Effects of Calcium Gluconate on the Utilization of Magnesium and the Nephrocalcinosis in Rats Fed Excess Dietary Phosphorus and Calcium

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Summary The effects of calcium gluconate on the utilization of magnesium and nephrocalcinosis in male Wistar rats made magnesium-deficient by adding excess dietary phosphorus (1.195 g of phosphorus/100 g of diet) and calcium (1.04 g of calcium/100 g of diet) were compared with the effects of calcium carbonate. The effects of dietary magnesium concentration on the magnesium status and nephrocalcinosis were also examined. Adding excess dietary phosphorus and calcium decreased the apparent magnesium absorption ratios and the concentrations of magnesium in the serum and femur and increased the deposition of calcium in the kidney, and the low magnesium condition (0.024 g of magnesium/100 g of diet) aggravated the deposition of calcium and the low magnesium status. The apparent magnesium absorption ratios and femur magnesium concentration in the rats fed a calcium gluconate diet (an equimolar mixture of calcium gluconate and calcium carbonate was used as a source of calcium) were significantly higher than in the rats fed a calcium carbonate diet (only calcium carbonate was used as a source of calcium), irrespective of dietary magnesium concentration. Dietary calcium gluconate lessened the accumulation of calcium in the kidney and increased the serum magnesium concentration compared with dietary calcium carbonate, when the rats were fed the normal magnesium diet (0.049 g of magnesium/100 g of diet) but not the low magnesium diet. We speculate that the increased utilization of magnesium by feeding the calcium gluconate diet to a limited extent prevented the low magnesium status and the severity of nephrocalcinosis caused by adding excess dietary phosphorus and calcium.

Key Words calcium gluconate, calcium carbonate, magnesium, phosphorus, rat, absorption, deficient, nephrocalcinosis
The Recommended Dietary Allowance (RDA) value for calcium is 600 mg for adults in Japan (1), but the typical calcium intake of adults in Japan is lower than the RDA for calcium (2). This discrepancy between the perceived need for calcium and actual calcium intake has encouraged companies to market a variety of calcium preparations and calcium-enriched foods (3). A number of investigators have compared the usefulness of various calcium sources (4–11).

On the other hand, some experimental data indicate that increased intake of calcium impairs intestinal magnesium absorption (12–14) and that magnesium deficiency in rats is associated with nephrocalcinosis (15–17). However, few reports have been published on the effect of different kinds of calcium supplements on magnesium utilization (18–21). Previously, we examined the effects of various calcium supplements on the utilization of magnesium and found that the apparent absorption of magnesium was higher in rats fed calcium gluconate than in rats fed calcium carbonate or other calcium sources (unpublished data). The purposes of this study were to compare the effects of calcium gluconate on the utilization of magnesium with the effects of calcium carbonate and to examine the effects of these calcium sources on the degree of nephrocalcinosis in rats made magnesium-deficient by adding excessive dietary phosphorus and calcium (13).

METHODS

Diets. The ingredients of the diets are given in Table 1. We prepared four kinds of test diets which contained 1.195 g of phosphorus/100 g of diet and either

|                | L Mg | N Mg |
|----------------|------|------|
|                | CaCO₃ | Gluconate | CaCO₃ | Gluconate |
| Casein         | 20    | 20    | 20    | 20        |
| DL-Methionine  | 0.3   | 0.3   | 0.3   | 0.3       |
| Corn oil       | 5     | 5     | 5     | 5         |
| Mineral mixture | 3.5  | 3.5   | 3.5   | 3.5       |
| Vitamin mixture | 1   | 1     | 1     | 1         |
| Choline bitartrate | 0.2 | 0.2   | 0.2   | 0.2       |
| Cellulose powder | 5   | 5     | 5     | 5         |
| Sucrose        | 60.088 | 57.109 | 52.569 | 57.068  | 52.528 |
| MgO            | 0.082 | 0.041 | 0.041 | 0.082    | 0.082 |
| CaCO₃          | 1.3   | 1.3   | 1.3   | 1.3       |
| Ca gluconate   | 0     | 0     | 0     | 0         |
| KH₂PO₄         | 1.75  | 5.25  | 5.25  | 5.25      |
| K₂CO₃          | 1.78  | 0     | 0     | 0         |

1Mineral mixture was prepared according to the AIN-76 formulation but CaHPO₄ and MgO were substituted for sucrose. 2Vitamin mixture was prepared according to the AIN-76 formulation.
0.024 g of magnesium/100 g of diet (low magnesium diet (LMg)) or 0.049 g of magnesium/100 g of diet (normal magnesium diet (NMg)) with either calcium carbonate or an equimolar mixture of calcium carbonate and calcium gluconate as a source of calcium (1.04 g of calcium/100 g of diet). Phosphorus was added in the form of potassium dihydrogen phosphate and the diets were balanced for potassium with potassium carbonate. We prepared the standard diet to compare the effects of test diets which contained excess phosphorus and calcium on magnesium metabolism. The standard diet contained 0.398 g of phosphorus, 0.049 g of magnesium and 0.52 g of calcium/100 g of diet (the levels in the AIN-76 diet (22)). Calcium carbonate was used as a source of calcium for the standard diet.

**Animals.** Thirty 4-week-old male Wistar rats (Japan SLC, Inc., Shizuoka, Japan) were housed in individual stainless steel cages in a temperature-controlled (24 ± 1°C) room with 60 ± 5% humidity and a 12-h light–dark cycle. After a 7-day adaptation period in which all rats were fed the standard diet, they were separated into five groups of six animals having similar mean body weight. Each group was fed one of the experimental diets and deionized water ad libitum for 28 days. Food consumption was recorded every third day. The rats were weighed once a week.

**Sample collection and analyses.** Rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (40 mg/kg body weight) and blood samples were taken from the aorta ventralis to determine the concentration of magnesium by using commercial kits (magnesium B-test, Wako Pure Chemical Industries, Osaka, Japan). Right kidneys and femurs from the right leg were excised and cleaned of adhering matter. Fecal samples collected from day 12 and 26 for 3 days during each period were cleaned of foreign adhering matter, dried to a constant weight and ground to a fine powder. Tissues and fecal samples were examined for magnesium, phosphorus and calcium content, after wet ashing as described previously (23), by using an inductive coupled plasma emission spectrometer (ICPS-2000; Shimadzu Ltd., Kyoto, Japan). The apparent absorption ratio was calculated on the basis of the following formula:

\[
\text{Apparent absorption (\%)} = \left(\frac{\text{intake} - \text{fecal loss}}{\text{intake}}\right) \times 100.
\]

**Histopathological examination of kidneys.** Left kidneys were collected and fixed in 10% buffered formalin, and processed for light microscopy. Paraffin-embedded tissues were sectioned, and routinely stained with hematoxylin and eosin (H-E) and von Kossa stains.

**Statistical analysis.** All data were analyzed by one-way analysis of variance. When a significant F ratio was found, individual means were compared by Tukey's test. The level of significance was considered to be \( p < 0.05 \).

**RESULTS**

**Growth of the rats**

Adding excess dietary phosphorus and calcium greatly reduced the growth rate...
and food intake of rats fed LMg diets, but the growth rate was only slightly reduced in rats fed calcium gluconate (Table 2). Figure 1 shows that a typical symptom of magnesium deficiency, red ears, was seen after 8 days among rats fed the LMg-calcium carbonate diet. By day 20, all rats fed the LMg-calcium carbonate diet showed this symptom. Among the rats fed the LMg-calcium gluconate diet, the symptom began to be observed on day 20, and at the end of the study five rats showed the symptom. Among the rats fed the NMg-calcium carbonate diet, only one had the symptom on day 21. We did not observe the symptom among the rats fed the standard diet or the NMg-calcium gluconate diet during the examination period.

**Magnesium utilization**

Table 3 shows the magnesium absorption and the magnesium concentrations in

|                          | Standard | LMg CaCO₃ | Ca gluconate | NMg CaCO₃ | Ca gluconate |
|--------------------------|----------|-----------|--------------|-----------|--------------|
| Initial body weight (g)  | 115±6.1a | 115±5.2   | 115±3.8      | 115±3.7   | 115±4.1      |
| Final body weight (g)    | 252±9.1a | 209±9.5b  | 230±4.4c     | 245±14.7ac| 246±9.4ac    |
| Food intake (g)          | 451±29a  | 370±21b   | 394±18nc     | 432±26nc  | 439±18a      |
| Feed efficiency ratio    | 30.3±1.3a| 25.4±1.9b | 29.4±1.7a    | 30.0±1.9a | 29.9±3.0a    |

1 Values are M±SD for six rats. Within the same row, means having different superscript letters differ significantly (p<0.05).
the femur and serum. Adding excess dietary phosphorus and calcium reduced the apparent magnesium absorption ratios and magnesium concentrations in the femur and serum. Apparent magnesium absorption ratios and the femur magnesium concentration in rats fed the calcium gluconate diet were higher than those in rats fed the calcium carbonate diet, irrespective of the magnesium concentration of the diet. The serum magnesium concentration was also higher in the rats fed the NMg diet containing calcium gluconate than that containing only calcium carbonate as a source of calcium. Rats fed the LMg diet did not show this difference.

**Kidney mineralization**

Table 4 shows the concentrations of magnesium, phosphorus and calcium in the kidney. Adding excess dietary phosphorus and calcium increased kidney wet weight and the concentrations of phosphorus and calcium. Dietary calcium gluconate in the NMg diet significantly lessened the accumulation of phosphorus and calcium and raised the magnesium concentration in the kidney compared with dietary calcium carbonate. When the rats were fed the LMg diet, however, dietary calcium gluconate did not alter the kidney wet weight or the concentrations of phosphorus, calcium and magnesium.

| Table 3. Apparent absorption and femur and serum concentrations of magnesium in rats fed experimental diets for 28 days.1 |
| --- |
| **| Standard | LMg | NMg |
| | | CaCO₃ | Ca gluconate | CaCO₃ | Ca gluconate |
| Apparent absorption ratio | | | | | |
| 12–14 days (%) | 65.4±2.3a | 33.4±3.0b | 49.7±5.2c | 34.3±4.9b | 50.6±3.4c |
| 26–28 days (%) | 57.1±5.8a | 31.4±3.5b | 45.4±10.6ac | 30.7±5.8b | 43.3±7.3c |
| Femur Mg (mg/g, dry) | 2.22±0.09a | 1.08±0.10b | 1.40±0.07c | 1.50±0.01a | 2.19±0.08a |
| Serum Mg (mg/100 ml) | 1.72±0.23a | 0.98±0.06b | 0.96±0.08b | 1.12±0.04b | 1.48±0.05c |

1 Values are M±SD for six rats. Within the same row, means having different superscript letters differ significantly (p<0.05).

| Table 4. Kidney weight and concentrations of magnesium, phosphorus and calcium in rats fed experimental diets for 28 days.1 |
| --- |
| **| Standard | LMg | NMg |
| | | CaCO₃ | Ca gluconate | CaCO₃ | Ca gluconate |
| Wet (g) | 0.97±0.04a | 1.17±0.10b | 1.31±0.10b | 1.25±0.10b | 1.14±0.13b |
| Mg (µg/g, wet) | 207±3a | 195±8b | 213±8a | 234±20b | 268±36b |
| P (mg/g, wet) | 2.81±0.06a | 8.64±1.28b | 8.29±1.02b | 8.20±0.89b | 5.65±1.60c |
| Ca (mg/g, wet) | 0.08±0.01a | 13.25±2.53b | 11.67±1.41b | 12.24±1.74b | 6.40±3.34c |

1 Values are M±SD for six rats. Within the same row, means having different superscript letters differ significantly (p<0.05).
Fig. 2. Regenerated epitheliums (*) and tubular dilatation (calcified tubuli, arrowheads) in the kidney (H-E, ×24).
Fig. 3. Calcification in cortico-medullary junction and cortical surface of the kidney (von Kossa, ×24).
Microscopical observation of kidney calcification

In kidney tissue of rats fed excess phosphorus and calcium diets, regenerated epithelia in the cortical surface and dilatation of the renal tubuli were observed by light microscopy (Fig. 2), and the LMg diet promoted regeneration of the epithelia. The degree of the regenerated epithelia of the cortical surface was weaker in the rats fed the calcium gluconate diet than in the rats fed the calcium carbonate diet, irrespective of the magnesium concentration of the diet.

Figure 3 shows the deposition of calcium in the cortico-medullary junction and the cortical surface of the kidney, which occurred in rats fed an excess phosphorus and calcium diet, and the LMg diets promoted the deposition of calcium. Dietary calcium gluconate as compared with calcium carbonate reduced the deposition of calcium.

DISCUSSION

In terms of osteoporosis, adequate intake of calcium is thought to be important (24), and there are many kinds of calcium supplements and calcium-enriched foods (3). Several investigations of their bioavailability as a source of calcium have been reported (4–11). On the other hand, clinical and experimental studies have shown that calcium inhibits magnesium absorption (12–14). However, to our knowledge, there are fewer experimental data focused on the effects of different kinds of calcium sources on magnesium metabolism (18–21). In our study, excess dietary phosphorus and calcium intake reduced the apparent absorption of magnesium and the concentration of magnesium in the femur and serum, as shown by other investigations (12–14, 17, 25). We compared the effect of calcium gluconate on magnesium utilization with the effect of calcium carbonate in these experimental conditions. The rats fed calcium gluconate diets absorbed magnesium more efficiently than did rats fed calcium carbonate diets (Table 3), and as shown in Fig. 1 the appearance of a typical symptom of magnesium deficiency which was characterized by auricular peripheral hyperemia was also delayed in the rats fed a calcium gluconate diet as compared with the rats fed a calcium carbonate diet. These data suggest that the inhibitory effect of calcium on magnesium utilization differed with the calcium source used in this study.

Brink et al. reported that increased intake of calcium and phosphorus stimulates the formation of an insoluble calcium-magnesium-phosphate complex in the intestine and affects magnesium absorption (14). As postulated by Heijnen et al., the increased production of volatile fatty acids and the related pH reduction is associated with the increase in the magnesium content in the liquid phase in the ileum when rats are fed lactulose, and this increase in the solubility of magnesium might increase the amount of magnesium available to be absorbed (26). Furthermore, several authors showed that indigestible carbohydrate increases magnesium absorption in the small intestine and/or the large intestine (27–30). Calcium gluconate consists of calcium and gluconic acid. Asano et al. reported that gluconic
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acid stimulated the growth of bifidobacteria in the intestine (31). This finding suggests that some part of gluconic acid may be undigested in the small intestine and fermented by intestinal bacteria. Although the pH and magnesium distribution in the intestinal lumen were not determined in our study, the stimulatory effect of calcium gluconate on magnesium absorption may be associated with the increased solubility of magnesium. Further study is needed to confirm this.

Nephrocalcinosis is a disorder involving intratubular deposition of calcium phosphate in the cortico-medullary junction of the kidney (32). It is reported that nephrocalcinosis is readily caused by feeding phosphorus at dietary levels exceeding certain critical levels, which depend on the dietary magnesium content (17). Masuyama et al. also reported that increasing the intake of magnesium decreased the calcium concentration in the kidney (33, 34). In our study, adding excess dietary phosphorus and calcium caused a higher concentration in the kidney and the deposition of calcium in the cortico-medullary junction and the cortical surface of the kidney, and the low magnesium diet aggravated the deposition of calcium. As shown in Table 4 and Fig. 3, dietary calcium gluconate in the NMg diet lowered the accumulation of calcium and phosphorus and reduced the deposition of calcium. The magnesium absorption ratio and the concentration of magnesium in the serum and femur also increased in the rats fed the NMg-calcium gluconate diet (Table 3).

We speculate that the utilization of magnesium increased by feeding the calcium gluconate diet weakened the severity of nephrocalcinosis.

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