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Absorption and safety of alendronate, a nitrogen-containing bisphosphonate, after intrapulmonary administration in rats

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ABSTRACT

Alendronate, a nitrogen-containing bisphosphonate, has been used as a first-choice drug for the treatment of hypercalcemia and osteoporosis. In the present study, we examined the absorption and safety of alendronate after intrapulmonary administration in rats. The bioavailability (BA) of alendronate after intrapulmonary administration was 47% at a dose of 5 mg/kg, while the BA after oral administration was only 2.9% at a dose of 50 mg/kg in rats. Plasma calcium level, an index of the pharmacological effect of alendronate, was effectively reduced after intrapulmonary administration of alendronate. Furthermore, alendronate continuously reduced the increase in plasma calcium levels for 9 days after a single intrapulmonary administration in rats with 1α-hydroxyvitamin-D_3-induced hypercalcemia. Intrapulmonary administration of alendronate also effectively suppressed the decrease in bone mass in a rat model of osteoporosis. Alendronate significantly increased the activity of lactate dehydrogenase (LDH) in bronchoalveolar lavage fluid (BALF), indicating that pulmonary mucosal damage was induced by intrapulmonary administration of alendronate. However, co-administration of superoxide dismutase (SOD) with alendronate completely suppressed the alendronate-induced increase in LDH activity in BALF, while maintaining sufficient pulmonary absorption and therapeutic effects of alendronate in rats with 1α-hydroxyvitamin-D_3-induced hypercalcemia. These findings indicated that the lung is a promising, noninvasive alternative route for the delivery of alendronate in the treatment of bone diseases.

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1. Introduction

Bisphosphonates (BPs), a class of drugs characterized by a PCP backbone, are physiological regulators of calcification and osteoclastic bone resorption (Schenk et al., 1986; Fleisch, 1987; Lambrinoudaki et al., 2006; Van Beek et al., 2003). A number of BPs are widely used for the treatment and prevention of bone diseases and disorders of calcium metabolism, including osteoporosis, Paget's disease, and hypercalcemia (Ezra and Golomb, 2000; Drake et al., 2008). Alendronate sodium (sodium 4-amino-1-hydroxybutylidene-1, 1-bisphosphonate trihydrate), a nitrogen-containing bisphosphonate, has been the most prescribed drug worldwide for the treatment of hypercalcemia and postmenopausal, male, and glucocorticoid-induced osteoporosis (Sato and Grasser, 1990).

The oral bioavailability (BA) of alendronate is only approximately 0.9–1.8%, due to its high polarity and hydrophilicity (partition coefficient: $<1 \times 10^{-4}$), and food can strongly suppress the intestinal absorption of alendronate (Porras et al., 1999). Although some groups reported that absorption enhancers, such as ethylenediaminetetraacetate (EDTA), enhanced the intestinal absorption of alendronate, absorption enhancers that effectively increase the intestinal absorption of alendronate tend to cause concomitant intestinal damage (Janner et al., 1991). In addition, oral administration of alendronate has been associated with mucosal damage, including gastritis, gastric ulcer, and erosive esophagitis (Graham, 2002; Sener et al., 2004; Naniwa et al., 2008). Accordingly, patients with osteoporosis should sit up or walk for more than 30 min after oral administration to prevent these adverse effects of alendronate, leading to poor compliance and reduced quality of life (QOL) in elderly and bedridden patients using alendronate (Kamatari et al., 2007). Although previous studies reported that taurine effectively prevented the alendronate-induced mucosal damage associated with oral administration, a large amount of taurine is needed for the prevention of mucosal damage (Sener et al., 2005a,b). Consequently, alendronate is sometimes administered by parenteral injection in clinical use due to the low absorption and adverse effects associated with oral administration (Ezra and

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Golomb, 2000). However, injections cause pain, local side effects, and allergic reactions. Therefore, it is highly desirable to develop new delivery systems that improve the compliance and QOL in these patients.

In our previous study, we focused on transdermal delivery as a noninvasive alternative route for the administration of alendronate, and we successfully developed a new transdermal delivery system for alendronate using a new type of hydrophilic patch to improve compliance and QOL of patients during treatment for bone diseases. We demonstrated that the transdermal permeation of alendronate observed using our patch system was sufficient for the treatment of hypercalcemia and osteoporosis in rat models (Kusamori et al., 2010). Pulmonary delivery also appears to be a suitable approach to improve compliance and QOL of patients with bone disease because pulmonary delivery is known to be the most promising of the noninvasive alternative routes (including nasal, buccal, rectal, ocular, vaginal, transdermal, and pulmonary delivery) for the absorption of poorly absorbed drugs (Yamamoto et al., 2001). It was reported that proteins, peptide drugs, and low molecular water-soluble drugs, which are all poorly absorbed through the gastrointestinal tract and other topical sites, are absorbed well through the lung because of the large surface area of the alveolar epithelium and the short distance to blood pathway (Morita et al., 1993). Furthermore, the pulmonary route can avoid the adverse effects associated with intestinal absorption, and patients will not be required to sit up or walk after administration. Because of these properties of pulmonary delivery, the lung may be a promising, noninvasive alternative route for the delivery of alendronate. However, the pulmonary absorption and safety of alendronate have not been examined so far.

In this study, we evaluated the pulmonary absorption and therapeutic potential of alendronate after intrapulmonary administration in rats. The therapeutic potential of intrapulmonary delivery of alendronate was investigated using rat models of hypercalcemia and osteoporosis. Finally, the effect of alendronate delivered via intrapulmonary administration on the pulmonary mucosal damage was also evaluated in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (8 weeks old, 240–260 g) and female Sprague–Dawley (S.D.) rats (9 weeks old, 200–220 g) were purchased from Japan SLC, Inc. (Shizuoka, Japan). Animals were maintained under conventional housing conditions. All animal experiments were conducted in accordance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocols for animal experiments were approved by the animal ethics committee at Kyoto Pharmaceutical University.

2.2. Materials

Alendronate sodium trihydrate was obtained from Toronto Research Chemicals Inc. (North York, Canada). Trichloroacetic acid (TCA), calcium chloride, sodium dihydrogenphosphate dehydrate, hydrochloric acid, sodium hydrate, disodium ethylenediaminetetraacetate (Na_2EDTA), fluorescamine, dichloromethane, 1α-hydroxyvitamin-D_3 (1α-(OH)D_3), dimethylsulfoxide (DMSO), paraformaldehyde, heparin sulfate, recombinant human superoxide dismutase (SOD), bovine catalase, and L-cysteine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were obtained commercially as reagent-grade products.

2.3. Absorption studies

The pulmonary absorption of alendronate was evaluated according to the methods outlined by Enna and Schanker (1972). Briefly, male Wistar rats were anesthetized by intraperitoneal injection of sodium pentobarbital (32 mg/kg). After the animal had been secured on its back on an animal board, the trachea was exposed through a longitudinal incision along the ventral aspect of the neck. The trachea was then cut transversely, halfway through, between the fourth and fifth tracheal rings caudal to the thyroid cartilage. A section of polyethylene tubing (i.d., 1.5 mm, o.d., 2.5 mm, 2.5 cm in length), which served as a tracheal cannula, was inserted through the tracheal incision caudally for a distance of 0.6 cm so that 1.9 cm of the cannula protruded from the trachea. Animal body temperature was maintained at 37 °C with a 40 W incandescent heat lamp and the use of a reflector suspended over the animal at a distance of about 25 cm during the experiment. Alendronate in phosphate-buffered saline (PBS) (100 μl) at 37 °C was injected into the lungs through the obtuse needle of a calibrated 250-μl syringe (Microliter® no. 725, Hamilton Co., Reno, USA). The needle was inserted through the tracheal cannula to a depth of 2.5 cm below the tracheal incision for the injection. At this distance of insertion, the tip of the syringe needle was located 1–2 mm above the bifurcation of the trachea. The solution was injected over a period of 1–2 s while the rat was maintained at an angle of 80°. Immediately thereafter, the tubing was withdrawn completely and 45 s after administration the animal was returned to an angle of 10°. The jugular vein was exposed and blood samples (200 μl) were collected into heparinized syringes at predetermined time intervals up until 4 h post drug administration. Samples were immediately centrifuged to obtain the plasma fractions (100 μl), which were kept in ice until use. In a certain experiment, alendronate in PBS was intravenously administered into the caudal vein by bolus injection in order to calculate the pharmacokinetic parameters. The concentration of alendronate in each plasma sample was analyzed using the RP-HPLC method as reported previously (Wong et al., 2004).

2.4. Pharmacokinetics analyses

The peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) of these compounds were determined directly from their plasma concentration–time profiles. The concentration of alendronate in the plasma after intravenous injection was analyzed using the nonlinear least-squares program MULTI (Yamaoka et al., 1981). The area under the plasma concentration–time curve (AUC) after intravenous injection was calculated based on a two-compartment model. The AUC after oral or intrapulmonary administration was calculated by numerical integration using a liner trapezoidal formula and extrapolation to infinite time based on a monoexponential equation (Yamaoka et al., 1978).

2.5. Hypercalcemia experiment

1α-(OH)D_3 (2.5 μg/kg/day) was administered intraperitoneally to ovariectomized female S.D. rats during the experiment to establish a hypercalcemia model induced by this treatment (Azuma et al., 1995). Six days after the first 1α-(OH)D_3 administration, alendronate was administered intrapulmonarily at a dose of 5 mg/kg using the method described above. The transversely cut trachea was then repaired with surgical sutures after the intrapulmonary administration. Alendronate was administered orally to a separate group of 1α-(OH)D_3-treated rats at a dose of 5 mg/kg. At predetermined intervals, blood was collected from the cervical vein of both groups of rats under ether anesthesia. Heparin sulfate was used as an anticoagulant. Plasma was obtained from the blood by centrifugation. Plasma calcium concentrations were determined by

the Calcium E test Wako (Wako Pure Chemical Industries, Osaka, Japan) according to the manufacturer's instructions.

2.6. Osteoporosis experiment

Female S.D. rats were subjected to an ovariectomy (OVX) to establish the postmenopausal osteoporosis-like state (Xiang et al., 2006). Immediately after the OVX operation, alendronate was administered intrapulmonarily to OVX rats at a dose of 5 mg/kg using the method described above. The transversely cut trachea was then repaired with surgical sutures after the intrapulmonary administration. Eight weeks after the OVX operation, the tibia bone structures of these rats were evaluated by histological examination as reported previously (Kusamori et al., 2010).

2.7. Evaluation of pulmonary damage

The effects of alendronate on pulmonary tissue were evaluated by measuring the activity of lactate dehydrogenase (LDH) in bronchoalveolar lavage fluid (BALF) as reported previously (He et al., 2007). Briefly, a solution of alendronate at a concentration of 2.5 or 12.5 mg/ml in PBS or a mixture of alendronate and an additive (SOD, L-cysteine, or catalase) was administered through the rat trachea at a volume of 0.4 ml/kg using the method described for the absorption studies. Four hours later, the rat was bled from the abdominal aorta under pentobarbital anesthesia. After perfusion of the lung with physiological saline via the pulmonary artery, BALF was recovered with PBS (4×4 ml). The recovered fluids were centrifuged at 200 g for 7 min at 4 °C. The activities of LDH were determined using an assay kit LDH CII (Wako Pure Chemical Industries, Ltd.) according to the manufacturer's instructions. BALF was also taken from rats with treatment of PBS in the control study.

2.8. Histological examination

Lung tissues 4 h after intrapulmonary administration of alendronate and tibiae 8 weeks after the OVX operation were excised and subjected to histological examination. Tissue samples were fixed with 4% buffered paraformaldehyde and embedded in paraffin blocks; 5-μm thick sections were cut from the paraffin blocks. The sections from the lung were stained with hematoxylin and eosin to observe pulmonary injury and tibia structures by light microscopy.

2.9. Statistical analyses

Results are expressed as the mean ± SE, and differences were statistically evaluated by one-way analysis of variance followed by the Student–Newman–Keuls multiple comparison test at a significance level of $p < 0.05$.

3. Results

3.1. Plasma concentrations of alendronate after intrapulmonary administration

Fig. 1 shows the plasma concentration–time profiles of alendronate after intravenous, oral, and intrapulmonary administration in rats. The pharmacokinetic parameters of alendronate after administration via the three different routes are summarized in Table 1. Alendronate was rapidly eliminated from the plasma after its intravenous injection in rats. The concentration of alendronate in plasma increased rapidly after intrapulmonary administration and reached a maximum level of approximately 1900 ng/ml 0.3 h after administration. In contrast, the concentration of alendronate in plasma increased gradually after oral administration. The BA of alendronate after intrapulmonary administration was 47%, while the BA after oral administration was only 2.9%.

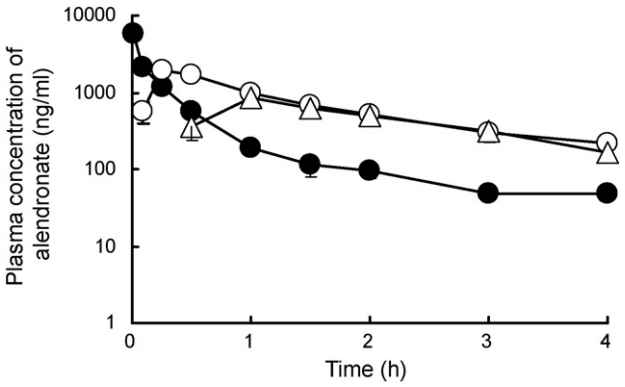

Fig. 1. Plasma concentration–time profiles of alendronate after intravenous, oral, and intrapulmonary administration in rats. ○, intrapulmonary administration (5 mg alendronate/kg); ●, intravenous administration (1 mg/kg); Δ, oral administration (50 mg/kg). Results are expressed as mean ± SE of four to seven rats.

3.2. Therapeutic effects of alendronate after intrapulmonary administration in hypercalcemia

We evaluated the therapeutic potential of alendronate after intrapulmonary administration using rat models of hypercalcemia (Fig. 2A). Our present study showed that the plasma calcium levels in rats increased significantly 5 days after the first 1α-(OH)D_3 administration; therefore, 6 days was selected as an initial time point for the evaluation of the therapeutic effects for treatment of hypercalcemia of alendronate after intrapulmonary administration. Six days after the first administration of 1α-(OH)D_3 and the concomitant increases in plasma calcium levels, alendronate was delivered via intrapulmonary administration, and plasma calcium levels were reduced for 9 consecutive days during which plasma calcium reached normal levels. The suppressive effect of plasma calcium levels with the intrapulmonary administration of alendronate was slightly higher than those with oral administration.

3.3. Effects of alendronate on OVX-induced osteoporosis after intrapulmonary administration

Micrographs of stained sections of bone tissue from the right tibia 8 weeks after OVX operation in rats are shown in Fig. 2B. In this study, we focused the growth plate (arrow in Fig. 2B) to evaluate the effect of alendronate on osteoporosis because the growth plate plays key roles in endochondral bone formation during the creation of new bone (Gafni et al., 2002). The width of the growth plate and the density of bone structure in OVX rats were reduced relative to that in control rats when observed 8 weeks after the OVX operation, indicating that osteoporosis was induced by the OVX operation. Alendronate after intrapulmonary administration prevented the decrease in the width of the growth plate and the density of bone structure after OVX operation.

3.4. Effect of alendronate on the membrane damage to the lung tissues

We evaluated the effect of intrapulmonary administration of alendronate on the pulmonary membrane by measuring the activities of LDH in BALF (Fig. 3) and conducting a histological examination (Fig. 4). Intrapulmonary administration of alendronate significantly increased the LDH activity in BALF in a dose-dependent

Table 1
Pharmacokinetic parameters of alendronate after delivery via three distinct routes.

	Intravenous injection	Oral administration	Intrapulmonary administration
Dose (mg/kg)	1	50	5
C_{max} (ng/ml)	–	834 ± 83	1928 ± 167
T_{max} (h)	–	0.8 ± 0.1	0.3 ± 0.02
$AUC_{0\to\infty}$ (ng h/ml)	1369 ± 62	1978 ± 114	3226 ± 237
BA (%)	–	2.9 ± 0.2	47 ± 3.5

Results are expressed as mean ± SE of four to seven rats.

manner (Fig. 3a). Moreover, infiltration of inflammatory cells, necrotic and/or damaged cells, perivascular edema and localized intra-alveolar bleeding were observed in stained histological sections of pulmonary tissue from rats that had received the alendronate-only treatment (5 mg/kg) via intrapulmonary administration (Fig. 4). These results indicated that pulmonary membrane was damaged by the intrapulmonary administration of alendronate. Co-administration of catalase had little effect on the elevation of LDH activity in BALF (Fig. 3d). In marked contrast, the elevation of LDH activity was completely suppressed by co-administration of SOD or L-cysteine in a dose-dependent manner (Fig. 3b and c). A low dose of SOD (20,000 IU/kg; 200 μmol/kg) completely suppressed the alendronate-induced pulmonary damage (Fig. 4); therefore, SOD rather than L-cysteine was used in subsequent experiments testing the absorption and pharmacological effects of alendronate delivered via intrapulmonary administration in the presence of an anti-oxidant.

3.5. Effect of SOD on absorption and pharmacological effects of alendronate delivered via intrapulmonary administration

The plasma concentration–time profiles of alendronate co-administered with SOD via intrapulmonary delivery to rats are shown in Fig. 5A. The plasma concentrations of alendronate after intrapulmonary co-administration with SOD increased rapidly and reached maximum levels at 0.3 h. The BA of alendronate after intrapulmonary co-administration with SOD was 33 ± 0.9%; these findings were close to those observed after intrapulmonary administration of alendronate alone. Furthermore, we evaluated the effect of SOD on the pharmacological effects of alendronate after intrapulmonary administration in 1α-$(OH)D_3$-induced hypercalcemia model rats (Fig. 5B). The 1α-$(OH)D_3$-induced increase in the plasma calcium levels after intrapulmonary administration of alendronate with SOD was reduced significantly and the plasma calcium levels were similar to those observed after intrapulmonary

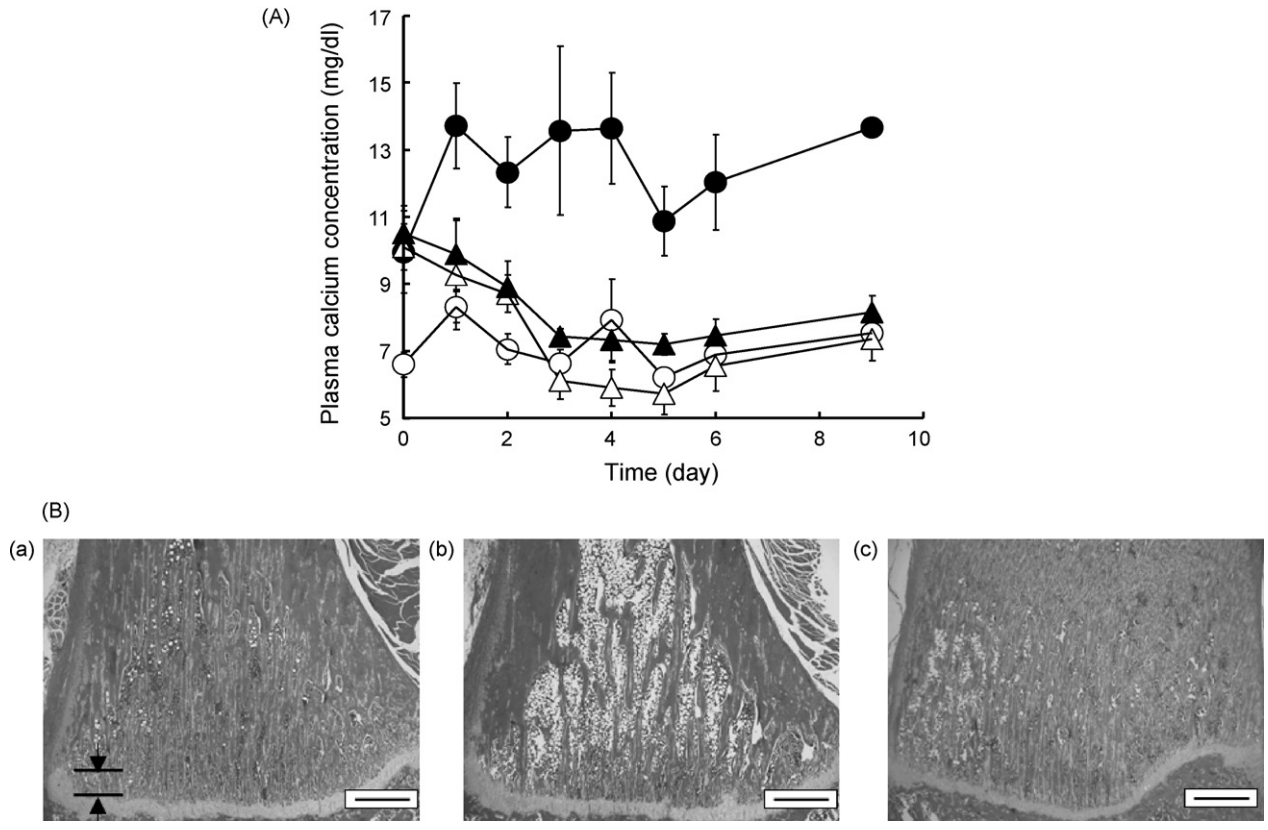

Fig. 2. The pharmacological effects of alendronate on bone diseases after intrapulmonary administration. (A) Effect of alendronate on the plasma calcium levels in 1α-$(OH)D_3$-induced hypercalcemic rats. Alendronate was administered 6 days after first 1α-$(OH)D_3$ administration. ○, naive; ●, 1α-$(OH)D_3$; Δ, 1α-$(OH)D_3$ + intrapulmonary administration of alendronate (5 mg/kg); ▲, 1α-$(OH)D_3$ + oral administration of alendronate (5 mg/kg). Results are expressed as mean ± SE of six to eight rats. (B) Effect of alendronate on the width of the growth plate (arrow) and the density of bone structure after intrapulmonary administration of alendronate in rats with OVX-induced osteoporosis. The micrographs depict stained sections of the bone tissue from the right tibia of female rats. Scale bar: 200 μm. (a) Sham; (b) OVX; (c) intrapulmonary administration of alendronate (5 mg/kg) immediately after OVX operation. All groups were evaluated 8 weeks after a sham or OVX operation.

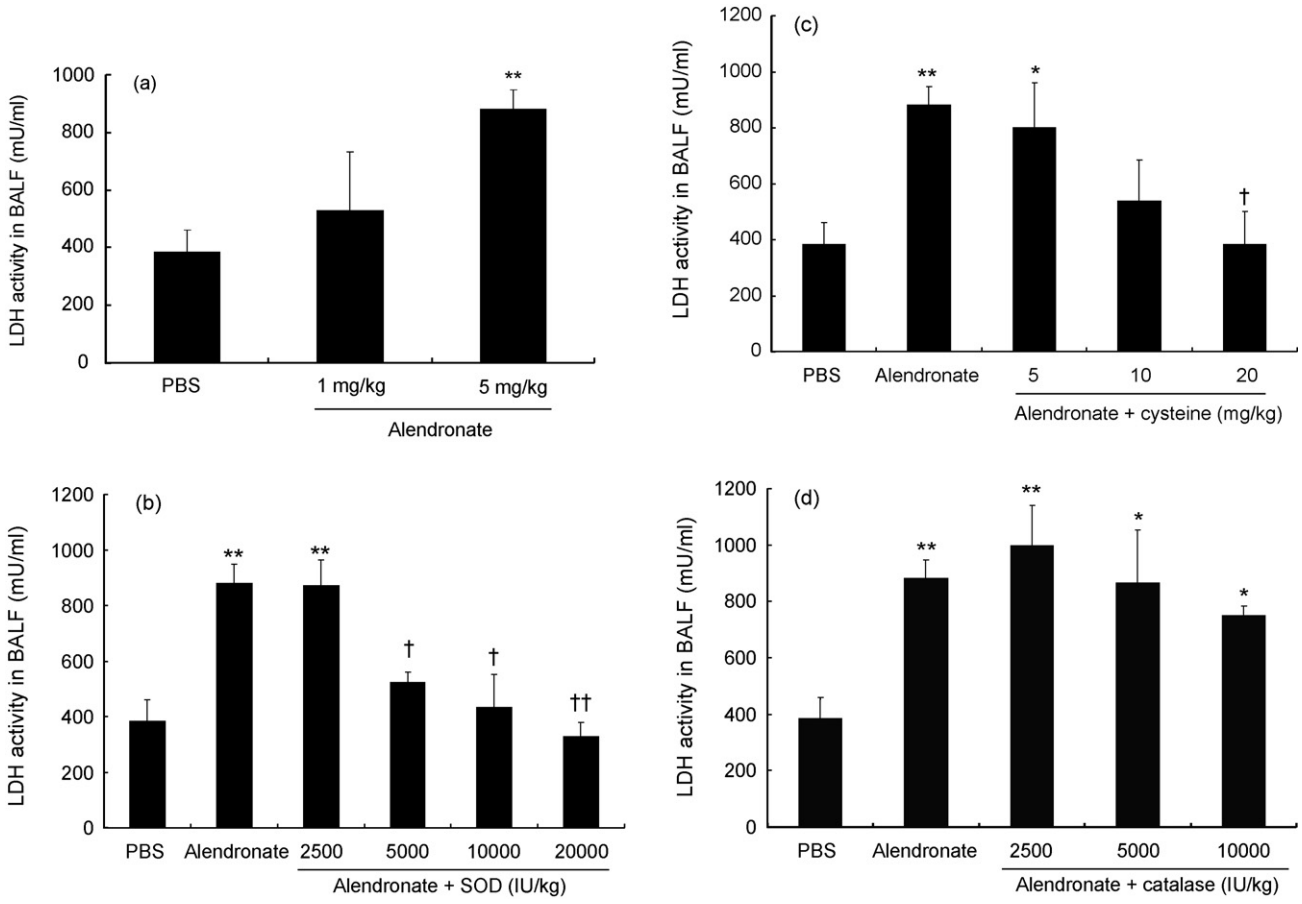

Fig. 3. The activities of lactate dehydrogenase (LDH) enzyme in BALF 4 h after intrapulmonary administration of alendronate with or without an additive. (a) alendronate-only (1 and 5 mg/kg), (b) alendronate (5 mg/kg) + SOD, (c) alendronate (5 mg/kg) + L-cysteine and (d) alendronate (5 mg/kg) + catalase. Results are expressed as the mean ± SE of four to six rats. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with PBS group. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ compared with alendronate group.

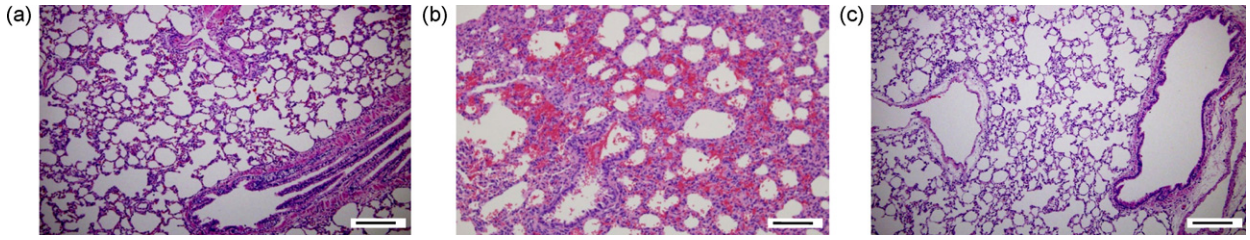

Fig. 4. Histological micrographs of the lung of rats with the intrapulmonary administration of various solutions. (a) PBS, (b) alendronate-only (5 mg/kg), (c) alendronate + SOD (5 mg alendronate/kg, 20,000 IU SOD/kg). Scale bar: 200 μm.

administration of alendronate alone. These results indicate that co-administration of SOD did not affect the pulmonary absorption or pharmacological efficacy of alendronate deliveries via intrapulmonary administration.

4. Discussion

Although many groups report that alendronate has therapeutic benefits in clinical use, the therapeutic potential of alendronate is limited by its low absorption and adverse effect when delivered via oral administration (Ezra and Golomb, 2000). Some groups attempted to improve the absorption of BPs using noninvasive alternative routes, but little attention has been paid to the systemic evaluation of the absorption and adverse effects of alendronate in these studies (Ezra and Golomb, 2000; Choi et al., 2008; Sutton et al., 1993). In the present study, we demonstrated that the BA of alendronate after intrapulmonary administration (approximately 47%) was 16-fold higher than that after oral administration (approximately 2.9%) in rats, and the alendronate-induced pulmonary damage could be completely suppressed by the co-administration of SOD. As far as we know, this is the first report to demonstrate and quantify the absorption of and tissue damage associated with alendronate delivered via intrapulmonary administration *in vivo*.

Previously, we reported that among various noninvasive drug delivery routes (including the small intestine, large intestine, nasal cavity, and buccal cavity), the lung was the best site for absorption of many water-soluble drugs with a wide range of molecular weights (Yamamoto et al., 2001). The higher drug absorption in the lung is probably due to the fact that the pulmonary tissue has a large epithelial surface area and the route to the blood vessels is shorter (0.1–5 mm) than in other tissues. Sutton et al. attempted to improve the alendronate absorption using a nasal route for delivery, but the BA of alendronate delivered via the nasal mucosa

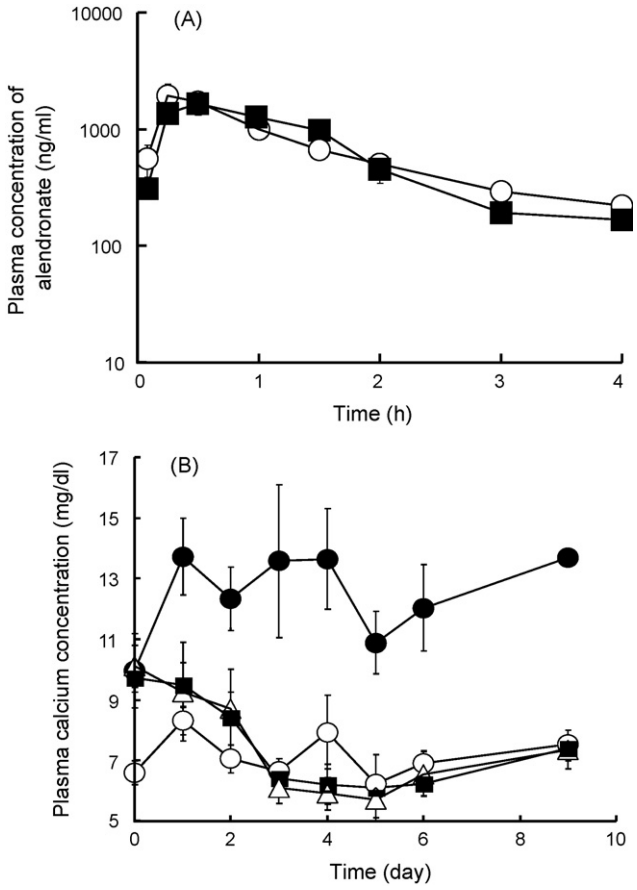

Fig. 5. Effect of SOD on the pulmonary absorption and pharmacological effect of alendronate delivered via intrapulmonary administration. (A) Plasma concentration–time profiles of alendronate after intrapulmonary administration in rats. ○, intrapulmonary administration of alendronate-only (5 mg/kg); ■, intrapulmonary administration of alendronate with SOD (5 mg alendronate/kg, 20,000 IU SOD/kg). The results are expressed as mean ± SE of four to seven rats. (B) Plasma calcium levels after intrapulmonary administration 6 days after first 1α-(OH)D_3 administration in rats. ○, naive; ●, 1α-(OH)D_3; Δ, 1α-(OH)D_3 + intrapulmonary administration of alendronate-only (5 mg/kg); ■, 1α-(OH)D_3+ intrapulmonary administration of alendronate with SOD (5 mg alendronate/kg, 20,000 IU SOD/kg). The results are expressed as mean ± SE of six to eight rats.

was approximately 7.3–22% in rats and dogs (Sutton et al., 1993). Although we also reported the transdermal permeation of alendronate using our patch system, the BA in rats was approximately 8.7% after a 24-h application of our alendronate patch (Kusamori et al., 2010). These results, together with our results of the pulmonary absorption, indicate that the lung is the most promising noninvasive alternative route for increasing the BA of alendronate. It was reported that the pulmonary absorption of drugs decreased with increasing in the molecular weights of the drugs (Yamamoto et al., 2001). Because alendronate is a low molecular weight compound, the pulmonary absorption of alendronate was quite high, and it was easily absorbed through the lung. The absorption of alendronate from the gastrointestinal tract has been proposed to occur primarily by a paracellular route rather than a transcellular route because alendronate is a highly hydrophilic drug that is charged at physiological pH (Ezra and Golomb, 2000). Therefore, the majority of the bioavailable alendronate may be absorbed from the lung via a paracellular route. Cocquyt et al., reported that alendronate was not metabolized, and excretion occurs exclusively through the kidney and was either excreted promptly or sequestered in bone for a long time (Cocquyt et al., 1999). It was also reported that disposition of alendronate after intravenous administration was linear in the wide range in rats and clinical study (Lin et al., 1992; Cocquyt et al., 1999). Furthermore, it was demonstrated that repeated intravenous administration did not alter the pharmacokinetic behavior of alendronate (Cocquyt et al., 1999). These pieces evidences indicate that disposition of alendronate would be linear in the wide range after its intrapulmonary administration. It was reported that there were species differences of drug absorption from the lung among various animals (Schanker et al., 1986). However, the lung is known to be a promising noninvasive alternative route of poorly absorbable drugs in humans (Patton et al., 1999). Therefore, alendronate may be effectively absorbed from the lung after its inhalation in clinical setting, although further studies are needed to control the individual variation of inhalation.

We evaluated the therapeutic and preventive effects of alendronate after its intrapulmonary administration in rat models of hypercalcemia and osteoporosis. BPs, including alendronate, are widely used for the regulation of osteoclastic bone resorption in the treatment of hypercalcemia and osteoporosis because osteoclastic bone resorption is known to play key roles in the pathogenesis and progress of both of these bone diseases (Graham, 2002). In the present study, the 1α-(OH)D_3-induced increase in the plasma calcium level was reduced significantly 3 days after the intrapulmonary administration of alendronate, which was in good agreement with the previously reported results of intravenous injection (Azuma et al., 1995). In the present study, the suppressive effect of plasma calcium levels with the intrapulmonary administration of alendronate was slightly higher than those with oral administration. It was reported that the BA was associated with the duration rather than the strength of pharmacological effect after the administration of alendronate (Khan et al., 1997). Therefore, we think that the pharmacological effect would be observed for a longer period of time after intrapulmonary administration than after oral administration in the later time point after the administration. We also clearly demonstrated that the alendronate administered intrapulmonarily showed preventive benefits in an osteoporosis rat model. These results strongly support the conclusion that alendronate absorbed from the lung regulates osteoclastic bone resorption and leads to therapeutic or preventive benefits in the treatment of hypercalcemia and osteoporosis.

As described above, alendronate has been associated with mucosal damage, including gastritis, gastric ulcer, and erosive esophagitis (Graham, 2002; Sener et al., 2004; Naniwa et al., 2008). Therefore, we evaluated the effect of alendronate on the pulmonary membrane after intrapulmonary administration in rats. In the present study, we confirmed that intrapulmonary administration of alendronate-induced pulmonary membrane damage. However, because the BA of alendronate after intrapulmonary administration was very high, the dose of alendronate can be reduced to minimize the adverse effects of alendronate. Nevertheless, the lung is the essential respiration organ for gas exchange, and a method for the prevention of any alendronate-induced pulmonary membrane damage should be established in clinical use.

Lichtenberger et al. reported that alendronate competitively displaced phosphatidylcholine from the surface of the mucosal layer, possibly triggering the alendronate-induced mucosal damage; nevertheless, a detailed mechanism of the alendronate-induced mucosal damage is not firmly established (Lichtenberger et al., 2000). Recently, it was reported that anti-oxidants such as taurine effectively prevented the alendronate-induced intestinal mucosal damage associated with oral administration (Sener et al., 2005a,b). We also reported that alendronate-induced skin damage was completely suppressed by the addition of butylhydroxytoluene (BHT), an anti-oxidant (Kusamori et al., 2010). Furthermore, reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, and hydroxyl radical among others, generated from inflammatory cells, such as macrophage and neutrophils, are known to contribute

to the various inflammatory processes through the NFκB (a transcription factor) activation (Bowie and O'Neill, 2000).

Based on these results, we hypothesized that alendronate-induced pulmonary membrane damage would be associated with ROS generated from activated alveolar macrophage and neutrophils. Therefore, we selected anti-oxidants, such as SOD, catalase, and L-cysteine, as additives to prevent damage to the pulmonary membrane (Koltover, 2009). Our present study showed that the alendronate-induced pulmonary membrane damage was not affected by the co-administration of catalase, a specific hydrogen peroxide scavenger, whereas it was completely suppressed by the co-administration of SOD, a specific superoxide anion scavenger, and L-cysteine, a non-specific reactive oxygen species scavenger. These results indicated that superoxide anion, but not hydrogen peroxide, was the predominant ROS contributing to the pathogenesis and progress of alendronate-induced pulmonary membrane damage. Co-administration of SOD with alendronate completely suppressed the alendronate-induced increase in LDH activity in BALF, while maintaining the high levels of pulmonary absorption and the therapeutic effects of alendronate in 1α-(OH)D_3-induced hypercalcemia rats (Fig. 5A and B). These results indicate that the co-administration of SOD with alendronate is a promising approach for the prevention of alendronate-induced pulmonary damage during the pulmonary delivery of alendronate.

5. Conclusion

In conclusion, we demonstrated that the BA of alendronate after intrapulmonary administration (approximately 47%) was 16-fold higher than that after oral administration (approximately 2.9%) in rats. Alendronate administered intrapulmonarily showed high therapeutic or preventive benefits in the treatment of rat models of hypercalcemia and osteoporosis. Co-administration of SOD with alendronate completely suppressed the alendronate-induced damage to the pulmonary membrane, and it did not inhibit the pulmonary absorption and therapeutic effects of alendronate in a rat model of hypercalcemia. These findings indicate that the lung is a promising, noninvasive alternative route for the delivery of alendronate in the treatment of bone diseases.

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