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Vitamin A deficiency increases hepcidin expression and oxidative stress in rat

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Abstract

Objective: The interaction between vitamin A and iron status has been widely reported; however, the exact mechanism involved in this interaction has not been well characterized. The present study investigated the mechanism involved in tissue iron accumulation and changes in the oxidative status in vitamin A–deficient rats.

Methods: Rats were treated with a control diet, a vitamin A–deficient diet, or a vitamin A/iron-deficient diet for 57 d. The animals were sacrificed; the blood, liver, and spleen were collected for biochemical analysis. Analysis of variance or Mann-Whitney tests were used to compare groups and Pearson's or Spearman's tests to investigate the bivariate correlation.

Results: Vitamin A deficiency increased liver hepcidin mRNA and iron spleen concentrations; however, iron deficiency in vitamin A–deficient rats deeply inhibits liver hepcidin mRNA expression and significantly increases divalent metal transporter-1 mRNA levels. Liver ferroportin and hereditary hemochromatosis gene mRNA levels did not change in either treatment. In the vitamin A–deficient groups, liver carbonyl protein increased, whereas catalase and glutathione S-transferase activities decreased, suggesting that vitamin A protects the liver against protein oxidation. A significant positive correlation was found between lipid oxidative damage and iron concentration in the liver and spleen ($r = 0.611$, $P = 0.007$; $r = 0.558$, $P = 0.025$, respectively).

Conclusion: These results suggest that vitamin A maintains iron homeostasis by the modulation of liver hepcidin expression. The increase of lipid peroxidation in vitamin A deficiency seems to be iron dependent, whereas protein oxidation is not.

Keywords: Vitamin A deficiency; Iron; Hepcidin; Oxidative stress

Introduction

Vitamin A and iron deficiency are the most prevalent micronutrient deficiencies in the world. It is estimated that, in developing countries, 43 million children younger than 5 y have vitamin A deficiency [1], and in South and Central America iron deficiency anemia may affect more than 50% of children and pregnant women [2]. Previous studies had suggested a close association between vitamin A and iron metabolisms. Despite the controversies about the positive or negative effect of vitamin A deficiency on hematopoiesis, iron tissue accumulation has been widely reported in vitamin A–deficient animals and cells cultures [3–6], suggesting that vitamin A deficiency diminishes iron mobilization and/or increases absorption [7].

The versatility of iron as an oxidizing/reducing agent is essential for metabolism. However, iron can catalyze oxidative reactions, such as the Fenton reaction, that leads to reactive oxygen species production and, consequently, in-

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creases oxidative stress in living organisms [8]. To avoid this potential adverse effect, mammals developed an efficient protein system that allows safe iron efflux from reserves to functional tissues. Several proteins are involved in iron metabolism, such as the divalent metal transporter-1 (DMT-1), a membrane transporter; transferrin (Tf), a serum transporter; cellular Tf receptor (TfR); ferroportin-1, an exportation protein; ferritin, a storage protein; and a hereditary hemochromatosis gene (HFE). The liver plays a major role in body iron homeostasis because it secretes hepcidin, a 25–amino acid peptide that negatively regulates intestinal iron absorption and tissue release [9,10]. A metabolic molecular regulation of iron absorption by hepatic hepcidin was recently proposed, where Tf and HFE compete for TfR1 binding. In a normal or overloaded iron status, diferric Tf binds with high affinity to TfR1, avoiding HFE/TfR1 binding. Once free, HFE signals to the hepatocyte nucleus, increasing hepcidin expression. Hepcidin is then secreted in plasma and promotes enterocyte ferroportin internalization and degradation, leading to iron accumulation in the enterocyte, which downregulates DMT-1 expression and decreases iron absorption [11,12]. This upregulator signal is abrogated when iron status is low and HFE binds to TfR1.

Preliminary results obtained in our laboratory have shown that vitamin A–deficient rats accumulate iron in the spleen and have a higher oxidative status. Considering the regulatory role of retinoic acid on many genes, we thus hypothesized that vitamin A would regulate the biosynthesis of proteins involved in iron homeostasis and that the widely reported antioxidant effect of vitamin A [13–15] could be actually an indirect effect on iron tissue accumulation. For this purpose, we investigated the effect of vitamin A deficiency on hepcidin, DMT-1, ferroportin, and HFE gene expressions in the liver of vitamin A–deficient animals. In addition, to evaluate if the change on oxidative status observed in vitamin A–deficient rats was correlated with iron tissue stores, a group of rats deficient in vitamin A and iron was included.

Materials and methods

Animals and diets

Eighteen male Wistar rats (Animal Laboratory, University of Brasília, Brasília, Brazil), 21 d old with a mean body weight of 52.5 ± 3.1 g, were housed individually in stainless-steel cages, under a 12-h light cycle, at a room temperature of 23 ± 2°C. The animals had free access to water; food access was only during the dark cycle. The animal care and use committee of the University of Brasília approved the experimental protocol.

Animals were distributed into three experimental groups with six animals per group. The control group received an AIN-93G diet [16], where the vitamin A content (4000 IU/kg of diet) was replaced by an equivalent amount of β-carotene (14 400 μg/kg of diet); the vitamin A–deficient group (−VA) received an AIN-93G without any source of vitamin A; and the iron/vitamin A–deficient group (−VA/−Fe) received an AIN-93G without any source of vitamin A and only 12.2 mg of iron/kg of diet.

The animals were weighed weekly and food intake was recorded daily. After 57 d of feeding the experimental diets, the animals were killed by cervical dislocation and blood was collected in tubes containing 7.0% ethylene-diaminetetraacetic acid (21 μL/mL of blood). The liver and spleen were excised, washed in cold 0.9% NaCl, dried in paper towels to remove excess saline, and rapidly frozen in liquid nitrogen (N_2) and stored at −70°C.

RNA extraction, treatment, and quantification

Extraction of total RNA from liver was done using TRIzol reagent (Invitrogen Inc., Canada). Briefly, 100 mg of tissue was homogenized in 1 mL of TRIzol using an Ultraturrax homogenizer, UKA, Deutschland, Germany. After chloroform extraction, RNA from the aqueous phase was precipitated with isopropyl alcohol and dissolved in water treated with diethylpyrocarbonate. RNA samples were treated with DNase (Promega Corporation, Madison, WI, USA): a 10-μL sample of RNA in water was transferred to an Eppendorf tube and 10 μL of RQ1 RNase-Free DNase 10× reaction buffer, RQ1 RNase-Free DNase (1 U/μg RNA), and nuclease-free water to a final volume of 100 μL were added. The mixture was incubated at 37°C for 30 min and reaction was stopped by the addition of 10 μL of RQ1 DNase stop solution and incubated at 65°C for 10 min to inactivate the DNase. RNA was then re-extracted with TRIzol reagent as described above, quantified by measuring ultraviolet absorbance at 260 nm, and purity assessed by calculating the optical density at 260/280 nm.

First-strand cDNA synthesis

Ten micrograms of total RNA was used for cDNA synthesis using an ImProm-II Reverse Transcription System (Promega Corporation). Oligo(dT) primers were added to total RNA and denaturated at 70°C for 5 min. Improm-II Reverse Transcriptase was added to the samples and further incubated at 42°C for 50 min. The reaction was stopped by incubating at 70°C for 15 min.

Reverse transcriptase polymerase chain reaction

Complementary DNA from liver samples was subjected to reverse transcriptase polymerase chain reaction (RT-PCR) in a PTC-100 Programmable Thermal Controller MJ-Research, Waltham, MA, USA. The PCR temperature/time profile used was an initial denaturation at 94°C for 1 min, followed by denaturation at 94°C for 30 s, annealing at 50°C for 40 s, and extension at 72°C for 40 s for 17, 27, 27, and 23 cycles for hepcidin, HFE, DMT-1, and β-actin gene,