Inflammation interferes with the assessment of vitamin A status in magnesium deficiency

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Abstract. Hyporetinemia is observed in several pathological conditions including a primary deficiency of vitamin A and has also been reported to accompany inflammatory diseases. Experimental magnesium (Mg) deficiency in rodents is accompanied by an inflammatory syndrome. The present study was designed to determine whether the acute phase response in Mg-deficient rats can modify vitamin A status. Clinical symptoms of acute phase response were observed in Mg-deficient rats and were accompanied by a reduction in plasma retinol and of plasma retinol binding protein (RBP). Mg deficiency in rats resulted in hyporetinemia without a significant decrease in liver retinol reserves. Consequently, the data strongly suggest that the decrease in plasma retinol concentration, resulting from the level of its binding protein, is related to the inflammatory effect of Mg deficiency. These results point to the possible interference of Mg deficiency on the use of plasma retinol as an indicator of vitamin A status.

Key words: magnesium, vitamin A, retinol, RBP, acute phase

Vitamin A is important for normal vision, gene expression, reproduction, embryonic development, growth and immune function. Vitamin A deficiency is a cause of significant morbidity and mortality worldwide [1]. Serum retinol is the most commonly used indicator of vitamin A status [1]. Hyporetinemia is observed in several pathological conditions including a primary deficiency of vitamin A, protein-energy malnutrition. Hyporetinemia has also been reported to accompany inflammatory diseases [2-4].

Magnesium (Mg) deficiency plays an essential role in a wide range of fundamental cellular reactions and it is not surprising that an increasing number of clinical disorders have been found to be associated with Mg deficiency [5, 6]. Mg deficiency can easily be produced in rodents by dietary depletion and it is accompanied by an inflammatory syndrome characterized by leukocyte and macrophage activation, release of inflammatory cytokines and acute phase proteins [5-8]. The decrease of several negative phase proteins completes the classical pattern of the acute phase response. This is accompanied in the liver by a decrease in the level of mRNA coding for retinol binding protein (RBP) [6]. However, the consequence of Mg-deficiency-induced inflammation on retinol transport has not been studied. The present study was undertaken to determine whether the acute phase response in Mg-deficient rats can modify vitamin A status.

Material and methods

Male weaning Wistar rats (Iffa-Credo, L’Arbresle, France) weighing 61 ± 3 g were randomly divided into control and Mg-deficient groups. The institution’s guide for the care and use of laboratory ani-
mals was used. The rats were housed in wire-bottomed cages in a temperature-controlled room (22°C) with a 12 h dark (20.00 h-8.00 h) and 12 h light period. They were pair-fed with the appropriate diets using an automatic feeding apparatus and maintained on the experimental diet. Distilled water was provided ad libitum. The diets contained (g/kg): 200 casein, 650 sucrose, 50 corn oil, 50 alphacel, 3 DL-methionine, 2 choline bitartrate, 35 modified AN-76 mineral mix (ICN Biomedicals, Orsay, France). Mg oxide was omitted from the mineral mix in the Mg-deficient diet. The Mg concentrations of the diets, determined by flame atomic absorption spectrometric analysis (Perkin Elmer 400, Courtaboeuf, France), were 30 and 950 mg/kg respectively.

Fasted animals anesthetized with sodium pentobarbital (40 mg/kg, i.p.) were killed by aorta exsanguinations. Blood was collected into heparinized tubes. Plasma after low-speed centrifugation (2 000 x g for 15 min) was stored at - 80°C for analyses. The liver was removed after blood sampling and stored at - 80°C.

Plasma Mg was determined by flame atomic absorption spectrometric analysis (Perkin Elmer 400, Courtaboeuf, France), and plasma total RBP and retinol were measured by using a nephelometric method (model BN 100, Behring SA, Marburg, Germany) with homemade antibodies and by HPLC as described previously [9, 10], respectively.

Results are expressed as means ± SEM. The statistical significance of differences between groups was assessed using the Student's t unpaired test (GraphPad Software, Inc. La Jolla, CA, USA). Results were considered significant at p < 0.05.

Results and discussion

The mean plasma Mg concentration was severely reduced in Mg-deficient rats as compared to controls (table 1). A characteristic allergy-like crisis with erythema, hyperaemia and oedema occurred in Mg-deficient rats, as previously described [5-7]. As compared to controls, Mg-deficient rats presented lower concentrations of plasma retinol and of plasma RBP (table 1). The mean concentration of retinol in the liver did not significantly differ between control and Mg-deficient rats (table 1).

Recent studies underline the importance of the immuno-inflammatory processes in the pathology of acute Mg deficiency [5-7]. The increase in the expression and/or plasma levels of positive acute phase proteins: α2-macroglobulin, α1-acid glycoprotein, complement, haptoglobin, fibrinogen, has been observed in the response to dietary Mg deficiency [6-8]. These changes have been related to the increased interleukin 6 concentrations [6, 7]. At the same time the expression and/or levels of several negative acute phase proteins decrease, such as albumin, apolipoprotein E and RBP [6, 7]. It is generally assumed that the liver shifts resources to the rapid synthesis of positive acute phase proteins needed for host defence and thus reduces the synthesis of less critical proteins. Thus, the significant reduction in the concentration of retinol and RBP in plasma and of RBP mRNA in the liver strongly implies that hyporetinemia in Mg-deficient rats is most likely caused by a reduction in the hepatic synthesis of RBP and subsequent secretion of a reduced amount of the holo-RBP complex into the plasma.

In this study Mg deficiency resulted in a state of hyporetinemia in rats whose vitamin A status was normal at the onset of the experiment, and whose liver retinol reserves were not significantly affected by Mg deficiency. Moreover, the reduction in plasma retinol was accompanied by a reduction in plasma RBP, associated with clinical symptoms of an acute phase response occurring in Mg-deficient rats. Consequently, the decrease in plasma retinol concentration was undoubtedly the consequence of the inflammatory effect of Mg deficiency. Thus, attention has to be paid to the possible interference of Mg deficiency in the use of plasma retinol as an indicator of vitamin A status. Other experiments are needed to assess the consequences of Mg deficiency of long duration on vitamin A status.

Table 1. Magnesium and vitamin A status in control and Mg-deficient rats (8-10 days of deficiency).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Mg-deficient</th>
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<tr>
<td>Plasma Mg (mmol/L)</td>
<td>0.81 ± 0.04</td>
<td>0.14 ± 0.01**</td>
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<tr>
<td>Plasma retinol (μmol/L)</td>
<td>1.896 ± 0.199</td>
<td>1.202 ± 0.135*</td>
</tr>
<tr>
<td>Plasma total RBP (μmol/L)</td>
<td>1.354 ± 0.060</td>
<td>0.820 ± 0.021**</td>
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<tr>
<td>Liver retinol (μmol/g)</td>
<td>17.45 ± 1.67</td>
<td>13.70 ± 0.87</td>
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Values are means ± SEM of 6-10 values per group; significantly different, * p < 0.01; ** p < 0.001.
References


