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RED BLOOD CELL METABOLISM IN THE BLACK RHINOCEROS (*DICEROS BICORNIS*)

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Abstract

The red blood cells of the black rhinoceros exhibit metabolic characteristics that are radically different from other mammalian species that have been studied. Either alone or in combination, deficiencies of intracellular ATP and certain enzymes result in erythrocytes that are extremely susceptible to oxidant stress and subsequent lysis. Additionally, there is a notable deficiency of catalase which may also contribute to the haemolytic syndrome and possibly to the syndrome of mucocutaneous ulcers. Further studies are underway to fully understand the significance of these findings for the unusual diseases noted in captive members of this species.

Zusammenfassung

Die Erythrozyten des Spitzmaulnashornes weisen Stoffwechseleigenschaften auf die sich von denen anderer untersuchter Säugetierspezies radikal unterscheiden. Entweder alleine oder in Kombination, führen Defizienzen an intrazellulärer ATP und an bestimmten Enzymen dazu dass die Erythrozyten für oxidatierenden Stress und nachfolgender Lysis sehr empfänglich sind. Hinzu kommt eine ausgesprochene Katalasedefizienz, die zum hämolytisches Syndrom und möglicherweise zum Syndrom der Schleimhautulzeration beitragen könnte. Es werden weitere Untersuchungen durchgeführt um die Bedeutung dieser Beobachtungen für die aussergewöhnliche Krankheiten bei in Gefangenschaft gehaltene Vertreter dieser Tierart zu deuten.

Résumé

Les érythrocytes du rhinocéros noir ont des caractéristiques métaboliques totalement différentes de celles des autres mammifères étudiés jusqu'à maintenant. Des déficiences en ATP intracellulaire seules ou en combinaison avec des déficiences d'enzymes spécifiques, rendent les érythrocytes extraordinairement sensibles au stress oxidatif et à une lyse subséquente. Additionnellement, une déficience marquée en catalase, peut contribuer au syndrome haemolytique et possiblement au syndrome des ulcères mucocutanés. Des recherches sont en cours afin d'éclaircir l'importance des observations faites relatives aux maladies inhabituelles chez les membres de cette espèce tenus en captivité.

Key Words
black rhinoceros, erythrocyte, red blood cell metabolism, haemolytic anaemia

Discussion

Growth of the captive population of black rhinoceroses (*Diceros bicornis*) has been limited by four diseases (haemolytic anaemia, mucocutaneous ulcerative syndrome, encephalomalacia, fungal pneumonia) whose etiology is poorly understood (5). Haemolytic anaemia ranks as the leading cause of death in the North American populations, accounting for approximately 40% of all mortalities (6).

Over 45 cases of chronic oral and/or skin ulcers have been noted in this population (8). Attempts to identify an underlying viral or autoimmune etiology have been unsuccessful (8). Additionally, four cases of massive encephalomalacia, involving primarily white matter, have been identified and despite extensive diagnostic evaluation, an etiology has not been identified (7). Finally, fungal pneumonia (usually *Aspergillus* sp.) has been identified in 8 black rhinoceros, suggesting the possibly of an immune deficiency (12).

Clinical similarities among black rhinoceroses undergoing acute haemolysis prompted efforts to identify a single common denominator for this syndrome, that is, a basic biochemical or metabolic defect that would allow a number of agents to initiate haemolysis. Given the similarities with metabolic induced haemolytic syndromes in man, most notably, a deficiency of glucose-6-phosphate dehydrogenase, studies of the red blood cell metabolism of this species were initiated (11).

These initial studies revealed that the general pattern of energy metabolism in the black rhinoceros departed radically from that of other known species (9,10,11). A number of enzymes showed increased activities relative to most other mammalian erythrocytes, whereas others were markedly decreased. Perhaps the most notable finding was that the levels of adenosine triphosphate (ATP) were reduced to a small fraction (often 5% or less) of the levels found in other mammalian erythrocytes. These findings appeared to be a species characteristic and no specific deficiencies were identified that distinguished those rhinoceroses experiencing haemolysis from those that did not. Although levels of G-6-PD were several times those of humans, several standard screening tests for G-6-PD deficiency were positive when healthy black rhinoceroses were tested. Red blood cell concentrations of reduced glutathione (GSH) were either equal (*D. b. michaeli*) or double (*D. b. minor*) the levels found in humans but testing with either acetylphenylhydrazine or ascorbic acid rapidly oxidised GSH. Thus, GSH maintenance was dependent on oxidant concentration (10).

Most mammalian erythrocytes depend on glucose and other simple sugars as substrate for the metabolic pathways that are essential to normal cell function. Anaerobic glycolysis via the Embden-Meyerhoff pathway generates ATP to sustain the cation pump and other biochemical reactions. If defective enzymes exist in this pathway, then ATP concentrations may decrease enough to produce premature cell death and chronic haemolytic anaemia (10).

The second metabolic pathway, known as the hexose monophosphate shunt (HMP) usually operates at a much lower capacity (5-10%) than the first, but can be stimulated 20- to 30-fold when needed to neutralise oxidants. Humans or animals with disorders of this pathway have no ill effects until they encounter sudden oxidative challenges. Under those conditions, if the system is impaired to a degree that a sufficient neutralising response to the oxidants is prevented, then the individual can undergo an acute episode of haemolysis.

Rhinoceros erythrocytes were found to be capable of glucose degradation, but they did not respond with increased glycolytic rates when challenged *in vitro* with oxidant stimuli that normally activate the HMP shunt in other mammals (9,10). This and other evidence suggests that the low ATP levels may be rate-limiting for the oxidant-induced acceleration of the HMP pathway.

Other deficiencies may also exist. The most significant which has been noted to date were catalase and glutathione S-transferase, which measured 2-3% and less than 1% respectively of human red blood cell activities. These deficiencies are potentially important as they are likely impair neutralisation of hydrogen peroxide, and potentially toxic intermediates generated by infections and the catabolism of plant nutrients.

Either alone or in combination, these unusual metabolic characteristics of the black rhinoceros red blood cell may create an impaired response to oxidants that is functionally equivalent to G-6-PD deficiency (9,10), even though the levels of that enzyme are elevated.

Given these findings, warning have been issued to avoid all agents that are known to initiate haemolysis in G-6-PD deficient humans. These include several classes of pharmaceuticals, for example, antimalarials, sulfonamides, sulphones, nitrofurans, acetanilid, chloramphenicol, vitamin K analogs and others (10). Also to be avoided are a number of chemical compounds that include cyclic hydrocarbons, for example, naphthalenes and phenols. The latter are of special concern since creosote has been used as a wood preservative around black rhinoceroses with apparent deleterious effects (1,4).

Dietary compounds that can cause haemolysis in humans and domestic animals, particularly horses, should also be avoided. These include fava beans (*Vicia faba*), wild onions (*Allium canadense*), oak

(*Quercus* spp.), and red maple (*Acer rubrum*) leaves. Additionally, infections, hypophosphataemia and acidosis may also initiate haemolysis. In the black rhinoceros, leptospirosis has been the infectious disease most often associated with haemolysis (3). Conditions that produce acidosis suppress red blood cell glycolytic activity and are likely interfere with the maintenance of ATP concentrations within the erythrocyte. Similarly hypophosphataemia can also suppress intracellular ATP levels. Hypophosphataemia has been noted in several ill black rhinoceroses (2). Both *in vitro* and *in vivo* studies have shown that phosphate supplementation increased erythrocytic ATP levels 10-20 fold (9,10). This appears to be a one promising approach to the treatment of haemolytic anaemia.

Further studies are addressing the significance of the catalase deficiency in black rhinoceroses. It is notable that it does not occur in the white rhinoceros (*Ceratotherium simum*), a species that in captivity does not share the unusual pattern of disease seen in the black rhinoceros (10).
Evolution of these marked variances in red blood cell metabolism may parallel that hypothesised for G-6-PD deficiency in man where it is an adaptive factor by conferring increased resistance to malarial infection. Since rhinoceroses are also affected by haemic parasites, similar protective mechanisms may have also evolved (10).

Further studies are ongoing to clarify the role that the unusual red blood cell metabolic pattern in this species plays in the pathophysiology of their diseases.

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