Original

Ultrastructural Study of the Age-dependent Effects of the Alendronate Sodium Hydrate Treatment on the Parathyroid Gland in Golden Hamsters

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Abstract: We examined the age-dependent effects of the alendronate sodium hydrate (alendronate) on the ultrastructure of the golden hamster parathyroid gland (PTG). At 1 year of age, the percent area of Golgi apparatus and lysosomes in alendronate-treated hamster PTG was significantly increased in comparison to control hamsters, while at 2 years of age, the percent area of lipid droplets was significantly decreased relative to control hamsters. These findings suggest that alendronate promotes the synthesis of hamster parathyroid hormone at 1 year but not 2 years of age. These results indicate that the sensitivity of PTG to alendronate is age-dependent.

Key words: Parathyroid gland, Hamster, Ultrastructure, Alendronate sodium hydrate

Introduction

It is widely accepted that bisphosphonates suppress osteoclast activity and affect calcium metabolism. The alendronate sodium hydrate (alendronate) is a therapeutic drug used for the treatment of hypercalcemia such as in malignant tumors1. However, few morphological studies have been conducted on the effects of alendronate on the fine structure of the parathyroid gland (PTG) in relation to calcium metabolism.

Here, we investigated ultrastructural changes in the hamster PTG at 1 and 2 years of age following administration of alendronate.

Materials and Methods

Ten 1-year-old and ten 2-year-old male golden hamsters were divided into two groups. The body weight of all animals was measured (Table 1). Five 1-year-old and five 2-year-old animals served as controls. The remaining five 1-year-old and five 2-year-old animals were intraperitoneally administered alendronate (0.17 mg/kg body weight) only once2. All animals were sacrificed at 1 week after the treatment under deep anesthesia following intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight) and the serum calcium levels of all animals were measured. The experimental protocols of this study followed the Guidelines for Animal Experiments at the School of Dentistry, Aichi Gakuin University. The PTGs from all animal groups were dissected, fixed in a mixture of 2.5% glutaraldehyde and 2.0% OsO4 in veronal-acetate buffer, pH 7.4, for 1 h at 4°C, dehydrated in a graded acetone series, and embedded in Epon 812. Ultrathin sections were cut using a Sorvall Porter-Blum MT-1 ultramicrotome (Du Pont Co., Wilmington, DE, USA), stained with uranyl acetate and lead salts, and examined under a JEM-1210 electron microscope (JEOL Ltd., Tokyo, Japan). For each hamster, 20 micrographs at a final magnification of 17,000 were randomly acquired from different regions of the PTG.

Tracing paper was placed over the micrographs and outlines of the cytoplasm, Golgi apparatus (vesicles, vacuoles and lamellae), cisternae of the rough endoplasmic reticulum (rER), lysosomes, large vacuolar bodies and lipid droplets were drawn. Quantitation of the areas of cytoplasm (total cell volume minus nucleus), cytoplasmic organelles and cytoplasmic inclusions was performed using a CanoScan 9900F scanner (Canon Inc., Tokyo, Japan) interfaced with an iMac PowerPC G4 computer (Apple Inc., Cupertino, CA, USA). Scans were saved as 8-bit TIFF files and imported into Adobe Photoshop (Adobe Inc., San Jose, CA, USA) and the NIH Image program (version 1.63, Wayne Rasband at the US National Institutes of Health, Bethesda, MD, USA) was used for analysis. Regions of the Golgi apparatus, rER, lysosomes, lipid droplets and large vacuolar bodies were expressed as a percentage of the cytoplasmic area. In addition, the number of secretory granule profiles per 100 μm2 in the cytoplasm was measured.

Statistical analysis

All data are presented as the mean ± SEM. Following the F-test, a Student’s t-test was used to calculate significant differences between the control and alendronate groups at the same age. Significance was accepted at p<0.05.

Results

Body weight

The mean body weight (g) of the control and alendronate groups is shown in Table 1. There was no significant difference between the control and alendronate groups at 1 and 2 years of age with regard to the body weight.

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Serum calcium level

The mean serum calcium level (mg/100 ml) of the control and alendronate groups is shown in Table 2. At 1 year of age, the serum calcium level in the alendronate group was significantly lower than that in the control group; however, no significant difference was observed at 2 years of age between the alendronate and control groups.

Electron microscopy analysis of PTG

Control groups. The parenchyma of the PTG at 1 and 2 years of age consisted of chief cells, which were oval or polygonal in shape and contained abundant free ribosomes and mitochondria. Chief cells displayed moderately tortuous outlines and were occasionally interdigitated intimately with adjacent cells (Figs. 1 and 2). The PTG morphology of the control hamsters in each group (Figs. 1 and 2) resembled that of normal hamsters, as reported earlier.

Experimental groups. At 1 year of age, the chief cells contained a well-developed Golgi apparatus consisting of small vesicles and large, dilated vacuoles compared to the control group (Fig. 3). The rER and lysosomes were well developed and randomly distributed in the cytoplasm (Fig. 3). Few secretory granules were present compared to the control group (Fig. 3). At 2 years of age, a few lipid droplets and many secretory granules were observed (Fig. 4). The morphology of the other

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**Table 1. The body weight of control and alendronate groups**

| Age (years) | Group   | Number of animals | Body weight (g) |
|-------------|---------|-------------------|-----------------|
| 1           | Control | 5                 | 174.8±11.2      |
| 1           | Alendronate | 5            | 166.9±4.6       |
| 2           | Control | 5                 | 139.0±4.0       |
| 2           | Alendronate | 5            | 140.4±3.8       |

Values are means ± SEM. *p<0.05 vs. control group.

**Table 2. Comparison of serum calcium levels between control and alendronate groups**

| Age (years) | Group   | Number of animals | Serum calcium levels (mg/100 ml) |
|-------------|---------|-------------------|---------------------------------|
| 1           | Control | 5                 | 11.13±0.10                     |
| 1           | Alendronate | 5            | 10.17±0.20*                    |
| 2           | Control | 5                 | 11.60±0.94                     |
| 2           | Alendronate | 5            | 11.47±0.49                     |

Values are means ± SEM. *p<0.05 vs. control group.

**Table 3. Comparison of organellar profiles on the PTG between control and alendronate groups**

| Age (years) | Group   | Number of animals | G | ER | Ly | L | V | S |
|-------------|---------|-------------------|---|----|----|---|---|---|
| 1           | Control | 5                 | 4.57±0.23 | 3.36±0.59 | 0.45±0.02 | 0.23±0.0 | 0.07±0.01 | 18.41±0.76 |
| 1           | Alendronate | 5            | 5.84±0.44* | 4.22±0.23 | 1.11±0.06* | 0.37±0.11 | 0.05±0.01 | 10.73±0.19* |
| 2           | Control | 5                 | 7.79±0.42 | 5.70±0.59 | 0.76±0.14 | 0.20±0.03 | 0.05±0.01 | 14.14±2.20 |
| 2           | Alendronate | 5            | 7.85±0.23 | 4.84±0.23 | 1.12±0.19 | 0.09±0.01* | 0.07±0.01 | 23.58±4.33 |

Values are presented as percentage of cytoplasmic areas; means ± SEM; G=the Golgi apparatus; ER=rough endoplasmic reticulum; Ly=lysosome; L=lipid droplet; V=large vacuolar body. Value of secretory granule (S) is presented as number of profiles per 100 μm² in the cytoplasm. *p<0.05 vs. control group.

**Figure 1.** Parathyroid chief cells of a control hamster at 1 year of age. The chief cells contain abundant free ribosomes, mitochondria, cisternae of rough endoplasmic reticulum (ER), Golgi apparatus (G) and secretory granules (arrows). Ly = lysosome. V = large vacuolar body. Inset: L = lipid droplet. Ly = lysosome. Scale bar = 1 μm.

**Figure 2.** Parathyroid chief cells of a control hamster at 2 years of age. Well-developed Golgi apparatus (G) are randomly distributed. ER = rough endoplasmic reticulum. Ly = lysosome. Scale bar = 1 μm.
cytoplasmic organelles at 1 and 2 years of age was similar to that of the control group each (Figs. 3 and 4).

**Morphometry of the PTG**

The results of morphometric measurements are shown in Table 3. Table 3 compares the PTG organelle profiles of the control and alendronate groups. At 1 year of age, the mean area of the Golgi apparatus and lysosomes in the alendronate group was significantly greater than in the control group. Further, the mean area of secretory granules in the alendronate group was significantly lower than in the control group. At 2 years of age, the mean area of lipid droplets in the alendronate group was significantly lower than in the control group. There were no differences between the control and alendronate groups with regard to the other cytoplasmic organelles.

**Discussion**

The serum ionized calcium concentration is known to regulate parathyroid hormone secretion\(^6\). Alendronate treatment reduces serum calcium levels, and the condition of hypocalcemia is attributed to a suppression of osteoclast function\(^5\). In this study, the serum calcium level in the alendronate group at 1 year of age was significantly reduced compared to the control group.

Parathyroid hormone (PTH) is synthesized in the rER, and is packaged into secretory granules in the Golgi apparatus. Secretory granules store and transport PTH to the cell surface, while other organelles (lysosomes, lipid droplets and large vacuolar bodies) are involved in regulating the overproduction of PTH. These organelles are well-known morphological parameters associated with the functional status of chief cells\(^6\).

The PTG of the alendronate group at 1 year of age showed well-developed Golgi apparatus and large numbers of lysosomes compared to the control group. The well-developed Golgi apparatus suggested an increased capacity for packaging of PTH\(^7\). Lysosomes in the PTG of domestic fowl increased during growth, and might function in the regulating the secretory process by limiting the overproduction of secretory granules\(^8\). These results are essentially similar to the observations which indicate an increase in the functional activity of the PTG of the isopropenol-treated golden hamsters, as described earlier\(^9,10\).

The number of secretory granules in parathyroid chief cells do not appear to be correlated with the functional state of the PTG, and it is thought that the number of secretory granules depends on the relationship between the synthesis, release and lysosomal digestion of PTH\(^11\). The present study demonstrated that the number of secretory granules in the alendronate group at 1 year of age was significantly lower than that of the control group. This is attributed to the accelerated release and lysosomal digestion of secretory granules compared to the control group.

Emura et al.\(^12\) suggested that the number of large vacuolar bodies of the PTG decreases with age and increase when the secretory activity of the PTG is suppressed in response to acute hypercalcemia. However, our data showed no significant differences between the control and experimental groups at 1 and 2 years of age. Additional research is needed to clarify the role of large vacuolar bodies in the PTG.

In the present study, the serum calcium level of the alendronate group at 2 years of age was not significantly different from the control group. Furthermore, there were no significant differences between the control and experimental groups at 2 years of age with regard to organelles besides lipid droplets. According to Castleman and Roth\(^13\), lipid droplets that originate from autophagosomes peak in resting chief cells and are reduced in the active state. In contrast, some authors have reported that active chief cells of the hamster PTG show an increase in lipid droplets\(^9\). Lipid droplets of the alendronate-treated group at 2 years of age were significantly lower compared to the control group. At present, a responsible mechanism is not clear, and additional investigation is required to clarify the role of lipid droplets in the PTG.

In conclusion, our ultrastructural study suggested that alendronate affected the PTG of hamsters at 1 year but not 2 years of age. Our results show that there is a difference in the effect of alendronate between 1 and 2 years of age. In general practice, alendronate doses are used according to the condition, but we have only studied one concentration. We selected the minimum dose close to that normally used in humans as a treatment for cancer-associated hypercalcemia. Increasing concentrations may have pharmacological effects even at 2 years of age. In general, the biological response to drugs is affected by age, but whether the effect of alendronate is affected by age needs to be
further investigated.

**Conflict of Interest**
The authors have declared that no conflict of interests exists.

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