報告番号	香大医博甲　第838号

様式１０７

学位論文審査の結果の要旨

令和　5年　6月　7日

審査委員	主　査	Katsuya Hirano	平野勝也	
	副主査	Tetsuo Minamino	南野哲男	
	副主査	Shinji Kosaka	小坂信二	
願出者	専攻	医学専攻	部門	（平成２７年度以前入学者のみ記入）
	学籍番号	19D724	氏名	JAHAN NOURIN
論文題目	Possible contribution of phosphate to the pathogenesis of chronic kidney disease in dolphins			
学位論文の審査結果	（合格）・　不合格　（該当するものを○で囲むこと。）			

〔要旨〕

This study aimed to investigate whether phosphate contributes to the pathogenesis of chronic kidney disease (CKD) in dolphins. Renal necropsy tissue of an aged captive dolphin was analyzed and *in vitro* experiments using cultured immortalized dolphin tubular (DolKT-1) cells were performed. In renal necropsy tissue analysis with a computed tomography scan, data showed medullary calcification in reniculi. Micro area X-ray diffractometry and infrared absorption spectrometry showed that the calcified areas were primarily composed of hydroxyapatite (calcium phosphate). *In vitro* experiments showed that treatment with both phosphate and calciprotein particles (CPPs) resulted in cell viability loss and lactate dehydrogenase release in DolKT-1 cells. However, treatment with magnesium markedly attenuated this cellular injury induced by phosphate, but not by CPPs. Magnesium dose-dependently decreased CPP formation. These data support the hypothesis that continuous exposure to high phosphate contributes to the progression of CKD in captive-aged dolphins. Our data also suggest that phosphate-induced renal injury is mediated by CPP formation in dolphins, and it is attenuated by magnesium administration.

Dissertation examination committee meeting was held on June 7, 2023. The following questions and answers were held.

<Dr. Tadashi Sofue >

- **Did you measure the level of FGF23 in dolphin plasma/serum?** →We did not measure FGF 23 in dolphin plasma/serum because still there is no established method available for measuring FGF 23 in dolphin plasma/serum.
- **In human CPP causes atherosclerosis. But you showed CPP in dolphin kidney medullary tissues? Why the localization of CPP is different in dolphin than human?** → As we focused on dolphin kidney, we mentioned CPP localization in kidney medullary tissues. It could be localized in other parts too.

<Prof. Tetsuo Minamino>

- **How much magnesium is required for an alive dolphin?** → We recommend the supplementation for magnesium but further investigation is needed to confirm the optimum dose for dolphin as they have large body mass and their physiology is different from human being.
- **If magnesium will be supplied, will it cause any side effects similar to hypermagnesemia?** → In human, hypermagnesemia has some side effects like diarrhea, nausea etc. but for dolphin there is no report regarding this issue. We do not know whether the effect will be similar as dolphin have large sized intestine with huge body mass.

<Prof. Shinji Kosaka>

- **How does phosphate decrease cell viability?** → This is our limitation that we could not indicate the precise mechanism of cell death caused by phosphate. Our investigations indicate cell death was not apoptotic and also not mediated by mitochondrial damage or disfunction.
- **Is there any possible cell death you can speculate?** → There are some other types of cell death e.g., ferroptosis, pyroptosis, parthantos could be induced by phosphate.
- **How magnesium interferes CPP formation?** → Magnesium inhibits the transition from primary CPP to crystalline secondary CPP.

<Prof. Katsuya Hirano>

- **Could you please clarify in this study which data is most important for CKD?** → We measured Creatinine and BUN but we are not sure these parameters are suitable to indicate CKD in dolphin, but we found higher serum phosphorous level in the dolphin plasma.
- **Did you confirm CKD in the dolphin from which you collected samples?** → No, we are not sure whether that dolphin has CKD. The presence of high phosphate level in diet, presence of renal calcification and declined nephron number due to old age may increase the risk of developing CKD in captive dolphins.
- **Does CPP formation itself induces renal cell damage or injury?** → CPP itself is not causing cell damage, when it transformed into secondary CPP it causes cell damage.
- **When you measured CPP formation in ex vivo/in vitro condition, what you exactly measured by absorbance 570nm?** → We measured turbidity of the medium. CPP are colloidal and then transformed into crystal. It would reflect by turbidity measurement of the experimental medium.

Based on the above questions and answers, the evaluation was conducted by the judges. This study examined whether phosphate contributes to the pathogenesis of chronic kidney disease (CKD) in dolphins. A diet rich in phosphate from fish and squid over many years may increase the risk of intratubular calcium phosphate (CPP) formation as they age. In the present study, analysis of renal tissue from an older dolphin revealed significant calcified lesions caused by the accumulation of calcium phosphate (hydroxyapatite). Further *in vitro* experiments using cultured dolphin tubular cells also demonstrated that CPPs were responsible for phosphate-induced renal injury in dolphin tubular cells, and magnesium inhibited CPP formation. The findings of this study are of great significance, as they demonstrate that captive dolphins are more likely to develop CKD due to higher phosphate levels as they age. Additionally, it is possible that magnesium supplementation potentially reduces the risk. Based on these reasons, the jury unanimously judged that this thesis was suitable for a doctoral dissertation in medicine and passed it.

掲載誌名	Scientific Reports　第 13 巻, 第 5161 号		
(公表予定) 掲載年月	2023 年 03 月	出版社（等）名	Nature publishing group

（備考）要旨は，１，５００字以内にまとめてください。