Interaction between Bisphosphonates and Mineral Water: Study of Oral Risedronate Absorption in Rats

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Bisphosphonates are antosteoporotic agents prescribed for patients with osteoporosis. Drug package inserts for bisphosphonate supplements indicate that their bioavailability is reduced by high levels of metal cations (Ca++, Mg++, etc.). However, standards for these cations in water used for taking risedronate have not been defined. Here, we examined the effect of calcium and magnesium in mineral waters on the bioavailability of the third-generation bisphosphonate, risedronate, following oral administration in rats. As risedronate is unchanged and eliminated renally, risedronate absorption was estimated from the amount excreted in the urine. Risedronate was dissolved in mineral water samples and administered orally at 0.35 mg/kg. Urine samples were collected for 24 h after dosing. Risedronate was extracted from urine using ion-pair solid-phase cartridges and quantified by HPLC with UV detection (262 nm). Cumulative recovery of risedronate was calculated from the amount excreted in the urine. The 24-h recovery of risedronate from evian® (0.32±0.02% [mean±standard deviation (S.D.)], n=4) and Contrex® (0.22±0.05%) mineral waters was significantly lower than that from tap water (0.47±0.04%, p<0.01). Absorption of risedronate in calcium chloride and magnesium chloride aqueous solutions of the same hardness (822 mg/L) was 54% (0.27±0.04%) and 12% (0.51±0.08%) lower, respectively, compared with ultrapure water; suggesting that absorption of risedronate declines as the calcium concentration of mineral waters increases. Consumption of mineral waters containing high levels of calcium (80 mg/L or above), such as evian® and Contrex®, is therefore not recommended when taking risedronate.

Key words risedronate; mineral water; drug interaction; urine excretion; rat

Osteoporosis is a systemic disease characterized by reduced bone mass and structural deterioration of bone tissue, leading to an increased risk of fracture. According to the 2011 Guidelines for the Treatment of Osteoporosis, the number of patients with osteoporosis in the rapidly aging population of Japan is increasing and is now estimated at about 13 million.1) The occurrence of a fracture significantly impacts a person’s daily activities, so pharmacotherapy for the prevention of osteoporosis is recommended for patients who are at high risk of bone fracture. A variety of drugs are available for treating osteoporosis, including calcium agents, active vitamin D3, raloxifene, and bisphosphonates (BPs). BPs exhibit particularly potent bone resorption inhibitory activity and are therefore the first-choice drug for treating osteoporosis.3) Because they inhibit resorption and thus increase bone mineral density, BPs play an important role in treating postmenopausal osteoporosis. The resorption-inhibiting activity of risedronate, a third-generation BP, is reported to be approximately 3000 times that of etidronate, a first-generation BP.2) Recently, risedronate began to be marketed for once per month (75 mg), in addition to daily (2.5 mg) and weekly (17.5 mg) dosing.

The variety of mineral waters sold and consumed in Japan has increased in recent years. Annual consumption of mineral water per person in Japan was 25.7 L in 2014 (approximately 3 times that of 15 years ago).3) The consumption of mineral waters in Japan is predicted to increase further in coming years. For orally administered BPs, the package inserts advise patients and health care workers that the bioavailability of the drug may be reduced when taken with mineral waters containing high levels of metal cations (Ca++, Mg++, etc.), as a result of chelation (Ajinomoto Co., Inc., Actonel® package insert [2013/2]). As the hardness of a mineral water depends on its calcium and magnesium content, differences in the hardness of mineral waters may affect the bioavailability of BPs. We previously reported that the absorption of alendronate decreases with increasing calcium concentration of mineral water consumed when taking that drug.4) However, standards regarding metal cations in mineral waters taken with alendronate have not been defined. The bioavailability of BPs after oral administration is very low (1–2%), and gastrointestinal absorption can be inhibited even further due to chelation of metal cations.5) Therefore, to improve the efficacy of BPs, it is necessary to determine the effects of interactions between other BPs and metal cations in detail. In this study, we examined how different levels of calcium and magnesium contained in various mineral waters or calcium and magnesium chloride aqueous solution affect the bioavailability of risedronate following oral administration in rats. Our results show which water is suitable for taking risedronate.

Absorbed risedronate is deposited in BP-hydroxyapatite without metabolism, and most risedronate (except that absorbed in bone) is rapidly excreted through the urine (Ajinomoto Co., Inc., Assessment of the metabolism of NE-58095-14C in the rat, Pharmacokinetics in postmenopausal women after oral administration of NE-58095 17.5 mg). In addition, as the plasma concentration of risedronate is very low, determining its concentration is extremely difficult unless highly sensitive chromatography methods are used. We therefore estimated the bioavailability of risedronate based on the amount excreted in urine over a given period of time.

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MATERIALS AND METHODS

Chemicals Risedronate and 1-octyltriethylammonium phosphate as an ion-pairing (IP) reagent were obtained from LKT Laboratories (St. Paul, MN, U.S.A.). Ammonium dihydrogen phosphate, calcium chloride, 1 M hydrochloric acid, and 1 M sodium chloride were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ethylenediamine-N,N,N′,N′-tetraacetic acid, disodium salt (EDTA/2 Na), as a chelating agent, and tetrabutylammonium bromide were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Methanol was obtained from Kanto Chemical Industries (Tokyo, Japan). 2-Pyridylacetic acid hydrochloride, as an internal standard (IS), etidronate (ca. 60% in water, ca. 4.2 mol/L), and ethylene glycol-bis(β-aminoethyl ether)-N,N′,N′,N′-tetraacetic acid (EGTA), used as a calcium scavenger, were obtained from Tokyo Chemical Industry (Tokyo, Japan). OASIS® HLB cartridges (Waters Corporation, Milford, MA, U.S.A.) were used for solid-phase extraction. Ultrapure water was generated using a Millipore Milli-Q Plus system (Merk Millipore, Billerica, MA, U.S.A.).

Water Samples

Beverages (Table 1a)
The mineral waters used in the study were “Amami no mizu” (Deep-Seawater, Ako Kasei Co., Ltd., Ako, Japan), evian® (imported and distributed by Ito En, Ltd., Tokyo, Japan; manufactured by Danone, Paris, France), and Contrex® (Suntory Beverage & Food Ltd., Tokyo, Japan [at the time of experiment]). House faucet tap water (drawn in Saitama, Japan) and ultrapure water were used as controls. The hardness of each water sample was measured using the titration method as reported in the Japanese Standard Methods of Analysis for Hygienic Chemistry.

Calcium and Magnesium Chloride Aqueous Solutions (Table 1b)
Aqueous solutions of calcium chloride (2.7, 8.2, 12.3 mM) and magnesium chloride (8.2 mM) were prepared. The calcium concentrations of the 12.3 mM calcium chloride solution and the Contrex® mineral water (469 mg/L) were very similar, as were the magnesium concentrations of the 8.2 mM magnesium chloride solution and “Amami no mizu” (Deep-Seawater) mineral water (215 mg/L). Calcium and magnesium chloride aqueous solutions were adjusted to pH 7.4 using 0.1 M potassium hydroxide. Ultrapure water was used as a control.

Study Design

Our study was conducted in accordance with the rules and regulations of the Subcommittee on Research Animal Care at Tokyo University of Science (Approval numbers Y12010). Male Wistar rats (9 weeks of age) used in the study were purchased from Sankyo Labo Service Corporation (Tokyo, Japan). Rats were fasted for 24 h and then a cannula (polyethylene tube, SP136, Natsume Mill, Tokyo, Japan) was inserted into the bladder. After a sufficient volume of urine was obtained, risedronate (dissolved in one of the 0.15 mg/mL water samples) was orally administered at a dose of 0.35 mg/kg body weight. This dose was determined based on weekly (17.5 mg) dosing divided by 50-kg in human body weight. Urine samples were collected at 0–3, 3–6, 6–9, 9–12, and 12–24 h after oral administration of risedronate and stored at −30°C until analyzed. The volume of urine collected was measured, and the cumulative urinary excretion recovery and rate of urinary excretion over the 24-h period after risedronate administration were calculated.

Sample Preparation Procedure

Calcium Phosphate Co-precipitation

Frozen urine samples were thawed at room temperature. After centrifugation (4°C, 2330 g, 5 min), the supernatant was removed and 10 µL of 1.25 mM calcium chloride solution was added. Next, 50 µL of 1 mM sodium chloride solution was added to induce the formation of a white precipitate (risedronate co-precipitates as a calcium chelate under alkaline conditions). Samples were then centrifuged for 10 min at 5000 g. The supernatant was removed, the precipitate was dissolved in 50 µL of 1 M hydrochloric acid, and the sample was diluted with 350 µL of ultrapure water. Next, 50 µL of 1 mM sodium chloride was added, the sample was centrifuged, and the supernatant was discarded. The steps described above were repeated twice. The precipitate pellet obtained after the second precipitation step was dissolved in 0.5 mL of 0.05 M EGTA and thoroughly vortex mixed; after which, 10 µL of IS (10 µg/mL) was added to each sample. After the addition of 4.5 mL of ultrapure water to each sample, 100 µL of 0.5 M IP reagent stock solution was added and the samples were mixed.

Ion-Pair Solid-Phase Extraction

OASIS® HLB extraction cartridges (30 mg, 1 mL) were conditioned with 2 mL of methanol followed by 1 mL of deionized water. The cartridges were then conditioned with 1 mL of 0.01 M IP reagent. After loading of the final samples obtained by calcium phosphate co-precipitation, the cartridges were washed with 1 mL of ultrapure water followed by 1 mL of water–methanol (95 : 5, v/v). Risedronate was eluted from the sorbent by drawing 1 mL of methanol, and the eluate was evaporated to dryness. Samples were reconstituted in 100 µL of buffered mobile phase solution (5 mM ammonium dihydrogen phosphate, 2 mM tetrabutylammonium bromide, 1.5 mM EDTA-2Na, 1 mM etidronate, pH 7.2)–methanol (88 : 12, v/v). A 20-µL aliquot of each sample was then injected into the HPLC system.

HPLC Analysis

The apparatus utilized for the liquid chromatographic procedure consisted of a degassing instrument (SSC-3215, Senshu Scientific Co., Ltd., Tokyo, Japan),

Table 1. Calcium and Magnesium Concentrations and Total Hardness of Water Samples

(a) Beverage

<table>
<thead>
<tr>
<th>Water samples</th>
<th>Calcium (mg/L)</th>
<th>Magnesium (mg/L)</th>
<th>Total hardness (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrapure water</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tap water</td>
<td>13</td>
<td>4</td>
<td>48</td>
</tr>
<tr>
<td>“Amami no mizu” (Deep-Seawater)</td>
<td>68</td>
<td>215</td>
<td>1067</td>
</tr>
<tr>
<td>evian®</td>
<td>83</td>
<td>20</td>
<td>289</td>
</tr>
<tr>
<td>Contrex®</td>
<td>469</td>
<td>77</td>
<td>1493</td>
</tr>
</tbody>
</table>

(b) Calcium and magnesium chloride aqueous solutions

<table>
<thead>
<tr>
<th>Water samples</th>
<th>Calcium (mg/L)</th>
<th>Magnesium (mg/L)</th>
<th>Total hardness (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7 mM Calcium chloride</td>
<td>110</td>
<td>0</td>
<td>274</td>
</tr>
<tr>
<td>8.2 mM Calcium chloride</td>
<td>331</td>
<td>0</td>
<td>822</td>
</tr>
<tr>
<td>12.3 mM Calcium chloride</td>
<td>496</td>
<td>0</td>
<td>1233</td>
</tr>
<tr>
<td>8.2 mM Magnesium chloride</td>
<td>0</td>
<td>200</td>
<td>822</td>
</tr>
</tbody>
</table>
mobile phase pumping system (SSC-461, Senshu Scientific Co., Ltd.), recorder (Chromatocorder 21, System Instruments Co., Ltd., Hachioji, Japan), column thermostat (SSC-2120, Senshu Scientific Co., Ltd.), UV detector (SSC-5410, Senshu Scientific Co., Ltd.), and ODS C18 column (4.6ϕ×250 mm, Pegasil ODS SP100, Senshu Scientific Co., Ltd.). The column temperature was maintained at 30°C. The mobile phase consisted of buffer (5 mM ammonium dihydrogenphosphate, 2 mM tetrabutylammonium bromide, 1.5 mM EDTA-2Na, 1 mM etidronate, pH 7.2)–methanol (88 : 12, v/v) and was pumped at a flow rate of 1.0 mL/min. The detection wavelength was 262 nm.

**Pharmacokinetic and Statistical Analyses** Cumulative urinary excretion risedronate recovery (%) was calculated based on the amount of risedronate excreted in the urine at each collection time, divided by the dosage. Urinary excretion rate was calculated as the percent excreted per hour. The elimination rate constant ($k_e$) was calculated using the sigma minus plot method. A $p$-value of <0.01 in the Bonferroni test was considered indicative of statistical significance. All data were expressed as the mean±standard deviation (S.D.).

**RESULTS**

Risedronate was administered orally with each water sample and was excreted rapidly, with approximately 80% excreted in the urine within 12h after administration. In the first 3h after administration, the mean urinary excretion rates for...
the “Amami no mizu” (Deep-Seawater), evian®, and Contrex® mineral waters were approximately 50% lower than those of ultrapure and tap water (Fig. 1).

Figure 2 and Table 2 show the change in cumulative urinary excretion recovery rate over the 24-h period after oral administration of risedronate for each water sample. The volume of urine collected per hour was similar for each water sample (0.23–0.28 mL/h), suggesting that urine volume had minimal effect on the urinary excretion rate of risedronate. Compared with the cumulative excretion recovery of risedronate in urine after administration of the drug dissolved in ultrapure water (0.58±0.08%), the cumulative excretion recovery values were significantly lower for all mineral water samples. The cumulative recovery rates for the evian® (0.32±0.02%) and Contrex® (0.22±0.05%) mineral water samples were significantly lower (32 and 53% lower, respectively) than the cumulative recovery rate for tap water (0.47±0.04%). The cumulative recovery rate of risedronate for “Amami no mizu” (Deep-Seawater) (0.38±0.03%) was not significantly different than that for tap water. Assuming that the pharmacokinetics of risedronate follow a 1-compartment model, the average $k_e$ was calculated as 0.14–0.19 h$^{-1}$ using the sigma minus plot method (Table 2).

When risedronate was administered orally with calcium chloride aqueous solution, the urinary excretion rate in the first 3 h after administration declined with increasing calcium concentration. Comparing calcium and magnesium chloride aqueous solutions of the same hardness (8.2 mM), the mean urinary excretion rate for the calcium chloride solution was about half that of the magnesium chloride solution in the first 3 h after administration (Fig. 3).

The cumulative excretion recoveries of risedronate in urine for the 8.2 mM calcium and magnesium chloride aqueous solutions were 0.27±0.04% and 0.51±0.08%, respectively (Fig. 4, Table 3), and the rates decreased with increasing calcium concentration. The volumes of urine collected per hour were 0.27 and 0.23 mL/h, and the average $k_e$ were calculated as 0.18 and 0.21 h$^{-1}$.

With respect to the calcium concentration of each water sample, the 24-h cumulative recovery rate declined as the

Table 2. Effect of Ultrapure, Tap, “Amami no Mizu” (Deep-Seawater), evian®, and Contrex® Mineral Waters on Cumulative Urinary Excretion Recovery of Risedronate after 24 h and the Elimination Rate Constant ($k_e$) after Oral Administration in Rats at a Dose of 0.35 mg/kg ($n=4$)

<table>
<thead>
<tr>
<th>Water samples</th>
<th>Cumulative urinary excretion recovery (%)</th>
<th>$k_e$ (h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrapure water</td>
<td>0.58±0.08</td>
<td>0.16±0.03</td>
</tr>
<tr>
<td>Tap water</td>
<td>0.47±0.04</td>
<td>0.16±0.03</td>
</tr>
<tr>
<td>“Amami no mizu” (Deep-Seawater)</td>
<td>0.38±0.03*</td>
<td>0.19±0.06</td>
</tr>
<tr>
<td>evian®</td>
<td>0.32±0.02***</td>
<td>0.14±0.03</td>
</tr>
<tr>
<td>Contrex®</td>
<td>0.22±0.05***</td>
<td>0.16±0.04</td>
</tr>
</tbody>
</table>

* $p<0.01$ vs. ultrapure water, ** $p<0.01$ vs. tap water.
calcium concentration increased to approximately 80 mg/L. At calcium concentrations above 80 mg/L, the cumulative recovery rate averaged approximately 0.25%, indicating that inhibition of absorption of risedronate had reached a plateau (Fig. 5, Table 3).

DISCUSSION

Taking risedronate with food or other medicines or with drinks other than water may inhibit absorption. It is therefore recommended that risedronate be taken with a glass of water immediately after waking and while the patient is in an upright position. Package inserts indicate that absorption of risedronate to rats (n=4) with Ultrapure Water (□), Tap Water (●), “Amami no Mizu” (Deep-Seawater) (▲), evian® (▲), Contrex® (□), or Various Calcium Chloride Aqueous Solutions (▲) once.

Table 3. Effect of Calcium and Magnesium Chloride Aqueous Solutions on Cumulative Urinary Excretion Recovery of Risedronate after 24 h and the Elimination Rate Constant (kₜ) after Oral Administration in Rats at a Dose of 0.35 mg/kg (n=4)

<table>
<thead>
<tr>
<th>Water samples</th>
<th>Cumulative urinary excretion recovery (%)</th>
<th>kₜ (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7 mM Calcium chloride</td>
<td>0.33±0.06*</td>
<td>0.22±0.07</td>
</tr>
<tr>
<td>8.2 mM Calcium chloride</td>
<td>0.27±0.04*</td>
<td>0.18±0.04</td>
</tr>
<tr>
<td>12.3 mM Calcium chloride</td>
<td>0.26±0.05*</td>
<td>0.17±0.08</td>
</tr>
<tr>
<td>8.2 mM Magnesium chloride</td>
<td>0.51±0.08NS</td>
<td>0.21±0.02</td>
</tr>
</tbody>
</table>

*p<0.01, N.S.: Not significant.

The cumulative urinary excretion recovery of risedronate dissolved in evian® and Contrex® mineral waters was significantly lower than the recovery in ultrapure or tap water. This finding indicates that the formation of risedronate–metal cation complexes reduces the drug’s bioavailability. The calcium concentration of “Amami no mizu” (Deep-Seawater) was nearly equal to that of evian® mineral water; however, the magnesium concentration was 10 times higher, resulting in total hardness values for the “Amami no Mizu” (Deep-Seawater) and evian® mineral waters of 1067 and 289 mg/L, respectively (Table 1a). The cumulative recoveries of risedronate in “Amami no mizu” (Deep-Seawater) and evian® mineral waters were 0.38±0.03% and 0.32±0.02%, respectively (Table 2, Fig. 2), suggesting that magnesium has a minimal impact on risedronate absorption in the gastrointestinal tract. These results were similar to those reported for alendronate.4) In examinations of calcium and magnesium chloride aqueous solutions of the same hardness, the cumulative recovery rates of risedronate were 54% (0.27±0.04%) and 12% (0.51±0.08%) lower, respectively, than that for ultrapure water (Table 3, Fig. 4). This finding showed that the bioavailability of risedronate is not significantly affected by magnesium but is affected by calcium.

BPs have a P-C-P structure and side chains.12) The calcium and magnesium binding affinities are affected primarily by the side chains, and calcium phosphate and magnesium phosphate seem to be produced.13) Calcium phosphate and magnesium phosphate solubility products of 2.1×10⁻³¹ and 1.0×10⁻²⁴ (mol/L)² suggest that calcium phosphate is more readily chelated than magnesium phosphate.14) Divalent cations differ with respect to their ability to form chelates, which in turn seems to be related to differences in the inhibition of drug absorption. The cumulative recovery rates of risedronate decreased as the calcium concentration increased, and reduced significantly for evian® (involved 83 mg/L as calcium concentration) and other waters containing higher level of calcium than evian®. Therefore, the rates decreased as the calcium concentration in the different water samples increased to approximately 80 mg/L, but at concentrations above 80 mg/L, the rates remained relatively stable. These results suggest that chelate formation saturates at a calcium concentration of approximately 80 mg/L. Thus, these findings suggest that the absorption of risedronate when taken with mineral water is primarily affected by the calcium concentration, with concentrations of 80 mg/L and above having a significant negative impact on absorption.

The calcium and magnesium concentrations of tap water vary with sampling location. The hardness of tap water in
our study was 48 mg/L, and the calcium concentration was 13.0 mg/L (Table 1a). The hardness of most tap water in Japan is within the range 30–80 mg/L, and the calcium and magnesium concentrations are thought to be in the range 3–20 mg/L and 1–8 mg/L, respectively. The tap water used in this study was therefore considered standard in terms of its calcium and magnesium concentrations.

Patients with osteoporosis are often advised to ingest sufficient amounts of calcium. More than 800 mg of calcium must be taken every day to prevent a reduction in bone mass in postmenopausal women and the elderly. These patients are typically advised to ingest foods, supplements, and mineral waters containing high levels of calcium. However, the findings of our study suggest that the absorption of risedronate, which is typically prescribed for osteoporosis, is inhibited when the drug is taken with water containing a high concentration of calcium. Consumption of mineral waters containing high levels of Ca\(^{2+}\) (approximately 80 mg/L or above), such as evian® and Contrex®, is therefore not recommended when taking risedronate.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES


