学位論文の要約

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Evaluation of the lead exposure situation in wild birds and identification of novel avian renal biomarkers

（国内に生息する鳥類における鉛汚染状況の解明と、鳥類種横断的に利用可能な新規腎障害マーカーの探索）

A decreasing number of birds is a serious problem worldwide, according to the Red List published by IUCN (International Union for Conservation of Nature) in 2017. Infectious diseases and habitat loss can be cited as some causes of this reduction in the number of birds, and chemical contamination is also a notable problem. Damage caused by pollution with metals, oil, and agricultural chemicals has been reported all over the world, and chemical contamination sometimes leads to mass deaths. In this study, I focused on lead (Pb) exposure in wild birds in Japan, and the necessity of novel renal biomarkers in birds.

Pb poisoning in raptors and waterbirds is an environmental pollution problem in Japan. Pb rifle bullets, Pb shot pellets, and fishing tackle are the main causes of Pb exposure in birds. Raptors mainly ingest fragments of Pb rifle bullets or shot pellets when consuming animals killed by hunters. Waterbirds also tend to accidentally ingest Pb from shot pellets or fishing sinkers when they swallow pebbles as gastroliths. Pb exposure causes neurological dysfunction, hematopoietic system dysfunction, immune suppression,

reproductive impairment, and with accumulation of Pb at very high levels, it eventually leads to death. The objective of this research was to evaluate Pb exposure in wild birds. The results showed that Pb contamination damage in birds occurred throughout the country. Pb stable isotope ratios showed that illegal use of Pb ammunition is still occurring in Hokkaido. Furthermore, in the areas of Honshu and Shikoku, where regulation of the use of Pb was restricted to only a limited number of areas and Pb exposure was not regarded as much of a problem, raptors such as the endangered golden eagle were exposed to Pb. In waterbirds, Pb shot was found inside the stomach, and there were high concentrations of Pb accumulated in the liver. Furthermore, as a result of analysis of Pb distribution in bone, it became clear that the Pb accumulation process differs depending on bone structure and the presence or absence of bone marrow. This study showed that bone specimens have valuable information about Pb exposure and might be useful in investigating and understanding mortalities suspected to be related to Pb poisoning.

In addition to Pb, there are many chemical substances, such as anti-inflammatory drugs and anti-cancer drugs, that cause kidney damage in birds. Birds have a special vascular system called the renal portal vein, where the renal blood flow rate is abundant, and the kidney is exposed to high amounts of chemicals via the blood. Renal disease due to these nephrotoxic chemical substances has been reported in many wild birds, but there is currently no early biomarker for kidney injury. Therefore, the objective in this study was to identify novel renal biomarkers in avian species. Acute kidney injury (AKI) includes acute interstitial nephritis (AIN) and acute tubular necrosis (ATN). Two different types of kidney injury models in chickens were created using nephrotoxic chemicals (diclofenac, which causes AIN, and cisplatin, which induces ATN). Kidney-specific disorders were confirmed by blood biochemical test and pathological examination, and in order to select nephropathy

marker candidates, analysis of plasma *N*-glycans (comprehensive analysis using the glycoblotting method) and transcriptome analysis of kidney samples (selection of candidate genes by exhaustive analysis using microarrays and quantitative real-time PCR analysis of each gene) were carried out.

The result of comprehensive glycan analysis in the search for biomarkers showed that there was an increase in glycan expressions after administration of 14 kinds of *N*-glycans in the diclofenac group. Many of these glycans are sialylated and have a non-fucosylated structure, and five of them are confirmed to have increased expression in human renal carcinoma. However, in the cisplatin-administered group, no changes in glycans were observed, and it is thought that differences in glycan expression appeared due to differences in diseased sites in the kidney. These results suggest that glycans may be useful as markers for AIN.

From the result of microarray analysis, 414 genes were up-regulated and 79 genes were down-regulated commonly in diclofenac- and cisplatin-treated chickens, and several genes were determined to be early renal injury marker candidates, reflecting both ATN and AIN.

In conclusion, there are many threatened species of birds, and many of them are dying due to the influences of human activity. Pb exposure in birds is one of those problems still occurring in the world, including Japan. In Hokkaido, damaged individual birds have appeared even after regulation of the use of Pb ammunition, and since damage has also occurred in other regions in Honshu and Shikoku, where surveys are extremely limited, Japan-wide investigation into birds' exposure to Pb and consideration of strengthening regulations on the use of Pb ammunition are required. At the same time, it is important to convey information on Pb contamination in birds and on the effectiveness of non-Pb bullets

to many citizens to reduce Pb exposure in avian species.

Regarding the identification of novel biomarkers, especially in avian species, translational research, which delivers original investigations from laboratories to clinical practices, is extremely limited worldwide. If a novel kidney injury marker is identified in birds and a simple kidney injury diagnosis kit is developed, it will be useful in treating early-stage renal disorders, monitoring treatment with medicines (such as therapeutic drugs for Pb poisoning) that cause nephrotoxicity as a side effect, and evaluation of environmental contamination by chemical substances that cause kidney damage. Thus, identification of an early biomarker for kidney injury can contribute to avian medicine and conservation of wild birds.