REGULATION OF IRON BALANCE IN FOUR SPECIES OF RHINOCEROSES

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Abstract

All rhinoceros species are endangered by encroachment and poaching in their natural habitats. In captivity, the health and longevity of African black (*Diceros bicornis*) and Sumatran (*Dicerorhinus sumatrensis*) species are further threatened by progressive development of pathologically extreme iron overloads.[2,4,5] By contrast, African white (*Ceroatotherium simum*) and Asian greater one-horned (Indian) (*Rhinoceros unicornis*) species exhibit no evidence of iron-storage disorder in captivity.[2,4,5] This distinct clinical disparity between browser and grazer rhinoceros species might be explained by evolutionary differences in the mechanisms by which they assimilate dietary iron and/or regulate its subsequent balance.[3]

Recent advances in studies of iron homeostasis have identified molecular mechanisms involved in the pathogenesis of human iron disorders. Since these regulatory pathways are highly conserved in mammals, those in rhinoceroses are likely to be similar. The phenotype of iron loading in black rhinoceroses is characterized by extensive iron deposition in various organs, and by serum transferrin saturations and ferritin concentrations that are markedly elevated over those of free-ranging black rhinos or captive white rhinos.[4,5] This disorder resembles certain forms of human hereditary hemochromatoses, which are now known to be caused either by deficiency of the key iron regulatory hormone hepcidin, or by resistance to hepcidin. Hepcidin controls iron homeostasis by blocking the absorption of iron from the diet, the release of iron from macrophages recycling old red blood cells and the mobilization of iron from stores in the liver. Hepcidin acts by causing the internalization and degradation of its receptor, ferroportin, the sole channel for egress of intracellular iron into plasma from enterocytes, hepatocytes, macrophages, and placental trophoblast.[3] In humans, hepcidin deficiency or resistance to hepcidin causes excessive iron absorption and deposition of iron in the liver and other tissues. Hepcidin *deficiency* results either from autosomal recessive mutations in the hepcidin gene itself or in genes encoding hepcidin regulators: hemojuvelin, transferrin receptor 2 (TfR2), and the hemochromatosis gene, *HFE*. Hepcidin *resistance* results from autosomal dominant mutations in the hepcidin receptor, ferroportin.

Our experimental approach compared regulatory gene sequences from both affected and unaffected rhinoceros species to search for possible underlying molecular defect(s). Previously, no differences were found in the sequences of rhinoceros HFE genes between affected and unaffected species,[1] but sequences for other iron related genes in rhinoceroses were not known. In this study, DNA was extracted from peripheral blood samples from all four available rhino

species, and genes encoding hepcidin, ferroportin, hemojuvelin, TfR2 and HFE were cloned and analyzed by PCR amplification based on conserved regions of related species (horse and dog).

Over half of the DNA sequences of these five genes have now been determined without identifying a specific cause for the apparent disparity in iron homeostasis among these four species. The sequence encoding the rhinoceros hepcidin was found to be identical between the black and white rhinos. We synthesized the rhinoceros hepcidin peptide and showed that it was active in an *in vitro* bioassay. However, the responsiveness of rhinoceros ferroportin to hepcidin modulation, as well as the level of expression of rhinoceros hepcidin, is still to be determined. Assays to measure hepcidin concentration in rhinoceros urine and sera, similar to methods used for human hepcidin, remain under development.

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