氏　　名	AHMAD BIKHARUDIN
授与した学位	博　士
専攻分野の名称	学　術
学位授与番号	博甲第７３７２号
学位授与の日付	令和７年９月２５日
学位授与の要件	医歯薬学総合研究科機能再生・再建科学専攻 （学位規則第４条第１項該当）
学位論文の題目	Co-precipitating calcium phosphate as oral detoxification of cadmium (リン酸カルシウム共沈殿を活用した消化管内カドミウム除去)
論文審査委員	鈴木　茂樹　教授　　江口　傑徳　准教授　　丸山　貴之　准教授

学位論文内容の要旨

Introduction

Osteophagia is a habit observed in wild herbivores, where they consume bones to obtain essential minerals such as calcium (Ca) and phosphorus (P) from nutrient-deficient environments and enhance the immune system and support healthy digestive functioning due to their nutritional composition. Bones consumed by animals enter the acidic stomach environment and then move into the small intestine, where they are neutralized by alkaline bile. Hydroxyapatite (HAp), a calcium phosphate (CaP) crystal that is the main mineral component of bone, can dissolve under acidic conditions and re-precipitate under alkaline conditions. Therefore, as the bone moves through the gastrointestinal (GI) tract, HAp dissolves in the acidic stomach and re-precipitates as crystalline or amorphous CaP in the small intestine after neutralization with the bile, which has beneficial environment in binding toxins. Previous studies showed that bone-eating or bone meals supplementation has been shown to be effective in detoxifying organic toxicants through encapsulation or adsorption, the detoxification of inorganic toxicants has not been investigated. Notably, Ca ions in CaP can be exchanged with heavy metal ions and co-precipitation can form heavy—metal—ion—substituted CaP from mixtures of those ions bydissolution/co-precipitation under GI tract. In this study, we investigated the effect of HAp dissolution and co-precipitation with Cd along the GI tract, comparing it to a commercial antidote, activated charcoal (AC), using both *in vitro* and *in vivo* oral administration studies. By mimicking the pH levels in the stomach and intestine, we demonstrated that HAp dissolution/co-precipitation enhances Cd removal in vitro. Furthermore, we proved that oral administration of HAp significantly prevents Cd accumulation in tissues and enhances Cd excretion in feces compared to AC.

Methods:

In vitro and *in vivo* studies were conducted in this study by mimicking pH levels under simulated GI tracts and using 6-week-old ICR male mice's GI tracts for Cd removal by dissolution/co-precipitation of calcium phosphate (CaP), respectively. Specifically for *in vivo* experiments, the study employed various dietary interventions especially normal diets, diets containing 2 wt% AC, diets with 20 wt% AC, diets with 2 wt% HAp, and diets with 20 wt% HAp. During the treatment, we simultaneously exposed the drinking water to 0 ppm (control group) or 100 ppm of Cd (Cd-treated groups). We systematically recorded the mice's daily feces excreted, food and water intake, and their weekly body weight during a four-week treatment period.

Results and discussions

The study's findings indicate that HAp has a maximum Cd-removal capacity of 410.06 mg/g, compared to AC (201.71 mg/g) by *in vitro* study. Our *in vitro* studies showed that co-precipitation of CaP from HAp in the GI tract model is significantly more efficient at removing Cd than AC under GI tract model. Acidic conditions increase Cd solubility, making Cd more accessible to HAp in the GI tract model. The dissolution of HAp in the GFM enhances surface complexation, leading to rapid HAp dissolution in highly acidic conditions and subsequent co-precipitation with Cd ions upon neutralization in the IFM. Co-precipitation of CaP from HAp thus attracts Cd ions, resulting in a higher adsorption capacity than HAp at neutral pH.

Similarly, for the *in vivo* studies, a co-precipitating CaP system in mice effectively prevented Cd accumulation in tissues and increased fecal Cd excretion compared to AC. Oral administration of HAp leads to CaP reformation in the intestine, improving the intestinal environment and increasing Cd excretion in feces, thereby reducing intestinal Cd absorption. Co-precipitating CaP acts as a blocking agent for Cd absorption, preventing Cd accumulation in tissues by decreasing Cd solubility in the stomach and inhibiting Cd absorption in the intestine. In contrast, orally administered AC removes toxins in their dissolved form through direct contact and may strongly acidify gastric pH, damaging the gastric epithelium. This damage increases Cd influx via Ca channels, promoting prolonged Cd accumulation in the stomach and higher Cd levels in surrounding tissues, such as the lung and spleen. The study also found that the AC-diet groups had noticeable changes in their plasma biochemical properties, which were linked to damage in the liver and kidneys, including serious cell death in the kidney tubes and disorganized kidney structures.

This study is the first to report a treatment that prevents heavy metals accumulation in

mice model by co-precipitating CaP along the GI tract using orally administered HAp. As a detoxifying agent, co-precipitating CaP from HAp shows promising results. It helps maintain plasma biochemical parameters, potentially acting as an antioxidant. Histopathological results also indicate that co-precipitating CaP protects liver and kidney tissue from Cd toxicity by modulating ALP and ALT levels, acting as an antioxidative defense system.

In summary, the co-precipitating CaP system in the GI tract is significantly more effective at removing Cd *in vitro* and *in vivo* than the commonly used antidote, AC. Furthermore, these findings are essential for designing new functional detoxification materials and potentially aiding disease treatment.

論文審査結果の要旨

[緒言] 野生動物は、必須ミネラルの摂取やその他の機能維持のために骨を摂取する骨食行動を示すことが知られている。消化管において、骨由来ハイドロキシアパタイト（HAp）はリン酸カルシウム（CaP）結晶であり、酸性の胃で溶解し、中性、アルカリ性の小腸で再沈殿することが想定される。このプロセスにおいて、CaPは消化管内で溶解および共沈殿によって重金属系毒素と結合し、除去できるという仮説を立てた。本研究では、消化管におけるHApの溶解およびカドミウム（Cd）との共沈殿の影響を、市販の解毒剤である活性炭（AC）と比較し検討した。
[材料および方法] 消化管内のpHレベルを模倣した*in vitro*でのCd回収実験を行った。また、6週齢のICR雄マウスを用いて、CaPの溶解/共沈殿によるCd除去を検討した。
*In vivo*試験では、通常食、飼料1食あたり2%または20%のACまたはHApを含む飼料を用いた。投与期間中、マウスは4週間、飲水を介して0 ppm（対照群）または100 ppm（Cd投与群）のCdに曝露された。生体各組織へのCd蓄積を組織染色で検討、また、組織沈着量や糞便中のCd量を原子吸光分析（GF-AAS）にて定量した。
[結果と考察] *In vitro*試験において、共沈CaPはACよりも高い最大Cd除去能を示した。酸性条件はCdの溶解性を高め、消化管モデルにおいてCdがHApにアクセスしやすくなる。胃液モデル（GFM）におけるHApの溶解は錯体形成を促進し、高酸性条件下でのHApの急速な溶解につながり、続いて腸液モデル（IFM）中での中和時にCdイオンとの共沈が起こった。このようにHApから派生するCaPの共沈はCdイオンを回収、ACよりも高い回収量を実現した。同様に、*in vivo*試験では、マウスにおいて共沈CaPシステムはACと比較して組織へのCd蓄積を効果的に抑制し、糞便中Cd排泄量を増加させた。共沈CaPはCd吸収の遮断剤として作用し、胃におけるCdの溶解度を低下し、腸管におけるCd吸収を阻害することで、組織へのCd蓄積を防ぐことが示された。

本研究では、HApなどCaPが糞便を介したCd排泄を促進することで、ACよりも効果的に組織におけるCdの蓄積およびCd誘発毒性を予防することを明らかにした。これは骨代謝における現象を活用し、歯科領域で研究が進むCaPを多様なヘルスケアに応用できることを示す重要な結果である。研究内容は、すでにエルゼビア社発行のJournal of Hazardous Materials (IF=12.0)に掲載され、国際的にも高い評価を受けている。以上のことから、審査委員会は本研究を博士（学術）としてふさわしいものと認めた。