| 報告番号 | 香大医博甲　第８３８号 |
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様式１０２

# 学位論文の内容の要旨

Summary of the Substance of Dissertation

| 専　攻<br>Major Field | 医学専攻 | 部　門<br>Department | |
|---|---|---|---|
| 学籍番号<br>Student No. | 19D724 | 氏　名<br>Name | JAHAN NOURIN |
| 論文題目<br>Thesis Subject | Possible contribution of phosphate to the pathogenesis of chronic kidney disease in dolphins | | |

（論文要旨）
Summary


## Background

As humanity enters a super-aging society, the number of patients with chronic kidney disease (CKD) is dramatically increasing. Although the pathophysiology of age-related CKD is likely due to multiple factors, the potential role of phosphate, which accelerates aging, has been recently attracting attention. In particular, the modern captive dolphin society is facing a super-aging population, and cases of deaths with declining kidney function have been often reported. In thus study, we aimed to investigate whether phosphate contributes to the pathogenesis of CKD in dolphins.

## Methods

Renal necropsy tissue of an aged captive dolphin was analyzed. Kidneys were removed and a computed tomographic (CT) scan and micro-CT scan were performed. Renal tissues were dissected. Dried tissues were also analyzed with microbeam X-rays and infrared absorption (IR) spectroscopy. In addition, *in vitro* experiments were performed in cultured dolphin proximal tubular (DolKT-1) cells. Cell viability, calcification, cytotoxicity, apoptosis, and mitochondrial function were quantified by the WST-1 assay, von kossa staining, LDH release, annexin V/propidium iodide double staining, and mitochondrial oxygen consumption, respectively. We evaluated calciprotein particles (CPPs) formation by measuring the absorbance.

## Results

An older dolphin in captivity died of myocarditis and other causes, but its renal function was within the normal range until shortly before death. In renal necropsy tissue, obvious glomerular and tubulointerstitial changes were not observed with hematoxylin and eosin staining. However, a CT scan and micro-CT scan showed many high-density areas in the medullary portion, which could be considered calcification. Von Kossa-positive staining was also observed in renal medullary tissue. Micro area X-ray diffractometry and IR spectrometry showed that the high-density calcified areas were primarily composed of hydroxyapatite. *In vitro* experiments showed that treatment with phosphate and

calciprotein particles (CPPs) resulted in cell viability loss and LDH release, but did not affect cell apoptosis and mitochondrial function in DolKT-1 cells. However, treatment with magnesium markedly attenuated the cellular injury induced by phosphate, but not by CPPs. Von Kossa staining showed that phosphate dose-dependently increased calcium deposition in DolKT-1 cells. Dense calcification was observed after 2 mM phosphate exposure for 3-7 days which was completely abolished by the addition of magnesium. Finally, we evaluated CPP formation by measuring the absorbance when concentrations of high phosphate (5 mM), high calcium (6 mM), and high magnesium (2 or 5 mM) were mixed together in the presence of fetal bovine serum. The 5 mM phosphate + 6 mM calcium group showed increased CPP formation from the initial time points and gradually increased over time. At each time point, magnesium significantly decreased CPP formation in a dose-dependent manner.

**Conclusions**

In conclusion, we analyzed necropsy renal tissue from an older dolphin with normal renal function and found that the medullary portion of reniculi tissue had marked calcified lesions due to calcium phosphate (hydroxyapatite) accumulation. Further *in vitro* experiments in cultured dolphin tubular cells showed phosphate-damaged tubular cells through the formation of CPP. The Inhibition of CPP formation by magnesium administration significantly attenuated the phosphate-induced dolphin tubular cell injury. These data suggest that dolphins are at increased risk of developing CKD due to phosphate accumulation in the kidney as they age.



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（備考）論文要旨は，日本語で１，５００字以内にまとめてください。
(Recital) Sum up the within １５００ letters.