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Amelioration of Cd-induced bone deterioration by orally administered calcium phosphate

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ABSTRACT

Cadmium (Cd) is a heavy metal that accumulates in the body, primarily through daily grain intake, and has a high affinity for bone, leading to skeletal diseases such as osteomalacia and fractures. Hydroxyapatite (HAp), a major bone mineral component, is highly pH-sensitive and is known to incorporate Cd, as observed in studies of Itai-itai disease. Based on these findings, we hypothesized that HAp could serve as an effective oral detoxification material for heavy metals. This study investigated the efficacy of orally administered HAp in inhibiting Cd-induced changes in bone physical and chemical properties, comparing its effects to those of activated charcoal (AC), a common detoxifying agent. Six-week-old male ICR mice were exposed to cadmium via drinking water containing $CdCl_2$ and subsequently given diets containing either HAp or AC for four weeks. Three-point bending tests, micro-CT analysis, and histological observations of the femurs demonstrated that mice receiving HAp exhibited improved mechanical strength and enhanced bone quality protection compared to the control and other Cd-treated groups. Activated charcoal also contributed to bone quality improvement at low concentrations, but its effect diminished at high concentrations. These results suggest that the oral administration of HAp may be a promising therapeutic strategy for suppressing cadmium-induced osteomalacia.

1. Introduction

Exposure to the heavy metal cadmium (Cd) has become a global health concern due to its high toxicity and long biological half-life. There are several routes by which Cd can be exposed to humans, including contamination of food and water, such as grains, tobacco smoke, and industrial activities, such as metal plating and mining processes (Genchi et al., 2020; Tchounwou et al., 2012; Charkiewicz et al., 2023; Torres et al., 2023; Pacyna & Pacyna, 2001). When Cd is taken into the body by ingestion or inhalation, it is particularly likely to accumulate in the liver and kidneys (Satarug, 2018; Tinkov et al., 2018). Cd is also known to cause skeletal disorders, such as osteomalacia, osteoporosis, and increased susceptibility to fractures (Faroon et al., 2012 Sep.; Ougier et al., 2021; Åkesson et al., 2014). An example of the long-term effects of Cd exposure is Itai-itai disease in Japan, which was reported to have been caused by Cd-contaminated rice, resulting in osteomalacia and osteoporosis, as well as bone fractures and severe kidney dysfunction (Wallin et al., 2024; Wang et al., 2003; Aoshima, 2012; Inaba et al., 2005). Although Cd concentrations in food and beverages are regulated, exposure to Cd remains a significant health issue, especially in developing countries with unregulated industrial Cd emissions and inadequate food safety monitoring. Therefore, the development of biocompatible materials to reduce Cd toxicity and maintain bone health is an urgent task.

Biomaterials have attracted attention for medical applications for decades due to their biocompatibility and promoting effect on tissue regeneration (Zhu et al., 2020; Montoya et al., 2021; Abraham et al., 2022). Calcium phosphate (CaP), including hydroxyapatite (HAp), is the main component of bone, has excellent biocompatibility, and is mainly used as a scaffold for bone regeneration (Liu et al., 2025; Wang et al.,

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2025; Jeong et al., 2019). Meanwhile, bone undergoes repeated degeneration and stabilization during development and growth, a process called bone remodelling (Takegahara et al., 2024; Rowe et al., 2023). The main mechanism of this remodelling is the dissolution of CaP due to a decrease in pH near local osteoclasts and the reprecipitation of CaP due to neutralization of the surrounding pH environment (Kollenda et al., 2020; Barone and Nancollas, 1978). In addition, several studies have reported that CaP not only promotes bone regeneration, but also exhibits a strong ability to adsorb heavy metals such as Cd through ion exchange and surface complexation mechanisms (Corami et al., 2008; Desalegn et al., 2023). In general, pH changes occur in the digestive tract, such as a decrease in pH in the stomach and neutralization of low pH in the intestine. These evidences indicate that CaP materials may function as bone protectors by reducing the systemic absorption of Cd in the digestive tract and inhibiting its accumulation in bone tissue. Our previous studies have demonstrated that oral administration of HAp, a type of CaP material, can provide a protective effect against Cd accumulation in various tissues, including the liver and kidneys (Bikharudin et al., 2025). However, the application of HAp as an oral therapeutic agent, especially its effect on the physicochemical properties of femoral bone quality induced by Cd, has not been fully studied.

In this study, we hypothesized that oral administration of HAp would suppress the accumulation of ingested Cd in bone and be effective in maintaining bone structure and function. In this study, we use an *in vitro* osteoblast model and an *in vivo* Cd-exposed mouse model to clarify whether HAp is effective in preventing bone tissue changes caused by Cd exposure, in comparison with activated charcoal (AC), a common detoxifier.

2. Materials and methods

2.1. Materials

All chemicals, unless specified, were of reagent grade and obtained from FUJIFILM Wako Pure Chemical (Osaka, Japan). HAp and AC were prepared followed by the previous study (Bikharudin et al., 2025) with particle sizes of AC and HAp is 3.115 and 2.368 μm, respectively (Supplementary Fig. 1). The specific surface area (SSA) value of HAp was 15.7 m^2/g, which was lower than AC (SSA, 1743.9 m^2/g). Cadmium chloride ($CdCl_2$, >98 %) was used as the Cd source for both *in vitro* and *in vivo* experiments. Murine calvarial osteoblast cell lines MC3T3E1 subclone 4 (CRL-2593) were purchased from ATCC (Manassas, VA, USA).

2.2. In vitro study: Protective effects of HAp and AC against Cd toxicity in cells

MC3T3-E1 cells were cultured in α-MEM supplemented with 10 % fetal bovine serum (FBS) and 1 % penicillin-streptomycin under standard conditions (37 °C, 5 % CO_2). The cells were seeded at a density of 1 × 10^4 cells/well in 96-well plates and allowed to adhere for 24 h before the treatment. Cell viability was assessed using a CCK-8 assay (Dojindo Molecular Technologies, Tokyo, Japan) to observe the effect of HAp and AC (0–10 mg/mL) on Cd (10 μM) removing. After incubating for 48 h, the suspension was collected to measure the concentration of Cd and *Ca* using an atomic absorption spectrophotometer (AAS, Z-9000, Hitachi, Tokyo, Japan). After the media was removed, 100 μL of culture medium containing 10 μL of CCK-8 solution was added to each well. The plate underwent an examination with a microplate reader (Multiskan GO, Thermo Fisher Scientific, Waltham, MA, USA) calibrated to 450 nm following a 2 h incubation period

2.3. In vivo study

2.3.1. Animal model and experimental design

Male ICR mice, 6 weeks old (SLC, Shizuoka, Japan) were randomly allocated into six groups (n = 7–10 per group):

Control: Standard diet devoid of Cd exposure.

Cd-treated groups were treated with 100 ppm Cd with different diet foods as follows.

- Cd group: Standard diet
- Cd + 2AC group: Food supplemented with 2 wt % of AC.
- Cd + 20AC group: Food supplemented with 20 wt % of AC.
- Cd + 2HAp group: Food supplemented with 2 wt % of HAp.
- Cd + 20HAp group: Food supplemented with 20 wt % of HAp.

The treatment was sustained for four weeks. Weekly records of dietary intake and body weight were kept. At the end of the experiment, femurs were dissected and fixed in 4 % paraformaldehyde (PFA) at 4 °C for further analysis. For the mechanical test, the femur was isolated in 0.9 % NaCl and immediately measured for a three-point bending test. All the animal procedures using mice were strictly in accordance with the Guidelines for Animal Experiments at Okayama University after the approval of the experimental protocol by Okayama University (OKU-2022,505).

2.3.2. Histological analysis

Femur sections were decalcified, embedded in paraffin, and stained with hematoxylin and eosin (H&E). The general morphology was analyzed using a conventional microscope (Nikon ECLIPSE Ti2, Tokyo, Japan).

2.3.3. Physico-chemical properties

Femur-fixed specimens were examined utilizing micro-computed tomography (micro-CT, SkyScan 1174, Aartselaar, Belgium) at a resolution of 6.5 μm to identify mineralized regions. Microstructural parameters, including trabecular thickness, bone volume fraction, and connectivity density, were analyzed to evaluate bone quality. The isolated femur was subjected to a three-point bending test using a universal mechanical testing system to evaluate bone strength (Ez-test; Shimadzu, Kyoto, Japan) fitted with a 500 N load cell at a span of 3.0 mm and a loading rate of 0.5 mm min^{-1}. Four measurements were performed for each sample.

The isolated femur was dry vacuumed at room temperature (RT) overnight and ground to obtain powder and characterized using X-ray diffraction (XRD, Miniflex 600, Rigaku, Japan) and attenuated total reflectance Fourier transform infrared ray spectrophotometer (ATR-FTIR, IR Affinity-1S, Shimadzu, Japan). XRD was analyzed using Cu-Kα (1.54 Å) irradiation at 40 kV and 200 mA. ATR-FTIR spectra were obtained using an instrument by directly pressing the materials onto a ZnSe prism. The outcome was evaluated utilizing spectrum analysis software (LabSolutions IR v2.13; Shimadzu).

2.3.4. Micro-structural analysis

Collagen orientation of isolated femur was evaluated using a birefringence method. Bone specimens were decalcified, embedded in paraffin, and sectioned at a thickness of 5 μm along the craniocaudal axis of the bone. The deparaffinized sections were observed using a two-dimensional birefringence measurement system (WPA-micro; Photonic Lattice, Miyagi, Japan) attached to an upright microscope (BX60; Olympus, Tokyo, Japan). Birefringence analysis was performed using WPA-VIEW software (version 2.4.2.9; Photonic Lattice), as previously described (Ishimoto et al., 2017).

The average size of bone apatite crystallites along the c-axis in isolated femur was calculated based on the Scherrer formula using the full width at half maximum (FWHM) of the (002) diffraction peak profile, obtained by a reflection-type microbeam X-ray diffractometer (D8 DISCOVER; Bruker AXS, USA). The analysis was conducted on bone specimens prepared from the 50 % mid-diaphysis of the femur, cut proximally and distally with a thickness of 1500 μm. Cu-Kα radiation (1.54 Å) was used as the X-ray source, operated at 50 kV and 1 mA. The incident beam was collimated and slit to a diameter of 100 μm.

Diffraction rings were acquired over a 2θ range of 23° to 43° The observed FWHM was corrected using a silicon (Si) single crystal powder as the standard, allowing for the calculation of the intrinsic peak broadening.

Statistical analysis

We used GraphPad Prism software (version 10.0; GraphPad Software, *CA*, USA) to perform statistical analyses using the student's *t*-test or one-way ANOVA alongside Tukey-Kramer tests. The alpha level was established at 0.05.

3. Results and discussions

3.1. Effects of Cd exposure on physical and mechanical properties of femoral bone

The effects of cadmium (Cd) on bone tissue have long been known as a disease caused by Cd. In Japan, a well-known pollution incident called Itai-itai disease occurred in people who consumed rice grown in waters downstream of a copper mine (Nogawa et al., 2004). Recent reports from China and Taiwan have confirmed that Cd-induced bone loss in some areas is comparable to cases of Itai-itai disease (Chen et al., 2009; Liao et al., 2023). This suggests that the eating habit of rice in Asian regions is closely related to Cd exposure. First, we conducted a detailed study of the physical and mechanical properties of femoral tissue in mice exposed to Cd in drinking water. The Cd accumulations were significantly increased in the liver and kidney, which as primary organs for Cd deposition (Bikharudin et al., 2025). In the femur bone, Cd accumulation was also observed, indicating the decreasing bone properties based on micro-CT (μCT) and mechanical test results (Fig. 1). Despite only four weeks of Cd exposure, a significant decrease in cortical bone thickness was observed. Quantitative evaluation based on μCT data showed significant decreases in bone mineral density (BMD) (Fig. 1b) and bone mineral content (BMC) (Fig. 1c) in the Cd-treated group. Furthermore, a significant decrease in the thickness and size of the cortical bone was observed (Fig. 1d,e). The results of the three-point bending test showed a significant decrease in the cortical bone thickness and size, with the Cd-treated group exhibiting a strength of approximately 15.0 MPa compared to approximately 26.0 MPa in the control group (Fig. 1*g*). It has been reported that Cd inhibits osteoblast differentiation and increases osteoclast-mediated bone resorption in bone (Wan et al., 2023; Tang et al., 2025; Brzóska et al., 2005; Ma et al., 2021), which may have resulted in the loss of bone mass. Moreover, oral administration of AC or HAp showed prevention of Cd accumulation in these tissues, which indicates that both have a function as an antidote for removing Cd under the GI tract. Thus, oral administration of HAp showed more advantages than AC by preventing Cd accumulation and regulating biochemical parameters in the plasma effectively (Bikharudin et al., 2025). These results also indicate that oral administration of HAp has a dual effect of reducing Cd bioavailability while supporting mineral balance, especially for bone development.

3.2. Effect of Cd exposure on the microstructural properties of the femoral bone

To further investigate the effect of Cd exposure on the microstructural properties of the femoral bone, X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) were performed. In addition, the degree of orientation of bone collagen was evaluated using a

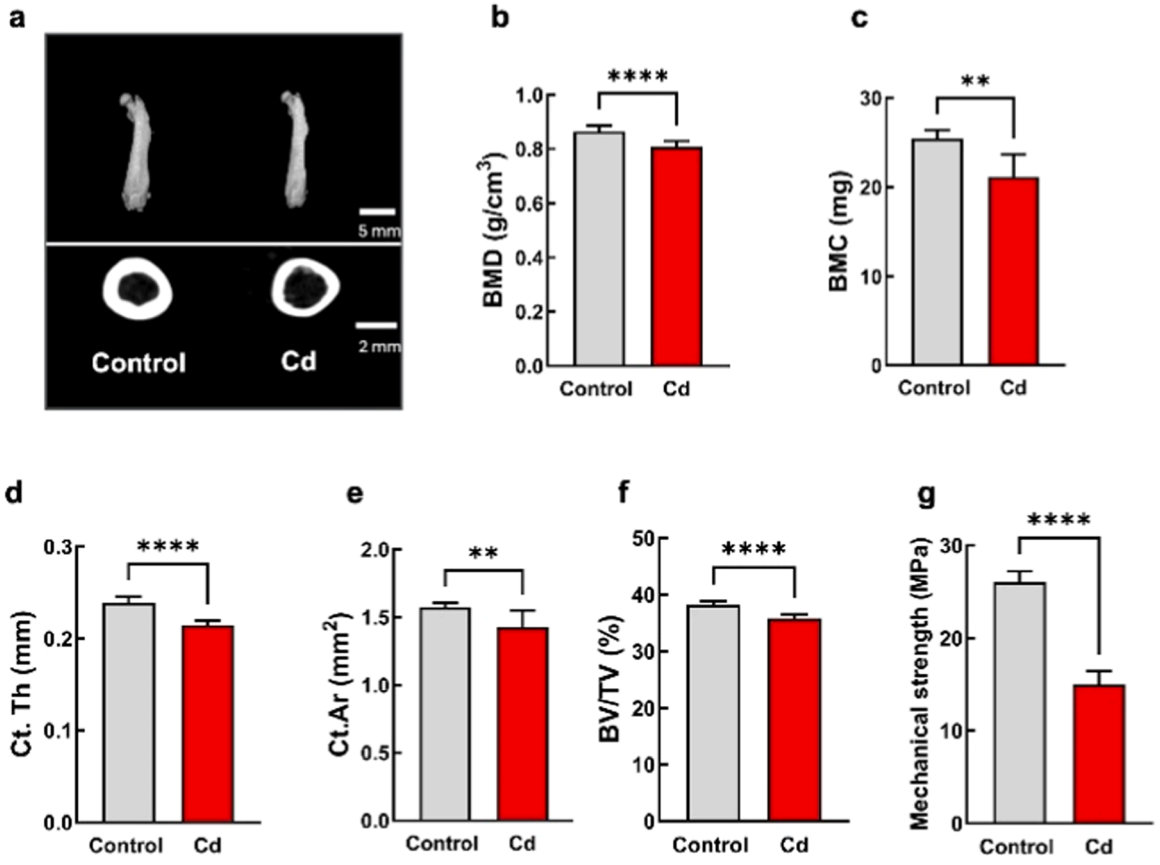

Fig. 1. Micro-CT of cortical bone and three-point bending test of femur exposed to Cd for four weeks. (a) Representative 3D images of cortical bone, (b) Bone mineral density (BMD), (c) Bone mineral content (BMC), (d) Cortical thickness (Ct. Th), (e) Cortical area (Ct. Ar), (f) Cortical bone/total volume ratio, and (f) mechanical strength of femur. Statistical analyses were performed with Student's *t*-test between control and Cd-treated; $^{**}p < 0.01$ and $^{****}p < 0.0001$.

birefringence microscope, and the degree of orientation of bone apatite was evaluated using small-angle X-ray scattering (SAXS). Collagen showed a uniaxial orientation along the long axis of the femur; however, this orientation did not differ between the Cd-treated and control groups. In addition, the c-axis orientation of bone apatite crystals in the Cd-treated group was highest in the center of the femur, as in the control group, and showed a tendency for the orientation to decrease toward the epiphysis (Fig. 2). H&E staining images of bone tissue showed no significant changes due to Cd, except for changes in cortical bone thickness (Supplementary Fig. 2).

On the other hand, XRD of powdered bone showed a decrease in crystallinity in the Cd-treated group. IR showed an increase in the peak due to C—O at around 1350 cm^{-1} in the Cd-treated group. The mechanism of this change is thought to be the inhibition of osteoblast differentiation and promotion of osteoclast differentiation by Cd, as well as the inhibition of bone apatite crystal growth due to Cd intervention. Cd competes with *Ca* ions during bone mineralization, inducing a decrease in bone *Ca* concentration (Ma et al., 2021; Christoffersen et al., 1988). This result is observed in Cd, whose ionic radius is close to that of *Ca* (Leão et al., 2020). It is speculated that repeated slight crystal distortion leads to peak broadening and increased replacement of carbonate ions.

3.3. Effect of cap on Cd-induced MC3T3-E1 cells

In this study, we hypothesized that oral administration of HAp suppresses Cd accumulation in mouse bones. As an *in vitro* verification of this, we examined whether the effects of Cd exposure would be reduced by adding HAp or AC to the culture environment of mouse-derived osteoblasts containing Cd. First, to examine the effects of Cd on cells, we performed cell culture experiments in the presence of Cd (Supplementary Fig. 3). At a Cd concentration of 0.1 μM, Cd treatment did not significantly affect cell viability. However, cell viability decreased significantly at 1 μM or higher, and most cells became nonviable at 10 μM or higher. These results indicate the high cytotoxicity of Cd.

Next, HAp and AC were added to cell culture medium containing Cd to examine the Cd recovery effect in a cell-free environment. The quantitative results are shown in Supplementary Fig. 3. By adding HAp and AC, more than 95 % of Cd (0.01–10 μM) present in the medium was recovered at all Cd concentrations. In this experiment, no significant difference was observed in the amount of Cd recovered between HAp and AC. In addition, the amount of *Ca* dissolved into the medium was shown to be significantly higher in the HAp-added group than in the AC-added group. Next, cell culture was performed with HAp and AC added in the presence of 10 μM Cd, at which the cells almost died. As a result, the cell viability was restored by the addition of HAp and AC, and this was concentration-dependent. Furthermore, when 10 mg/mL or more of HAp or AC was added, HAp further improved the cell viability, while the addition of AC suppressed the cell viability. Since there have been many reports on the cytotoxicity of high concentrations of AC (Guo et al., 2022; Strauss et al., 2021; Piccirillo et al., 2013), the results of this study are considered reasonable.

Previous studies have shown that CaP effectively immobilizes heavy metal ions in cell culture medium and reduces the toxicity level of bone-forming cells (Leão et al., 2020). The HAp-added group showed a dramatic improvement in cell viability, suggesting that HAp is a material with low cytotoxicity and excellent Cd removal.

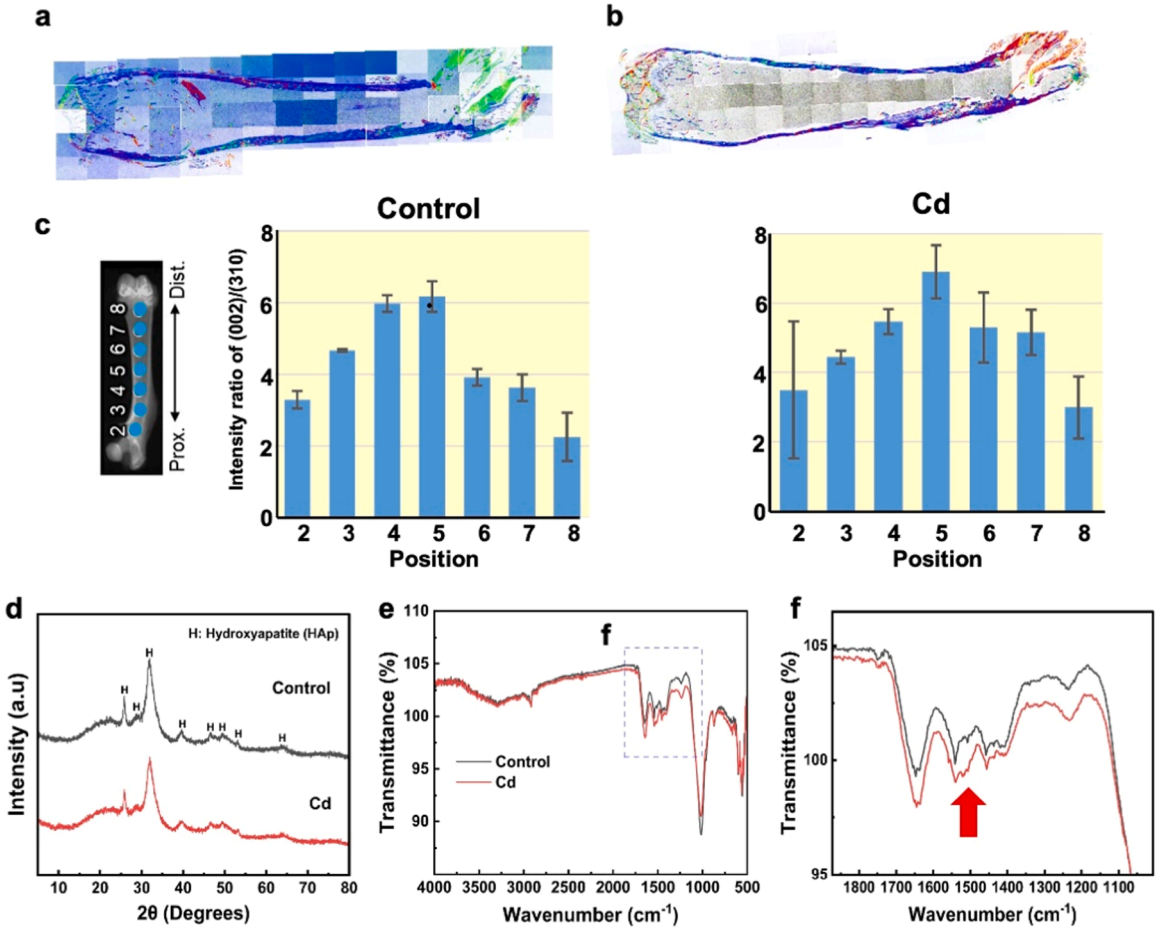

Fig. 2. Physico-chemical characteristics of femur after Cd-exposed for four weeks. (a) Infrared (IR) dichroism images of femur (a) control and (b) Cd. (c) Degree of apatite orientation of femur control and Cd groups by SAXS analysis. (d) XRD pattern and (e & F) FT-IR spectra of femur.

3.4. Improvement of physical and mechanical properties of Cd-exposed bone tissue by supplying HAp and AC

We investigated changes in the physical and mechanical properties of the femur when mice were fed HAp and AC in their diets under Cd exposure conditions. The results of μCT observations showed that the cortical bone thickness of mice fed HAp recovered to normal. This result was also evident from the quantitative results based on μCT data, and it was shown that the bone mineral density, cortical bone thickness, and total bone mass all approached normal values depending on the amount

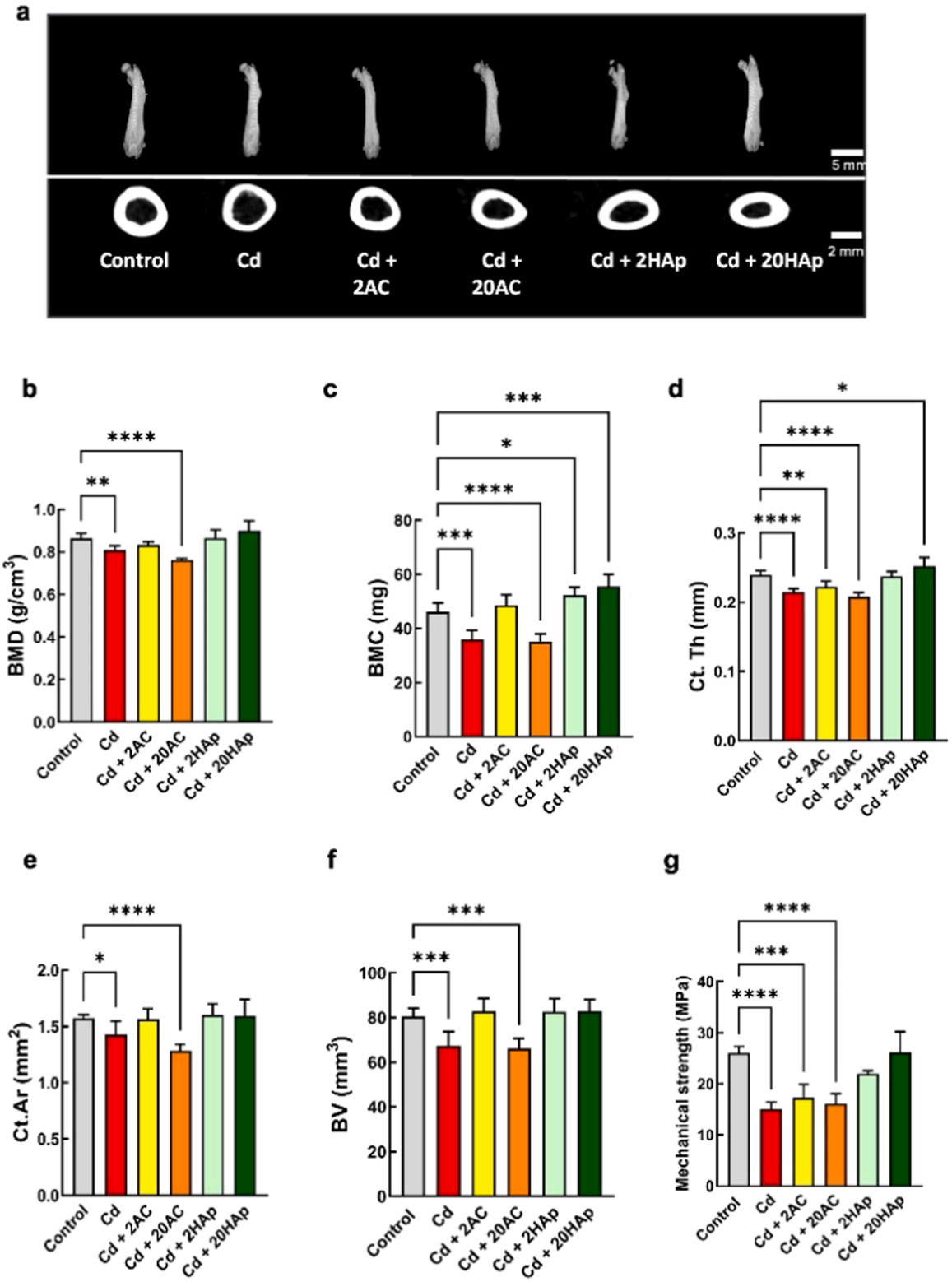

Fig. 3. Micro-CT analysis of cortical bone in femur mice treated with Cd and inorganic particles via oral administration for four weeks. (a) Representative 3D images of cortical bone of cortical bone in femur, (b) BMD, (c) BMC, (d) Ct. Th, (e) Ct. Ar, and (f) BV, and (g) mechanical strength. Statistical analyses were performed with one-way ANOVA and Tukey-Kramer tests between control, Cd-treated with AC and HAp; $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

of HAp added to the diet. On the other hand, in the group fed AC, the addition of 2 wt % AC showed a tendency to approach normal values, while the addition of 20 wt % AC showed a decrease in all indices compared to the 2 wt % AC group (Fig. 3). No significant changes were observed in the H&E staining images (Supplementary Fig. 4). The results of the bone strength obtained by the three-point bending test are shown in Fig. 3*g*

In this case, as in the μCT results, bone strength was restored to normal values by the addition of HAp. On the other hand, it was shown that while AC intake showed a tendency for improvement at low doses, it decreased bone strength at high doses. Importantly, the group with 2 % AC showed good improvements in bone strength, but about 40 % of the mice in the 20 % AC group died during the experiment. Histopathological analysis could not be performed on these deceased mice because vital organs like the liver and kidney were already severely damaged after death and were unsuitable for examination. This is an important limitation of the current study. The mortality suggests the significance of elevated serum ALP (Bikharudin et al., 2025) and body weight loss (Supplementary Fig. 5), which may cause hepatotoxicity and malnutrition. Previous toxicological studies have indicated that AC impairs essential nutrient absorption and induces stress on the liver and kidneys in rodent models (European Medicines Agency/Committee for Medicinal Products for Human Use 2010; Dungkokkruad et al., 2021). Offor et al. (2017) demonstrated that AC influenced biochemical levels in rats exposed to lead, suggesting possible implications for liver and kidney function (Offor et al., 2017). Our findings corroborate these reports, indicating that determining the cause of death in mice necessitates histological analysis of organs collected at the time of death, in conjunction with liver function markers including ALT, AST, and bilirubin levels. Future research should encompass a thorough analysis, including necropsy and biochemical assessments at various time points and across different doses of AC, to investigate the mechanisms underlying AC-induced mortality.

Moreover, although both *in vitro* HAp and AC had similar Cd adsorption, the function of oral administration of HAp and AC for the *in vivo* model showed differences due to the complexity of the environment and systemic GI tract. AC is an effective nonspecific adsorbent that can bind Cd in the gut, thereby reducing its absorption and partially preventing Cd-induced bone toxicities. However, AC can also absorb essential nutrients, including calcium, phosphate, zinc, and vitamins, which may disrupt the mineral balance and impair the bone homeostasis during the treatment (Juurlink, 2016; Bonilla-Velez et al., 2017). In addition, our findings showed that a high dose of AC elevated the systemic toxicities, increasing the mortality during the treatment, suggesting that excessive oral administration of AC may compromise organ functions (Kubiak-Mihkelsoo et al., 2025). In contrast, HAp under the GI

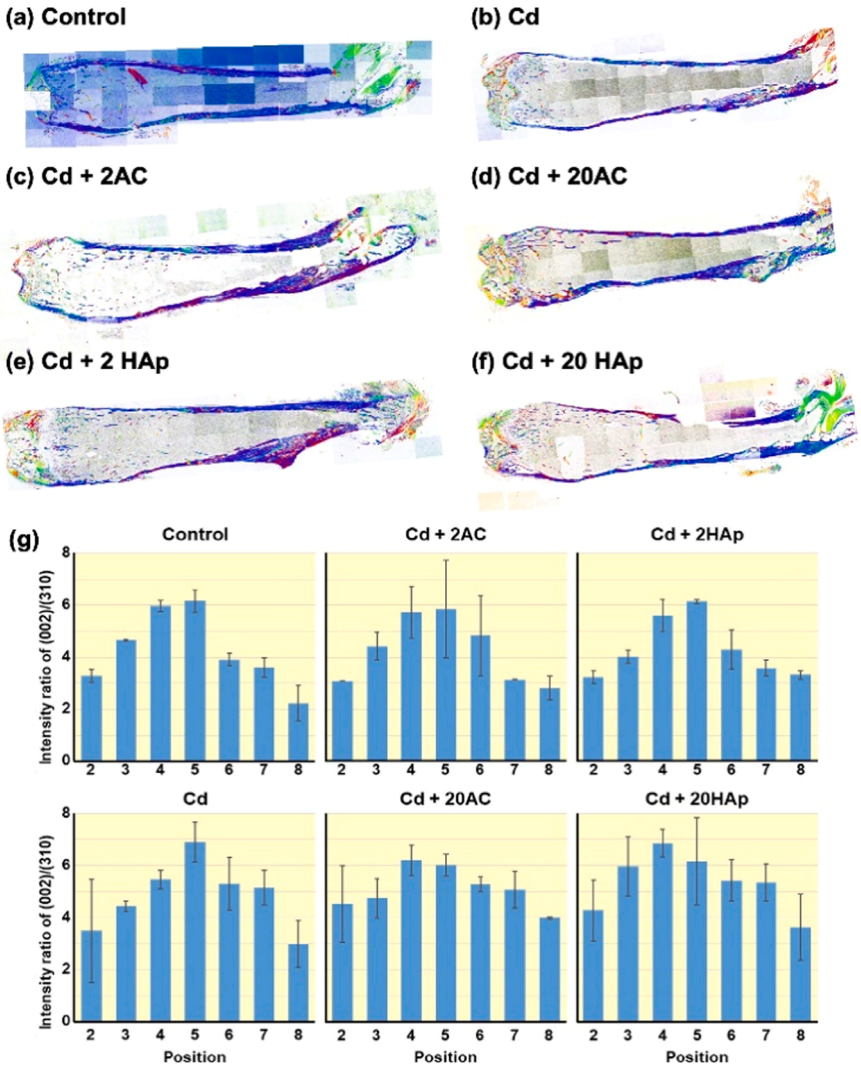

Fig. 4. Imaging of collagen orientation of (a) control, (b) Cd, (c) Cd + 2AC, (d) Cd + 20AC, (e) Cd + 2HAp, and (f) Cd + 20HAp. (g) Degree of apatite orientation of femur control and Cd groups by SAXS analysis of control, Cd, Cd + 2AC, Cd + 20AC, Cd + 2HAp, and Cd + 20HAp. White and red show that the orientation is parallel to the long axis of the cortical bone, and blue shows that orientation is perpendicular to the long axis of the cortical bone.

tract not only acts as a binder of Cd through ion exchange and phosphate complexation by dissolution-precipitation, but it also provides support for Ca^{2+} and PO_4^{3-} ions that could maintain bone homeostasis. These ions are critical regulators of bone remodeling and mineralization (Mitić et al., 2024; Chen et al., 2024), and their release may support maintaining bone homeostasis under Cd exposure. The structural similarity of HAp to natural bone mineral also makes it more biocompatible and osteoconductive, which could make its protective effect against Cd-induced bone deterioration even stronger. Thus, HAp offers additional bone-specific benefits *in vivo* that are insufficient by AC, indicating that oral administration of HAp is a more promising candidate for translational application for bone protection.

Results of bone collagen orientation using a birefringence microscope showed that the highest collagen orientation was observed in the diaphysis in both the HAp and AC-administered groups, with a tendency for orientation to decrease toward the epiphysis. This trend did not differ significantly from that observed in control or Cd-treated bone (Fig. 4). The decrease in bone crystallinity in the Cd-treated group, as observed by XRD of powdered bone, was improved by oral administration of HAp, as was the increase in peaks due to C—O observed by IR (Supplementary Fig. 6).

Previous experiments have shown that HAp ingested orally dissolves due to pH changes in the digestive system, and HAp and dicalcium phosphate dihydrate (DCPD) precipitate in the intestine. Furthermore, it has been demonstrated that HAp effectively captures more than about 95 % of Cd in the digestive system (Bikharudin et al., 2025). In mice that ingested HAp, blood *Ca* concentration did not change even at high concentrations, and it was confirmed that most of the ingested HAp was excreted from the body in feces (Bikharudin et al., 2025). These findings suggest that HAp intake may have a dual function of retrieving Cd in the digestive system and simultaneously maintaining blood calcium levels.

The oral dosage of HAp and AC using a mouse model in our study (2 or 20 %w/w per food) was selected to demonstrate the efficacy in preventing Cd-induced bone deterioration. These dosage levels are comparable to those in commercial HAp toothpastes, which typically contain 10–20 % HAp and are safe for human use (Limeback et al., 2023; Pawińska et al., 2023). The European Scientific Committee on Consumer Safety (SCCS) has confirmed that nano-HAp is safe in toothpaste up to 29.5 % and in mouthwash up to 10 % (Scientific Committee on Consumer Safety 2025; National Institute for Public Health and the Environment (RIVM) 2025). This clinical precedent supports the biocompatibility of HAp at high dose concentrations. Nevertheless, while these doses are feasible in oral care products, systemic dietary supplementation at 20 % of HAp would not be directly applicable to human clinical practice, where such high dietary supplementation would be impractical and potentially unsafe. Moreover, HAp has been applied clinically as a bone graft substitute and implant coating, while AC is administered in a single oral dose of 25–100 g for acute poisoning (Position statement and practice guidelines on the use of multi-dose activated charcoal in the treatment of acute poisoning 1999; Rashidi et al., 2022; Austin Health 2024; Eddleston et al., 2008). Our findings showed, therefore, that the translational use of HAp is as safe and effective as Cd-binding materials to prevent bone deterioration. Thus, future translational research should focus on optimizing lower-dose, clinically relevant doses and investigating the biosafety of HAp or HAp-based materials as oral therapeutic agents against Cd-related bone toxicities.

The limitation of our study is the lack of a high-dose HAp-only group (20 % HAp without Cd exposure). This precludes the complete exclusion of the possibility that HAp may affect bone physiology. Oral administration of HAp is generally considered safe, with minimal systemic absorption, and is extensively utilized in dental and orthopedic applications as a biocompatible, osteoconductive material (Limeback et al., 2023; Pawińska et al., 2023; National Institute for Public Health and the Environment (RIVM) 2025). Safety evaluations indicate that nano-hydroxyapatite is minimally absorbed by cells and does not cause systemic toxicity (National Institute for Public Health and the Environment (RIVM) 2025). Consequently, although we cannot entirely dismiss the possibility of independent effects of high-dose HAp, the bone-protective results observed are more convincingly attributed to diminished Cd bioavailability and the mineral-supportive function of HAp. Subsequent investigations should include HAp-only controls to validate this interpretation. In addition, HAp is made from naturally abundant *Ca* and P as raw materials, and its synthesis is cheap and easy. In addition, its size and surface modification are easy to control, and various surface properties and shapes can be controlled. Its biosafety has also been demonstrated in many long-term implantation studies. This time, the effect on Cd recovery was shown, however, similar effects can be expected for heavy metals other than Cd. Due to these many advantages, Cd detox materials made from HAp could become an important option for biocontrol in the pre-disease stage, a need for which will increase in the future.

4. Conclusion

In conclusion, our findings indicate that CaP supplementation could prevent Cd-induced bone deterioration by lowering Cd levels and modulating bone mineralization compared to AC. The multifunctional orally administered CaP as a detoxifying agent and its bone-protective properties have led to the design of safe, food-grade biomaterials to prevent heavy metal exposure and larger applications of CaP for regenerative medicine.

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The authors declare no competing interests.

Ethical statement

All animal experimental procedures were conducted in accordance with the Guidelines for Animal Experiments at Okayama University after the approval of the experimental protocol by Okayama University (OKU-2022505). Six-week-old ICR male mice were housed under controlled temperature and humidity, with a 12-h light/dark cycle and *ad libitum* access to food containing a normal diet, a hydroxyapatite diet, and an activated charcoal diet. At the same time, they were exposed to 100 ppm of cadmium via drinking water for four weeks. All animals were sacrificed using an overdose of isoflurane to collect the tissues for further analysis.

CRediT authorship contribution statement

Ping-chin Sung: Writing – original draft, Investigation, Formal analysis, Data curation. **Ahmad Bikharudin:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Masahiro Okada:** Validation, Supervision, Investigation, Conceptualization. **Randa Musa:** Investigation. **Kenta Uchida:** Investigation. **Akihisa Otaka:** Investigation. **Tadaaki Matsusaka:** Investigation. **Aira Matsugaki:** Investigation. **Takayoshi Nakano:** Investigation. **Takuya Matsumoto:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2025.101482.

Data availability

Data will be made available on request.

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