics have been developed to prevent or treat the hemolytic syndrome. Comparative studies of red cell biochemistry, enzymology and metabolism indicate that extremely low catalase activity is the principal feature distinguishing the black rhinoceros from three other species which are rarely, if ever, affected by these disorders. A hemorrhagic diathesis, perhaps initiated by infectious agents, may represent an additional new, *non*hemolytic anemia syndrome. We postulate that relative catalase deficiency and/or other impairments in neutralization of reactive oxygen species in diverse tissues, (such as leukocytes, vascular endothelium, neural and connective tissue elements), may represent a common abnormality responsible for any or all of these disorders.

Resumen

Varias enfermedades similares, de alta morbilidad y mortalidad, han tenido un gran impacto en el programa de crianza en cautiverio del rinoceronte negro africano (*Diceros bicornis*). Estas enfermedades incluyen un desorden ulcerativo mucocutáneo, leucoencefalomalacia congénita, susceptibilidad a la neumonía micótica y episodios de anemia hemolítica agudos. Este último trastorno parece estar relacionado con algunas de las características metabólicas poco usuales de los eritrocitos de los rinocerontes, los cuales difieren radicalmentede la mayoría de los mamíferos, particularmente en relación con la generación de ATP y el metabolismo anti-oxidante. Estrategias basadas en las características de estas células rojas han sido desarrolladas para prevenir o tratar el síndrome hemolítico. Estudios comparativos de los eritrocitos en cuanto a su bioquímica, enzimología y metabolismo indican que la baja actividad de la catalasa es el principal rasgo distintivo del rinoceronte negro con las otras tres especies que, muy excepecionalmente, son afectadas por esas enfermedades. Una diátesis hemorrágica, posiblemente iniciada por un agente infeccioso, puede representar un nuevo y adicional síndrome de anemia no hemolítica. Nosotros proponemos que esa relativa deficiencia

de catalasa y/o otros deterioros en la neutralización del oxígeno reactivo en diversos tejidos como leucocitos, endotelio vascular, elementos del tejido conectivo y neural, pueden representar una anormalidad común responsable de algunos o todos esos desórdenes.

Overview

Captive African black rhinoceroses (*Diceros bicornis)* are commonly affected by several clinically disparate diseases of unknown etiology, often with high mortality. As recently reviewed by Miller,[3-4] these include acute episodic hemolytic anemia, mucocutaneous ulcerative disease, fungal pneumonia and leukoencephalomalacia, none of which affects other rhinoceros species to any significant degree. Studies at the UCLA Hematology Research Laboratory have focused on the hemolytic syndrome that has now been documented in at least 47 instances affecting 39 animals with a 75% mortality rate. Hemolytic anemia occurs suddenly as a primary disease in otherwise healthy rhinoceroses, often associated with exposure to drugs or chemicals, and it also occurs as a secondary complication in other disorders, including infection and ulcerative disease. Since this hemolytic syndrome has become the leading cause of death within the captive population, extensive investigations have been undertaken to determine its etiology. These have effectively excluded autoimmune mechanisms,[1] erythrocyte membrane defects or hemoglobinopathies.[2] Studies of the metabolic capacities of rhinoceros erythrocytes, however, have revealed a number of extraordinary differences compared to other mammalian red cells.[5,7,9,11,13] Some of these unusual features, either alone or in combination, might be responsible for premature hemolysis, since they appear to reflect a pattern of impaired red cell capacity to neutralize oxidant compounds and free radicals that are generated during many physiological and most pathological processes.[5,7,9-12]

One of the most unusual biochemical characteristics of rhinoceros erythrocytes initially observed was a dearth of high-energy phosphate that is essential for many metabolic reactions, ATP concentrations being only 2-5% of those found in human and other mammalian red cells.[5,11,13] This led to an hypothesis that ATP deficiency might be the biochemical lesion responsible for premature hemolysis in black rhinoceroses under oxidant duress.[5] Rationale for that hypothesis derived from the known dependence of mammalian red cells on phosphorylation of glucose by ATP and diversion of its product through an ancillary metabolic pathway, the hexose monophosphate shunt (HMPS), in direct response to the cells' need to neutralize peroxides and reactive oxygen species. Additionally, an extremely common deficiency of the first enzyme of that pathway, glucose-6-phosphate dehydrogenase (G-6-PD), produces a hemolytic syndrome in humans that is clinically identical to that affecting black rhinoceroses.[5] That hypothesis, however, has now been effectively refuted by collaborative studies with Prof. Eric H. Harley of the University of Cape Town in which we have demonstrated complete independence between HMPS flux rates and intracellular ATP concentrations.

Nonetheless, extremely low reserves of ATP in rhinoceros red cells may be the proximate cause for eventual failure of the membrane cation pump with consequent water influx and cell lysis. Studies in humans have shown a direct correlation between serum phosphate and red cell ATP concentrations, and hypophosphatemia with decreased intracellular ATP has been associated with premature hemolysis both in humans and in several rhinoceroses. This has led to a

rationale for phosphate supplementation to increase endogenous ATP concentrations in rhinoceros erythrocytes as a preventive measure or for therapeutic intervention.[6,8] At the Oklahoma City Zoo, intensive parenteral phosphate infusions in one black rhinoceros during an acute hemolytic episode were associated with progressively increasing red cell ATP, cessation of hemolysis, and return of hematocrit to normal levels (45%) from a nadir of 16%. At the Dallas Zoo, high-phosphate dietary supplements in another rhinoceros with severe mucocutaneous ulcerative disease were associated with increased red cell ATP levels and no evidence of hemolysis. Similar supplementations have been attempted in other animals with a variety of disorders, and elevated ATP concentrations have been documented.

These experiences substantiate conclusions drawn from in vitro experiments and illustrate the importance of avoiding or correcting hypophosphatemia to prevent hemolysis resulting from further depletion of marginal reserves of red cell ATP. Since glycolysis is critical to ATP generation in mammalian erythrocytes, avoidance of any condition that inhibits glycolysis, such as acidosis, also constitutes an important preventive measure. As we have previously stressed, the most effective prevention remains an avoidance of agents known to increase the potential for oxidant production.[5-12] These include several classes of drugs, such as sulfonamides, antimalarials, sulfones, nitrofurans, chloramphenicol, acetanilid, and possibly vitamin C and vitamin K analogues, as well as a number of chemical compounds, particularly those containing cyclic hydrocarbons such as naphthalene and phenols, and especially creosote, which may have direct hepatotoxic effects as well as potential capacity to initiate hemolysis. Given the hemolytic effects of certain plants, such as wild onion, oak and red maple leaves in horses and other animals, the possibility of similar effects in rhinoceroses must be considered in design of captive diets. Additionally, the frequent association of *Leptospirosis* infection with earlier cases of hemolytic anemia supports a continuing recommendation for vaccination, although occasional adverse reactions to the vaccine have been observed.

Recent Studies

Recently, we have had an opportunity to study specimens from three black rhinoceroses at zoos in Denver, Fort Worth, and Fossil Rim with a number of clinical and laboratory findings in common that may represent a new *non*hemolytic anemia syndrome, possibly a hemorrhagic diathesis. Each had profound loss of red cell mass with packed cell volumes as low as 13%, but laboratory data and clinical observations did not support a hemolytic process or external blood loss. The possibility of internal hemorrhage was supported by low plasma proteins and pronounced swellings involving legs, shoulders, chest and neck, but there was no evidence of a coagulopathy by conventional criteria. In one of these, Dr. Richard Montali of the National Zoo observed histopathologic evidence of a vasculitis with endothelial damage and diapedesis into soft tissue interstitia. The anemia component was eventually self-limited in all three rhinoceroses, as they responded to careful clinical management of their primary conditions (laminitis, post-partum infection). Therapy included phosphate supplementation as necessary to maintain normal or elevated serum phosphate and red cell ATP concentrations. This group of rhinoceroses is the subject of an intensive collaborative investigation to determine whether they share a common etiology, perhaps with infectious initiation.

Discussion

Our studies of comparative red cell metabolism have now been extended to include all extant species except the Javan rhinoceros, and similarities among them are far more common than differences. All have comparably low amounts of erythrocyte ATP, for example, supporting our experimental evidence that low ATP alone cannot account for hemolytic tendencies in black rhinoceroses. The most significant metabolic difference that we have so far observed between the black rhinoceros and other species is in their relative activities of red cell catalase, black rhinoceroses having by far the lowest (<2-5 %, compared to humans). Since catalase is perhaps the singularly most important enzyme in antioxidant metabolism, and its activity in mammalian erythrocytes exceeds all other enzymes by several orders of magnitude, it becomes tempting to postulate that its relative deficiency sets the black rhinoceros apart in terms of susceptibility to oxidant stress and the consequent induction of acute hemolytic crises.

It is also tempting to go one step further: since enzyme activities in erythrocytes often reflect their corresponding activities in other tissues, the possibility of catalase deficiency or otherwise defective antioxidant metabolism in vascular endothelium, leukocytes, neural and connective tissue elements, etc., should be considered when investigating other disorders affecting this species: namely, mucocutaneous ulcerative disease, impaired immunity with susceptibility to unusual infections, congenital leukoencephalomalacia, and a possibly new syndrome of hemorrhagic diathesis resulting in nonhemolytic anemia. It seems intuitively improbable that so many severe, clinically disparate disorders could occur with such prevalence in a single species without being related by some common etiology or mechanism. Collaborative studies, focused on catalase and other enzymes crucial to neutralization of reactive oxygen species, continue to test this hypothesis.

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