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Original Article

Effects of magnesium deficiency on purine-base metabolism in rats

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Summary

Molybdenum (Mo) is a constituent of Mo cofactor (MoCo) that is indispensable for the activity of molybdoenzyme such as xanthine oxidase (XO). This molybdoenzyme plays an important role on purine-base metabolism in the liver where Mo mainly exists as MoCo. Our previous experiment indicated that magnesium (Mg) deficiency increased Mo concentration in rat liver. We hypothesized that the increase in Mo reflects up-regulating expression of molybdoenzyme such as XO in the liver of Mg-deficient rats. We investigated the effect of Mg deficiency on the metabolism of purine-base in rats. Twelve growing male rats were given a control diet or an Mg-deficient diet for 4 weeks. Urine was collected every day during the last 5 days in the feeding trial. At the end of feeding trial, liver was collected, and the hepatic XO activity and the concentration of purine-base metabolites such as hypoxanthine, xanthine, uric acid, and allantoin were determined. Additionally, urinary excretion of purine-base metabolites was determined. Mg deficiency did not affect the XO activity and the concentration of purine-base metabolites in the liver. One the other hand, urinary excretion of uric acid and allantoin was significantly decreased by Mg deficiency. These results suggested that the increase in hepatic Mo concentration does not affect purine-base metabolism in the liver of Mg-deficient rat, but that Mg deficiency decreases urinary excretion of purine-base metabolites.

Introduction

Molybdenum (Mo) is a constituent of Mo cofactor (MoCo) that is indispensable for the activity of molybdoenzymes such as xanthine oxidase (XO)[1], aldehyde oxidase (AOX)[2], and sulfite oxidase (SOX)[3] in humans and animals.

The liver plays the central role of purine-base metabolism and XO is a key enzyme for purine-base metabolism in the liver[4]. XO oxidizes hypoxanthine to xanthine and further oxidizes xanthine to uric acid. Then uric acid is converted to allantoin by another enzyme, uricase in many animals except for humans[5]. During these reactions by XO, reactive oxygen species are generated, which induces oxidative stress[6].

We previously reported that magnesium (Mg) deficiency increases Mo concentration in the liver[7]. As mentioned above, XO induces oxidative stress in the liver. Mg deficiency is also known to induce oxidative stress in the liver[8]. Because Mo mainly exists as MoCo in the liver[9], the increase in Mo concentration possibly reflects increasing activity and/or expression of molybdoenzyme(s) in the liver. Therefore, it can be postulated that the activity of XO is increased by the elevated Mo level in the liver of Mg-deficient rats. Mo toxicity was reported to associate with the incidence of gout-like syndrome with elevated blood levels of Mo and uric acid[10] that is the primary end product of purine-base metabolism in humans. Thus, the increasing hepatic Mo possibly enhances purine-base catabolism.

In present study, we investigated the effects of Mg deficiency on the purine-base metabolism in rats.

Materials and Methods

Animals and diets

Twelve 4-week-old male Sprague-Dawley rats were purchased from SLC Japan (Shizuoka, Japan) and cared according to the Guide for the Care and Use of Laboratory Animals (Animal Care Committee, Kyoto University). They were randomly divided into two groups of six rats each and individually housed in metabolic cages fitted with urine separators in a room maintained at 24℃ and 60% humidity with 12-h light : dark cycle. All rats were fed the control diet, AIN-93G diet[11], for 6-day adaptation period, then divided into two groups given either the control diet or the Mg-deficient diet, AIN-93G-based diet with Mg-free AIN-93G-MX[11]. The control rats

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were pair-fed to match the average intake of the rats given the Mg-deficient diet for 4 weeks. All rats were allowed free access to demineralized water.

Sample preparation

Urine sample was collected every day during the last 5 days in the feeding trial and were stored at −80 ℃ until analysis. After feeding trial, the rats were exsanguinated under isoflurane anesthesia and the liver was quickly harvested and rinsed with chilled saline solution. The liver was stored at −80 ℃ until analyses.

Determination of xanthine oxidase activity

Hepatic XO activity was determined according to the method of Yoshihara et al.[12]. Briefly, liver sample was homogenized at 4 ℃ with saline solution (1 g of liver tissue in 9 mL saline) and centrifuged at 8,000 × g for 15 min at 4 ℃. The 0.2 mL supernatant was mixed with 2.2 mL of 0.136 M Tris-HCl buffer (pH 7.4), and the mixture was incubated at 37 ℃ with shaking for 3 min. After then, 0.6 mL of 0.3 mM xanthine solution, as a substrate of XO, was added to the mixture, and incubated at 37 ℃ with shaking accurately for 10 min. The mixture was quickly placed in ice-cold water at the end of incubation and the mixture was added with 1 mL of 20 % perchloric acid to terminate the reaction, and centrifuged at 8,000 × g for 15 min at 4 ℃. The supernatant was adjusted pH between 4 and 5 with 0.4 M potassium phosphate buffer (pH 7.8) and filled up to 15 mL with distilled water. Uric acid concentration in the supernatant with and without incubation was determined by the method shown below. The difference of uric acid concentrations was obtained between the supernatant with and without incubation.

The protein concentration in supernatant was spectrophotometrically determined with bicinchoninic acid using bovine serum albumin as the standard[13]. The xanthine oxidase activity was expressed as uric acid formed (μmol) per min per protein (g).

Determination of purine-base metabolites

The 0.5 mL of liver homogenates (1 g of liver tissue in 9 mL saline) was treated with perchloric acid (final concentration 0.5 M) and centrifuged at 8,000 × g for 10 min. The supernatants were neutralized to be pH between 6 and 7 with 0.4 M potassium phosphate buffer (pH 7.8) and filled up to 10 mL with distilled water. The treatment of urine sample was determined based on the method of Chen et al.[14].

Hepatic and urinary hypoxanthine, xanthine, uric acid, and allantoin content were simultaneously measured by HPLC based on the method of George et al.[15] for bovine urine. Briefly, the system consisted of HPLC (D-7000, Hitachi, Japan) equipped with a UV spectrophotometric detector and a reversed-phase column (Capcell Pak, UG120, 5 μm, 4.6 mm × 250 mm, Shiseido, Japan). The flow rate was 1 mL/min and the absorbance detector was set at 220 nm. The mobile phase consisted of 1 % acetonitrile in 10 mM potassium phosphate buffer (pH 4.7) (A) and 20 % acetonitrile in 10 mM potassium phosphate buffer (pH 4.7) (B), delivered isocratically (100 % A) for 13 min and then in gradient mode (100 % to 0 % A from 13 min to 26 min). The peaks of purine-base metabolite were identified by the retention times and quantified by comparison of the peak areas of the samples with those of authentic standards on a 20 μL injection.

Statistical analysis

All data are presented as the mean and standard error. Significant difference between the control group and the Mg-deficient group was evaluated by Student's t-test.

Results and Discussion

One of the control rats was excluded from the experiment because of severe polyuria.

Plasma Mg concentration was significantly lower ($P < 0.05$) in the Mg-deficient rats than in the control rats, hepatic Mo concentration was approximately 20 % higher in the Mg-deficient rats than in the control rats, the creatinine clearance was significantly lower ($P < 0.05$) in the Mg-deficient group than in the control group[16].

The activity of XO was not different between the control and the Mg-deficient group (Table 1). Mg deficiency did not affect the concentration of purine-base metabolites, i.e., hypoxanthine, xanthine, uric acid, and allantoin in the liver (Table 1). Because Mo mainly exists as MoCo in the liver[9], the increase in Mo concentration possibly reflects increasing molybdoenzyme(s) in the liv-

Table 1 The effect of Mg deficiency on xanthine oxidase activity and the concentration of hypoxanthine, xanthine, uric acid, and allantoin in rat liver

	Control	Mg D
	Unit[1]	
Xanthine oxidase activity	0.49 ± 0.12	0.43 ± 0.05
	μmol/g wet liver weight	
Hypoxanthine	1.11 ± 0.09	1.16 ± 0.07
Xanthine	1.76 ± 0.06	1.71 ± 0.05
Uric acid	0.93 ± 0.04	0.86 ± 0.03
Allantoin	9.41 ± 0.33	10.05 ± 0.68

1 Unit is expressed as μmol of uric acid formed per min per g protein in liver

er. Thus, the increasing hepatic Mo possibly enhances purine-base catabolism. On the other hand, the results of present experiment indicated that the increase in Mo concentration did not affect the activity of XO and purine-base metabolism in the liver of Mg-deficient rats. XO is responsible for production of reactive oxygen species and plays a role in several oxidative stress-related diseases[6]. Mg deficiency also induces oxidative stress in the liver[8]. The present experiment clarified that oxidative stress was not related to the activity of XO in the liver of Mg-deficient rats. Because the liver highly expresses other molybdoenzymes including AOX[17] and SOX[18], Mg deficiency possibly increase the expression of these molybdoenzymes, which is responsible for increasing Mo concentration in the liver. Otherwise, Mg deficiency possibly increases free MoCo without working as the coenzyme, or increases Mo in the form other than MoCo in the liver. Further experiment should be performed to clarify the form of Mo in the liver of Mg-deficient animals.

Although hepatic concentration of purine-base metabolites was not affected by Mg deficiency, urinary excretion of uric acid and allantoin was significantly lower in the Mg-deficient rats than in the control rats (Table 2). The concentration of xanthine and hypoxanthine was too low to measure in urine. We observed that the Mg deficiency reduced creatinine clearance in the same rats used in the present experiment[16], which was supported by a report showing the reduction of inulin clearance in Mg-deficient rats[19]. These results suggested that Mg deficiency decreased glomerular filtration rate, which may decrease urinary excretion of uric acid and allantoin.

Table 2 The effect of Mg deficiency on the excretion of urinary uric acid and allantoin in rats

	Control	Mg D
	μmol/day	
Uric acid	26.2 ± 2.0	13.7 ± 2.2**
Allantoin	295.1 ± 4.4	259.0 ± 12.8*

*P < 0.05, **P < 0.01; Significant difference compared to control group

In present study, we showed that Mg deficiency does not affect the activity of XO and the concentration of purine-base metabolites in the liver but suppresses urinary excretion of uric acid and allantoin. Mg deficiency may be a risk for gout through suppressing urinary excretion of uric acid in humans.

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