Magnesium concentration in the cerebrospinal fluid of mice and its response to changes in serum magnesium concentration

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Abstract. Magnesium (Mg) is essential for cell functions such as the transport of calcium and potassium ions, and modulates signal transduction, energy metabolism, and cell proliferation. Although mice have been used as models of various neurological diseases of humans, and for investigating the therapeutic effects of Mg, neither the normal concentration of Mg in cerebrospinal fluid (CSF), nor its response to alteration of the serum level of Mg has yet been reported. The present study investigated the normal Mg concentration in the CSF of C57BL/6J (B6) and ICR mice and its response to elevation of the serum Mg level in B6 mice. In B6 mice, the normal Mg concentration in the CSF was 0.89 ± 0.11 mM, being lower than that in serum, which was 1.38 ± 0.12 mM, whereas in ICR mice the corresponding values were 1.00 ± 0.12 mM and 1.10 ± 0.09 mM, respectively. No significant alteration was found in the CSF of B6 mice injected intraperitoneally with Mg, even though the serum Mg concentration was significantly increased.

Key words: blood-brain barrier, blood-CSF barrier, cerebrospinal fluid, magnesium, mice, serum

Magnesium (Mg) is essential for cell functions such as the transport of calcium and potassium ions, and modulates signal transduction, enzyme activities, nucleic acid and protein synthesis, energy metabolism, and cell proliferation, and protects biological membranes [1]. Mg deficiency has been reported to be correlated with skeletal maldevelopment [2], stroke [1, 3, 4], secondary tissue damage in brain trauma [5], ischemic heart disease, cardiac arrhythmia, atherosclerosis, hypertension and diabetes mellitus [1], and retinal function abnormality [6]. It is also suggested to play a role in the pathogenesis of Parkinson’s disease [7] and the parkinsonism-dementia complex (PDC) and amyotrophic lateral sclerosis (ALS) of Guam [8-10]. In relation to the pathogenesis of Parkinson’s disease and PDC, Mg deficiency over generations induces selective loss of dopaminergic neurons in the substantia nigra of rats [11].

Some clinical and experimental studies have shown that intravenous Mg administration exerts a neuroprotective effect in cases of traumatic brain injury, seizure, subarachnoid hemorrhage and cerebral ischemia in humans and animals [1, 3, 12, 13], and also lidocaine-induced seizures in rats [13]. These data indicate that consumption of a Mg-
deficient diet induces degeneration of certain neurons in the brain, whereas an elevated Mg concentration in serum has a therapeutic effect on neurons in the brain in certain pathological conditions.

On the other hand, a number of studies have reported that serum Mg enters the CSF, but that the ionic composition of the CSF remains remarkably constant during changes in blood Mg concentration in humans [14, 15] and animals [13, 16-21] under normal as well as pathologic conditions, or that only a slight increase in the CSF Mg level occurs in human patients with brain injury or in dogs with 2-4 times the normal level of serum Mg under otherwise normal conditions [22, 23]. However, some studies have indicated that the CSF Mg concentration is significantly increased in patients with preeclampsia [24] and in rats with hippocampal seizures [25], and that long-term Mg deficiency induces a decrease of the CSF Mg concentration [21].

Mice are used as models of various neurological disorders, and in studies of neuroprotective treatment using Mg. However, no measurements of Mg concentration in the serum, CSF or brain have been performed during chronic oral Mg administration to mice used in the Parkinson’s disease model [26], and no significant increase in the brain tissue concentration of Mg was reported during oral intake of Mg pidolate in SOD (superoxide dismutase)-1 transgenic (Tg) mice [19]. Also, no reports have indicated the normal concentration of Mg in the CSF, nor its response to elevation of the Mg level in serum. Here we report details of the normal Mg concentration in the CSF in C57BL/6J (B6) and ICR mice, and alteration of the Mg concentration in the CSF after intraperitoneal injection of Mg sulfate in B6 mice.

**Materials and methods**

**Animals**

The present study was conducted in accordance with the Guidelines for Experiment and approved by the Animal use and Care Committee of Tokyo Metropolitan Institute for Neuroscience, and adequate measures were taken to minimize pain and discomfort to the animals. We used male B6 (n = 27, CLEA Japan, Inc., Tokyo, Japan) and ICR mice (n = 11, CLEA Japan, Inc.) at 8 weeks of age, weighing 21-24 g and 32-37 g, respectively. The content of Mg in the diet for the mice used was 0.25 g/100 g.

**Experimental groups**

Normal levels of Mg in the serum and CSF were measured in both B6 (n = 7) and ICR mice (n = 11), and alterations of the Mg concentration were measured in the CSF and serum after intraperitoneal injection of Mg sulfate (Conclyte-Mg, MgSO₄·7H₂O, 1.23 g/kg body weight, Nipro Pharma, Osaka, Japan) to B6 mice (n = 40).

**Collection of samples from serum and CSF**

The animals were anesthetized with Nembutal (50 mg/kg, Dainippon Pharmaceutical Co., Ltd.). For collection of serum, a cut was made in the tail about 1-1.5 cm from the tip, and blood from the wound was collected in a polypropylene tube (catalog No. 430791, Corning, NY, USA) using polypropylene tips (catalog no. RS-200Y, Renover Science, Co., Ltd., Tokyo, Japan). The serum was then separated by centrifugation of the tubes at 1,500 rpm. CSF was obtained from the cisterna magna. After separating the skin and muscle in the neck, the CSF was aspirated by penetrating the exposed dura mater and the arachnoid membrane using a butterfly needle (27 G x 1/2, 0.4 x 13 mm, TOP Co., Tokyo, Japan). Samples were collected from a normal control, and from C57 mice at 20 min, 40 min, 2 h and 4 h after intraperitoneal injection of Mg sulfate. The samples were diluted 1,000-fold with milliQ water.

**Measurement of Mg concentration**

Mg concentration was measured using quadrupole ICP-MS (inductively coupled plasma mass spectrometry; 7500 Series, Agilent Technologies, Tokyo, Japan). Yttrium (Kanto Kagaku, Co., Ltd., Tokyo, Japan), for internal standardization, in 1% nitric acid was added to the samples and standards at a final concentration of 10 μg L⁻¹. The instrument was flushed with milliQ water before the introduction of each sample or standard. Operating conditions of the instrument for ICP-MS were as follows: ICP rf power, 1,500 W; cooling chamber temperature, 2°C; argon gas flow rate, plasma 0.7 L/min, carrier 0.35 L/min; hydrogen gas flow rate, 0.4; scanning mass, Mg m/z = 25, Y m/z = 89. Each sample and standard solution was subjected to measurement three times.

**Statistical analysis**

Data were analyzed by repeated-measures analysis of variance (ANOVA) with treatment group and day of testing as independent variables, followed by the
Table 1. Normal magnesium (Mg) concentration in plasma and cerebrospinal fluid and the response of Mg concentration in the CSF/brain tissue after systemic administration of Mg in various species reported previously.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Age</th>
<th>Sex</th>
<th>Normal Mg concentration</th>
<th>Response of Mg concentration in CSF/brain tissue after systemic Mg administration</th>
<th>Methods for Mg concentration measurement</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>C57BK/6J</td>
<td>8 weeks</td>
<td>M</td>
<td>1.38 ± 0.12 mM</td>
<td>No significant response in CSF</td>
<td>ICP-MS</td>
<td>Present study</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.89 ± 0.11 mM</td>
<td></td>
<td></td>
<td>Marie et al. 1983 [27]</td>
</tr>
<tr>
<td></td>
<td>ICR</td>
<td>8 weeks</td>
<td>M</td>
<td>1.10 ± 0.09 mM</td>
<td></td>
<td>ICP-MS</td>
<td>Present study</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00 ± 0.12 mM</td>
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<td></td>
<td>Pamphlett et al. 2003 [19]</td>
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<tr>
<td></td>
<td>SOD-1 Tg</td>
<td></td>
<td></td>
<td>2.8 ± 0.1 mg/dL</td>
<td></td>
<td>ICP-MS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A/J</td>
<td>adult</td>
<td>M</td>
<td>17000 μg/mL</td>
<td></td>
<td>ICP-MS</td>
<td></td>
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<tr>
<td></td>
<td>Balb/c</td>
<td></td>
<td>F</td>
<td>24284 mg/g wet weight</td>
<td></td>
<td>ICP-MS</td>
<td></td>
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<tr>
<td>Rat</td>
<td>Wistar</td>
<td></td>
<td>F</td>
<td>1.96 ± 0.24 mg/dL</td>
<td></td>
<td>AAS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sprague-Dawley</td>
<td></td>
<td>F</td>
<td>2.0 ± 0.1 mg/dL</td>
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<td>AAS</td>
<td></td>
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<tr>
<td></td>
<td>Long-Evans</td>
<td></td>
<td>F</td>
<td>2.4 ± 0.25 mg/dL</td>
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<td>Slight increase in CSF</td>
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<td></td>
<td>2.41 ± 0.14 mg/dL</td>
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</tr>
<tr>
<td>Guinea pig</td>
<td></td>
<td></td>
<td></td>
<td>0.97 mM</td>
<td></td>
<td>AAS</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.81 mM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td></td>
<td></td>
<td></td>
<td>1.35 mM/kg H₂O</td>
<td></td>
<td>Colorimetry</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1.33 mM/kg H₂O</td>
<td></td>
<td></td>
<td>Ames III et al. 1964 [31]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>White rabbit</td>
<td>adult</td>
<td></td>
<td>2.09 ± 0.36 mEq/L</td>
<td></td>
<td>AAS</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>0.72 ± 0.13 mEq/L</td>
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<td></td>
<td></td>
<td>0.90 ± 0.20 mEq/L</td>
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<tr>
<td>Dog</td>
<td>Mongrel</td>
<td></td>
<td></td>
<td>1.61 (1.33-2.0) mEq/L</td>
<td></td>
<td>HPLC</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>2.16 (1.03-2.9) mEq/L</td>
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<tr>
<td>Swine</td>
<td>Miniswine</td>
<td>2 &amp; 40-day</td>
<td></td>
<td>2.24 ± 0.12 mEq/kg H₂O</td>
<td></td>
<td>FS</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1.93 ± 0.06 mEq/kg H₂O</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>No significant response in brain tissue</td>
<td></td>
<td>PMRS</td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td></td>
<td></td>
<td></td>
<td>2.24 ± 0.12 mEq/kg H₂O</td>
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<td>ES</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1.93 ± 0.06 mEq/kg H₂O</td>
<td></td>
<td></td>
<td>Pappenheimer et al. 1962 [32]</td>
</tr>
<tr>
<td>Human</td>
<td>Normal</td>
<td>adult</td>
<td>F</td>
<td>1.44 ± 0.16 mg/dL</td>
<td></td>
<td>modified MBCP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>adult</td>
<td></td>
<td>1.69 ± 0.14 mEq/L</td>
<td></td>
<td>AAS</td>
<td></td>
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<td></td>
<td>2.29 ± 0.1 mg/dL</td>
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<td></td>
<td>Heipertz et al. 1979 [35]</td>
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<tr>
<td></td>
<td>Postop. patient</td>
<td>adult</td>
<td></td>
<td>1.91 ± 0.26 mg/dL</td>
<td></td>
<td>CPN</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.67 ± 0.44 mg/dL</td>
<td></td>
<td></td>
<td>Ko et al. 2001 [15]</td>
</tr>
<tr>
<td>Patients</td>
<td>18-89 y.o.</td>
<td>M/F</td>
<td></td>
<td>Mg²⁺: 0.92 ± 0.18 mM</td>
<td></td>
<td>ACCA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mg²⁺: 1.25 ± 0.14 mM</td>
<td></td>
<td></td>
<td>McKe et al. 2005 [22]</td>
</tr>
<tr>
<td>Hypertension</td>
<td>adult</td>
<td></td>
<td></td>
<td>0.58 ± 0.05 mM</td>
<td></td>
<td>EA</td>
<td></td>
</tr>
</tbody>
</table>

M: male; F: female; AAS: atomic absorption spectrophotometry; ACCA: automated clinical chemistry analyzer; CPN: chlorophosphonazo 3; EA: electrolyte analyzer; ES: emission spectrophotometry; FS: flame spectrophotometry; HPLC: high-performance liquid chromatography; ICP-MS: inductively coupled plasma mass spectrometry; MBCP: methythymol blue complexometric procedure; PMRS: phosphorus-31 magnetic resonance spectroscopy; postop: postoperative. Parentheses indicate range, and the numbers represent average ± SD.
Bonferroni post hoc test for multiple comparisons between groups using Excel software (Microsoft Ltd.). The level of statistical significance was set at $p < 0.05$. All values are presented as mean ± SD.

**Results**

Mg levels in the milliQ water were within the range $0.14 \pm 0.12$ mM ($n = 11$). Since these values were considered to be within an acceptable error range, no correction was made for the Mg concentration in the serum and CSF. Mg concentrations were $0.89 \pm 0.11$ mM in CSF ($n = 7$) and $1.38 \pm 0.12$ mM in serum ($n = 7$) from normal B6 mice, and $1.00 \pm 0.12$ mM in CSF ($n = 11$), and $1.10 \pm 0.09$ mM in serum ($n = 11$) from normal ICR mice (table 1). No significant alteration was found in the CSF of B6 mice that had been injected intraperitoneally with Mg, even though the magnesium concentration in the serum was significantly increased from $1.38 \pm 0.12$ mM (normal level; $n = 5$) to $8.68 \pm 1.85$ mM (20 min after injection; $n = 5$), to $7.58 \pm 0.67$ mM (40 min after; $n = 5$) and $3.56 \pm 0.96$ mM (2 hr after; $n = 5$), and decreased to the normal level at 4 h after (n = 5, figure 1).

**Discussion**

The present study clarified the normal concentration of Mg in the CSF and its response to changes in the serum concentration of Mg in mice. The normal Mg concentration in the CSF was $0.89 \pm 0.11$ mM in B6 mice and $1.00 \pm 0.12$ mM in ICR mice, and the Mg concentration in the CSF of B6 mice did not change in response to an increase in the concentration of Mg in serum. The Mg concentrations in B6 mice were $1.38$ mM in plasma but $0.89$ mM in CSF, and those in ICR mice were $1.10$ mM in plasma and $1.00$ mM in CSF. It is unclear why there was such a large difference in Mg concentrations between B6 mice and ICR mice, particularly with respect to the values in CSF and serum in B6, but not in ICR mice.

The Mg concentration in the plasma of mice was slightly higher [27-29] than that reported for other species (table 1). The Mg concentration in the CSF was lower than that reported in the serum of mice, guinea pigs [30], cats [31], and goats [32], but higher than that reported in the serum of rats [16, 25, 33], rabbits [34], dogs [23] and humans [14, 15, 22, 24, 35] (table 1). The reason for the differences in serum Mg concentrations among species is unclear.

With regard to the correlation of Mg levels between CSF and serum, it has been reported that the Mg concentration in CSF does not increase even if the serum concentration is about 200% of normal in humans [14, 15] and in animals [21]. In addition, the Mg concentration in brain tissue was reportedly not increased in newborn pigs after intravenous infusion of Mg sulfate [17] or in SOD-1 Tg mice after oral Mg supplementation [19] (table 1).

In contrast, a few studies have reported significant increases in the CSF concentration of Mg after an increase in the serum Mg concentration. In rats, after intraperitoneal injection of Mg sulfate, an increase in the CSF level of Mg was found, in parallel with a significant elevation of the serum level, and the levels of Mg in both the CSF and

**Figure 1.** Magnesium (Mg) concentration in cerebrospinal fluid (CSF) and serum after intraperitoneal injection of Mg. *$p < 0.01$; compared with basal.
serum decreased 2 hours later [25]. Oppelt et al. reported that serum Mg entered the CSF, and that the Mg concentration in CSF increases to 121% of the control level if the serum Mg concentration remains elevated for 3-4 hours [23]. Thurnau et al. reported that the CSF Mg level increased to 119% of the control after intravenous of magnesium sulfate in patients with pre-eclampsia, resulting in a concentration 400-500% of the normal one [24]. McKee et al. reported a slight but significant elevation of the Mg concentration in CSF after systemic Mg administration to patients who had suffered from various disorders [22]. On the other hand, changes in CSF Mg concentration have also been described in cases of severe long-term hypomagnesemia [21]. Our present results confirmed that, in normal mice, the CSF Mg concentration did not respond to an increase in the serum Mg concentration (table 1, figure 1).

With regard to the mechanism of Mg concentration homeostasis in the CSF, Oppelt et al. considered that, in the normal dog, the intact blood-CSF barrier was impermeable to Mg [23]. Hilmy et al. concluded that, in the normal rabbit, the brain was protected by the BBB and that a regulatory mechanism ensured that the CSF Mg level remained constant [18]. However, under conditions of brain damage, such as trauma, SAH or stroke, the mechanism responsible for maintaining CSF Mg homeostasis might be disrupted [36-41].

In mice, there have been some attempts to treat neurodegenerative disorders with Mg. In a murine model of amyotrophic lateral sclerosis (SOD-1 transgenic), oral supplementation of Mg elicited no effect, and the Mg concentration in brain tissue was not increased [19]. In a murine model with Parkinson’s disease, oral supplementation of Mg produced an improvement in behavior, although striatal dopamine was decreased [26]. When taken together, the present and previous findings suggest that, for the treatment of neurological disorders in murine models, direct Mg administration into brain tissue or the subarachnoid space/ventricles would be necessary.

Conclusion

Mg concentrations were 0.89 ± 0.11 mM in the CSF, and 1.38 ± 0.12 mM in the serum of normal 8-week-old male C57BL/6J (B6) mice, and 1.00 ± 0.12 mM in the CSF, and 1.10 ± 0.09 mM in the serum of normal 8-week-old male ICR mice. No significant alteration was found in the CSF of B6 mice injected intraperitoneally with Mg, even though the Mg concentration in serum was significantly increased.

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