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Changes of Electrolytes, Hematological Indices, and Cytokines following Dietary Magnesium Deficiency in Rats

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Magnesium (Mg) plays an essential role in physiological and metabolic reactions. Recently, there has been an increased interest in the role of Mg deficiency, particularly the relationship between serum Mg value and inflammatory response. This study was designed to determine the relationship between serum Mg deficiency with inflammatory response, electrolytes and hematological alteration over long-term periods. Sixteen male Sprague-Dawley rats were divided into two groups: control (n=8), and Mg deficiency group (MgD group, n=8). Chow and normal water (tap water) were regularly provided to the control group and Mg-depleted chow and third distilled water were regularly provided for 60 days to the MgD group. Body weights, Serum Mg, K^+, inorganic phosphorus (IP) and total iron binding capacity (TIBC) levels in the MgD group were lower than those of the control group ($P<0.05$). Granulocyte fraction and MCV, RDW and PDW levels were higher, whereas lymphocyte fraction, erythrocyte, hemoglobin and MCHC levels were lower in the MgD group than in the control group ($P<0.05$). MCP-1 and TNF-α levels in the MgD group were greater than those of the control group ($P<0.05$). In conclusion, the results of the present study suggest that Mg deficiency over a long-term period had not altered total leukocyte concentration in the blood, but had detrimental effects, including disturbances of electrolytes balance, disturbance of iron indices, potential anemia and elevation of pro-inflammatory cytokine. However, further studies should be performed to determine the relationship between serum Mg deficiency and major organ damage or alteration.

Key Words: Magnesium deficiency, Inflammation, Cytokine

INTRODUCTION

Magnesium (Mg) is the fourth most abundant extracellular cation and the second in intracellular space. The adult human body contains 21~28 g of Mg. It is subdivided into three major compartments of body: about 65% in the mineral phase of the skeleton, some 34% in the intracellular space, and only 1% in the extracellular fluid (Barbagallo and Domingez, 2007). The small intestine is the main site for Mg absorption, whereas Mg excretion is mainly performed through renal pathways. Serum Mg exists in the three forms: a protein-bound fraction (25% bound to albumin, 8% bound to globulin), a chelated fraction (12%) and the metabolically active ionized fraction (55%) (Saris et al., 2000). Mg plays an essential role in physiological and metabolic reaction, including energy metabolism, protein synthesis, nucleic acid synthesis, glucose utilization, fatty acid synthesis, cellular cytoskeleton, hormonal reaction and L-type calcium channel antagonist. Over 300 enzymatic reactions are dependent on Mg (Singh et al., 1997; Dacey, 2001; Swaminathm, 2003; Gums, 2004; Moe, 2008).

Mg deficiency, or a reduction in dietary intake of Mg, plays an important role in the etiology of diabetes, hypertension, congestive heart failure, alcoholism and eclampsia (Gums, 2004). Furthermore, Mg deficiency was a common problem in hospital patients, with a prevalence of about 10%. The prevalence of hypomagnesemia in critically ill

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patients was even higher (20~65%). Also, patients in an intensive care unit (ICU) were associated with increased mortality due to Mg deficiency (Chernow et al., 1989; Rubeiz et al., 1993; Swaminathan, 2003). However, Mg monitoring was overlooked in clinical states except for special events.

Recently, there has been an increased interest in the role of magnesium deficiency, particularly, the relationship between serum Mg value and inflammatory response. Clinical and animal experimental studies suggested a link between low Mg status and inflammation, including leukocytosis, leukocyte infiltration of several tissue, spleen size enlargement, elevation of acute phase protein, pro-inflammatory cytokine, pro-inflammatory neuropeptide and oxygen free radical production (Weglicki et al., 1992; Mak et al., 1997; Maier et al., 1998; Malpuech-Brugère et al., 2000; Guerrero-Romero and Rodriguez-Morán, 2006; Mazur et al., 2007). Most of the animal models with Mg deficiency that have studied the change of acute Mg decline during a short period (1~3 weeks), and alteration of inflammatory response and/or hematological indices following chronic Mg deficiency were not reported.

Thus, this study was designed to determine the relationship between serum Mg deficiency with inflammatory response, electrolytes and hematological alteration for long-term periods.

MATERIALS AND METHODS

Animals and experimental protocol

Male Sprague-Dawley rats (aged 5 weeks), weighed 90~100 g, purchased from Central Lab. Animal Inc. (Korea). Rats were housed in standard laboratory conditions with free access to food and water. All rats were housed on a 12/12 hours light/dark cycle at a temperature of 25℃ and humidity of 60%. After adaptation for 1 week, rats were randomly divided into two groups: control (n = 8), and Mg deficiency group (MgD group, n = 8). In the control group, chow and normal water (tap water) were regularly provided. In the MgD group, Mg-depleted chow and third distilled water were regularly provided for 60 days. The Mg-depleted diet chow contained (g/kg): casein (200), DL-Methionine (3), corn oil (50), sucrose (500), cornstarch (150), cellulose (50), choline bitartrate (2), AIN-76A vitamin mix (10), and Mg-free mineral mix (35) (Dyets Inc., USA). Control group rats were fed with Purina Lab. Rodent chow, which contained 0.16% of Mg content (Central Lab. Animal Inc., Korea). Rats of all groups had their body weight measured at the same time daily during the experimental period.

All rats were anesthetized with inhalation of ether after none per-oral (NPO) of 24 hours, and then placed on the rat-operating table (Dong Seo Science, Korea) in the supine position. A blood sample of 8~10 ml was taken directly from the heart. The amount of 1 mL was infused in an ethylene diamine tetraacetic acid (EDTA) tube for analyzing hematological indices. The remainder of the blood was centrifuged into serum or plasma at room temperature (RT), 2,000 g for 15 minutes, and stored at -70℃ for measuring serum Mg, iron indices and cytokines. All animal experiments were accepted by the Institutional Animal Care and Use Committee of the Catholic University of Pusan.

Serum Mg and electrolytes

Levels of Mg, total calcium (Ca), sodium (Na^+), potassium (K^+), and inorganic phosphorus (IP) in the serum were measured by an auto biochemical analyzer (Toshiba Co., Japan).

Iron indices

Iron (Fe) and total iron binding capacity (TIBC) levels in the serum were analyzed by a Hitachi 7600-210 (Hitachi Co., Japan) with Lqdia Fe (Asan Co., Korea). Unsaturated iron binding capacity (UIBC; UIBC = TIBC - serum iron) and transferrin saturation (transferrin saturation = serum iron / TIBC × 100) were calculated.

Hematology

One ml of whole blood was infused into an EDTA bottle for measuring CBC & differential count using an auto hematology analyzer (BC-2800 ver., Shenzhen Mindary Bio-Medical Electronics CO., Ltd., Germany).

Substance P and cytokines

The enzyme linked immunosorbent assay (ELISA)

Table 1. Comparison of body weights from control and magnesium deficiency in rats

Body weights (g)	Group	
	Control (n=8)	MgD (n=8)
Adaptation	187.12±2.09	182.82±1.27
After 2 weeks	286.62±6.48	246.60±4.24*
After 4 weeks	347.50±10.19	282.80±4.78*
After 6 weeks	407.25±18.00	305.25±5.14*
At sacrifice	406.50±13.50	293.87±6.40*

Data were expressed as the mean ± standard error (SE).
*, $P<0.05$ (compared with control group).
Abbreviation: MgD, magnesium deficiency.

Table 2. Comparison of serum Mg and electrolytes levels from control and magnesium deficiency in rats

Variable	Group	
	Control	MgD
Magnesium (mg/dl)	2.18±0.03	0.61±0.03*
Total calcium (mg/dl)	9.52±0.44	10.40±0.20
Na^+ (mmol/l)	156.62±0.98	157.75±0.72
K^+ (mmol/l)	5.37±0.37	4.07±0.16*
IP (mg/dl)	7.27±0.21	6.36±0.19*

Data were expressed as the mean ± SE.
*, $P<0.05$ (compared with control group).
Abbreviation: MgD, magnesium deficiency; IP, inorganic phosphorus; Na, sodium; K, potassium.

method was applied for measuring the substance P. Aprotinin (final concentration of 0.014 TIU/ml) was added into the serum, and then was analyzed by a Biotrak II micro plate reader (Biochrom Ltd., Austria) with a Parameter™ substance P assay kit (R & D systems, USA). The ELISA method was applied for measuring concentrations of Monocyte chemoattractant protein-1 (MCP-1), Tumor necrosis factor-α (TNF-α), Interleukin-6 (IL-6), and Interleukin-10 (IL-10) in the serum, and a Biotrak II micro plate reader (Biochrom Ltd., Austria) with a Thermo Scientific rat MCP-1 ELISA kit (Pierce Biotechnology, USA), a Thermo Scientific rat TNF-α ELISA kit (Pierce Biotechnology, USA), a Quantikine ®rat IL-6 immunoassay kit (R & D Systems, USA), and a Quantikine ®rat IL-10 immunoassay kit (R & D Systems, USA) were used.

Statistical analysis

Data were presented as mean ± SE (standard error). A Paired *t*-test was utilized for comparison of the difference between the control and MgD group (SPSS program). Statistical significance was accepted with $P<0.05$.

RESULTS AND DISCUSSION

Body weights in the MgD group were significantly lower than those of the control group during the rest of the experimental period except at the adaptation period ($P<0.05$, Table 1). It is considered that lower body weights are attributable to atrophy of skeletal muscle or defect of muscle formation. Mg is known to participate in energy metabolic processes including glycogen breakdown, fat oxidation, protein synthesis, ATP synthesis, and second messenger system (Shils and Rude, 1996). Nakagawa et al. (1976) reported that calcium and Mg deficiency induced atrophy of muscle in a rat model. As known, skeletal muscle mass is a part of body weight. A deficiency of a dietary Mg supplement can cause the malfunctioning of required energy conversions from the diet thus body weight loss may occur.

Serum Mg, K^+, and IP levels in the MgD group were significantly lower than those of the control group ($P<0.05$, Table 2). Mg is one of the most abundant elements in physiologic cation. Mg is required for the activation of numerous important enzyme systems, including those that involve adenosine triphosphate. This element is also essential for the transfer, storage and utilization of intracellular energy, for the metabolism of protein, carbohydrate, fat and nucleic acids, for the maintenance of normal cell membrane function and for neuromuscular transmission (Berkelhammer, 1985). Mg influences the balance between extracellular and intracellular potassium (Rayssiguier, 1977). Even though the MgD group had a normal level of serum K^+ that was obviously lower than the control group, Mg and potassium homeostasis are closely related. Hypokalemia is a frequent finding in patients with hypomagnesemia. Potassium depletion cannot be corrected until Mg depletion is corrected. The exact mechanism for the development of hypokalemia in Mg deficiency is not clear, but may be related to the dependence of Na^+, K^+-ATPase, Na, K-Cl co-transport, potassium channels and other transport processes of Mg (Ryan, 1993; Swaminathan, 2003). Inorganic phos-

Table 3. Comparison of iron indices from control and magnesium deficiency in rats

Variable	Group	
	Control	MgD
Iron (μg/dl)	84.63±6.73	61.37±18.95
UIBC (μg/dl)	361.50±17.77	333.00±14.18
TIBC (μg/dl)	446.12±17.54	394.37±12.00*
TS (%)	19.10±1.61	15.67±1.71

Data were expressed as the mean ± SD.
*, $P<0.05$ (compared with control group).
Abbreviation: MgD, magnesium deficiency; UIBC, unsaturated iron binding capacity; TIBC, total iron binding capacity; TS, transferrin saturation.

Table 4. Comparison of leukocyte variables from control and magnesium deficiency in rats

Variable	Group	
	Control	MgD
Actual count (10^3/μl)		
Total leukocyte	4.31±0.49	5.38±0.93
Lymphocyte	3.13±0.36	3.50±0.53
Monocyte	0.08±0.01	0.15±0.05
Granulocyte	1.08±0.13	1.73±0.38
Fraction (%)		
Lymphocyte	72.65±1.08	66.31±1.86*
Granulocyte	25.35±1.00	31.16±1.56*
Monocyte	2.00±0.14	2.52±0.36

Data were expressed as the mean ± SE.
*, $P<0.05$ (compared with control group).
Abbreviation: MgD, magnesium deficiency.

phorus (IP) is critical for numerous normal physiologic functions including skeletal development, mineral metabolism, energy transfer through mitochondria metabolism, cell membrane phospholipids content and function cell signaling, and even platelet aggregation. In the present study, the IP level in the MgD group was lower than in the control group, suggesting that hypomagnesemia may lead to a decrease of IP level. Hypomagnesemia-induced decline of IP concentration may cause skeletal muscle atrophy in addition to muscle weakness, hemolysis, impaired platelet and leukocyte function, and in rare cases, neurological disorders (Moe, 2008).

There were no significant differences in iron levels between the control and the MgD group. Also, unsaturated iron binding capacity (UIBC) levels and transferrin saturation value showed no significant differences between the control and the MgD group ($P>0.05$, Table 3), but those of the MgD group tended to be low concentrations compared with those of the control group. The serum total iron binding capacity (TIBC) levels in the MgD group were significantly lower than those of the control group ($P<0.05$, Table 3). The TIBC level in the MgD group was clearly decreased, indicating that hypomagnesemia may contribute to a disturbance of iron metabolism. That a deficiency of a dietary Mg supplement may cause a decline of iron indices has not been reported yet. Iron is an essential micronutrient, as it is required for adequate erythropoietic function, oxidative metabolism and cellular immune responses (Muñoz et al., 2009). Darreau et al. (2004) reported that the iron metabolism imbalance in chronic inflammatory states, elevated ferritin and decreased of serum iron, transferrin, and transferrin saturation. The results of this present study showed that a deficiency of dietary Mg could derive iron indices imbalance and cause potential inflammation. However, there needs to be further studies of the iron indices imbalance including iron storage and transport in dietary Mg deficiency.

There were no significant differences in the actual counts of total leukocyte, lymphocyte, monocyte and Granulocyte between the two groups ($P>0.05$, Table 4), but those of the MgD group tended to have higher counts compared with those of the control group. However, the MgD group had higher Granulocyte fraction and lower lymphocyte fraction than the control group ($P<0.05$, Table 4). Recent studies have shown that the characteristic allergy-like crisis of the Mg deficient rat was accompanied by leukocyte response and changes. In peripheral blood, the most prominent change is leukocytosis, which results from the increased number of polymorphonuclear leukocytes (Malpuech-Brugèsre et al., 2000; Mazur et al., 2007). But, total leukocyte concentration in the blood was not altered by Mg deficiency over a long-term in the present study. That this result is discordant with previous studies may be due to experimental period differences. Previous studies showed that leukocyte counts change in relation to acute Mg decline during short periods (1~3 weeks). This finding showed that was not affected total leukocyte changes following chronic Mg deficiency.

Erythrocyte counts were significantly lower, while mean

Table 5. Comparison of erythrocyte and platelet variables from control and magnesium deficiency in rats

Variable	Group	
	Control	MgD
Erythrocyte (10^6/μl)	9.52±0.15	6.33±0.20*
Hemoglobin (g/dl)	15.93±0.28	12.93±0.20*
Hematocrit (%)	49.98±0.83	41.57±0.61
MCV (fl)	52.56±0.32	66.03±1.47*
MCH (pg)	16.68±0.15	20.48±0.43*
MCHC (g/dl)	31.83±0.14	31.06±0.06*
RDW (%)	11.52±0.16	13.97±0.62*
Platelet (10^3/μl)	619.50±28.71	803.25±68.89
MPV (fl)	6.62±0.10	7.02±0.09
PDW (%)	15.11±0.08	15.57±0.04*

Data were expressed as the mean ± SE.
*, $P<0.05$ (compared with control group).
Abbreviation: MgD, magnesium deficiency; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; MPV, mean platelet volume; PDW, platelet distribution width.

Table 6. Comparison of substance P and cytokines levels from control and magnesium deficiency in rats

Variable	Group	
	Control	MgD
SP (pg/ml)	110.01±42.67	193.96±34.18
MCP-1 (pg/ml)	1940.63±87.16	2479.01±46.10*
TNF-α (pg/ml)	35.05±1.86	64.32±5.46*
IL-6 (pg/ml)	123.38±6.38	129.19±6.84
IL-10 (pg/ml)	16.80±3.84	16.90±4.39

Data were expressed as the mean ± SD.
*, $P<0.05$ (compared with control group).
Abbreviation: MgD, magnesium deficiency; SP, substance P; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; IL-10, interleukin-10.

corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red blood cell distribution width (RDW) levels were greater in the MgD group than in the control group ($P<0.05$, Table 5). Hemoglobin and mean corpuscular hemoglobin concentration (MCHC) levels in the MgD group were significantly lower than those of the control group ($P<0.05$, Table 5). Platelet counts in the MgD group tended to be high compared to the control group, but not statistically significant. The platelet distribution width (PDW) level was significantly higher in the MgD group than in the control group ($P<0.05$, Table 5). This study shows that red blood cell (RBC) count and hemoglobin consideration in the MgD group were lower, whereas MCV, MCH and MCHC levels in the MgD group were higher than in the control group, implying adverse effects of hypomagnesemia on the hematological homeostasis.

Piomelli et al. (1973) demonstrated that an Mg-deficient diet to rats for 4~5 weeks lead to anemia. By their findings, the erythrocytes were slightly smaller and flatter, and had a reduced hemoglobin component and a decreased osmotic fragility. Eilin and Tan (1977) reported that in rats Mg deficiency causes plaque formation on the erythrocyte membrane, altered erythrocyte membrane lifespan and anemia. Decreased RBC and hemoglobin levels together with elevated MCV, MCH and MCHC levels in this test mean potential anemia resulted from Mg deficiency. Actual platelet counts tended to be higher in the MgD than in the control group although there was no statistical significance. In platelet distribution width (PDW) level, the MgD group was significant higher than the control group. This finding indicates a change on platelet level following hypomagnesemia.

SP levels in the MgD group tended to be high compared with the control group, but not significantly ($P>0.05$, Table 6). MCP-1 and TNF-α levels in the MgD group were significantly greater than those of the control group ($P<0.05$, Table 6). But, there were not significant differences in IL-6 and IL-10 levels between the control and the MgD group ($P>0.05$, Table 6). SP is an 11-amino acid neuropeptide that functions as a neurotransmitter and as a neuromodulator. It belongs to the tachykinin neuropeptide family, one of the more potent vasodilators known. In addition, it also plays a major role in modulating inflammatory and immune responses. SP induces the production of cytokines, such as interleukin (IL)-1, IL-6 and tumor necrosis factor-α (TNF-α) in monocytes (Harrison and Geppetti, 2001). Weglicki and Phillips (1992) demonstrated a significant elevation of the neurokinin, substance P during the first week on the Mg-deficient diet. Thus, increased trend of SP together with elevation of TNF-α level represent evident inflammatory reaction by hypomagnesemia.

The present results are supported by Weglicki et al.

(1992) showing that an Mg deficit has a profound effect on the process of inflammation, causing high circulating amounts of interleukin and TNF-α. TNF-α is considered to be the most important mediators of cardiovascular disease. TNF-α directly decreases myocardial contractile function. The initial contractile depression induced by TNF-α is mediated by the activation of sphingomyelinase, which hydrolyzes the phospholipids sphingomyelin to ceramide. In addition, TNF-α has an effect on cardiac injury and evokes an increased rate of apoptosis, progression of congestive heart failure, synthesis of other cytokines and inflammatory response (Ferrari, 1999). Hypomagnesemia also produces chemokine, as a significant increase of monocyte chemoattractant protein-1 (MCP-1) in the MgD group. MCP-1 plays an important mediator in inflammatory response development, it is characterized by expression of pro-inflammatory cytokines, adhesion molecules, and importantly, chemokines that orchestrate the infiltration of leukocytes into damaged tissue area, expression of damaging agents and activation of complement system (Gerszten et al., 1999; Gerard and Rollins, 2001; Moser, 2001). Also, recent investigations have demonstrated that TNF-α and MCP-1 play a major role in the pathophysiology of renal function. TNF-α and MCP-1 promotes renal inflammation, thereby contributing to acute and chronic nephropathies. Renal endothelial cells and proximal tubular epithelial cells produce cytokines and chemokines that result in the infiltration of inflammatory cells into the interstitial of the kidney. Inflammatory cells in the kidney produce pro-inflammatory cytokines that may further increase inflammation in the kidney. Its production is stimulated by oxidative stress (Zager, 2005; Eardley et al., 2006). However, the present study did not determine the alternation of major organ and organ function chemical markers such as the heart and kidney. Therefore, there needs to be further study on the relationship pathologic alteration of major organ between elevations of cytokine and dietary Mg deficiency.

In summary, deficiency of dietary Mg for a long-term period showed that total leukocyte concentration in the blood was not altered, but may cause disturbances of electrolytes balance, disturbance of iron indices, potential anemia and elevation of pro-inflammatory cytokine. However, further studies are needed to show the relationship between serum Mg deficiency and major organ damage or alteration.

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