RF-085 A study on the bioaccumulation of mercury using the ecosystem of Yambaru area as a trial model（Abstract of the Interim Report）

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Total Budget for FY2008 9,100,000Yen

Key Words Mercury, bioaccumulation, Yambaru area, Primary hepatocyte culture system, Gene expression analysis

[Abstract]

Javan mongooses inhabiting in the forest region “Yambaru area” located in northern part of Okinawa island, Japan accumulated high level of mercury in their bodies. This fact suggested that possibility of high mercury exposure in this area. This study focuses on the understanding the distribution and dynamics of mercury in the ecosystems of Yambaru area using the two types of approaches aiming to elucidate mercury concentration mechanisms in the Javan mongoose which accumulated mercury with high levels.

One is the approach using the liver which is the main organ accumulating mercury in Javan mongoose bodies. We established a system for primary hepatocyte culture in Javan mongoose, which is based on methods of continuous recirculating perfusion of the rat liver. We could successfully apply autometallographic (AMG) technique to histological detection of mercury. We determined IC_{50} values for the hepatocytes from Javan mongooses and a rat in the response to various heavy metalsby MTT assay and explored key molecules of Hg metabolism and detoxication in Javan mongooses.

Another is the approach using mercury analysis of the animals distributing Yambaru area. Our results suggested that the background level of mercury in Yambaru area is relatively high when compared with mercury levels in the bodies of low tropic animals inhabiting in other areas. In addition, we also suggested that rare birds such as Okinawa woodpecker, Ryukyu robin and Okinawa rail and rare amphibia, Anderson's crocodile newt, accumulate mercury with relatively high concentration, and they take the major role of supply mercury to Javan mongoose.

1. Introduction

Javan mongoose inhabiting the northern part of Okinawa island called “Yambaru area” accumulates mercury (Hg) with high level in its organs and tissues, especially in the liver [1)]. It is well known that some marine mammals such as small cetaceans and sea birds such as albatross accumulated Hg with high levels which exceed the hazardous value for human. This concentration mechanism of Hg relates to Se that is a essential element for animals. However, because of the difficulties of duplicate experiment using the marine mammals and endangered sea bird species, this detoxification process of Hg relating Se is still unclear. Javan mongooses have a potentials to elucidate this subject of wildlife. This possibility might be the applied to identification of ecological risks of Hg in wildlife animals. In addition, they suggested that the Yambaru area is exposed the Hg with high level from atmospheric route or, at least, this area has certain amount of Hg and these amounts of Hg circulate in the ecosystems of Yambaru area.

2. Research Objective

This study aims to elucidate the mechanisms of Hg concentration in the Yambaru area and Javan mongoose which is a top predator and a symbol of biomagnification process of Hg in this ecosystem. Therefore, this study focuses on the understanding the distribution and dynamics of mercury in the ecosystems of Yambaru area using the two types of approaches aiming to elucidate mercury concentration mechanisms in the Javan mongoose which accumulated mercury with high levels. One is the approach using the liver which is the main organ accumulating mercury in Javan mongoose. We tried to establish a system for primary hepatocyte culture in Javan mongoose and determined IC_{50} values for the hepatocytes from Javan mongooses. In addition, we also explored key molecules of Hg metabolism and detoxication in Javan mongooses. Another is the approach using mercury analysis of the animals distributing Yambaru area.

3. Results and discussion

Approach 1) Using the liver which is the main organ accumulating mercury in Javan mongoose

We have established a system for primary hepatocyte culture in Javan mongoose, which is based on methods of continuous recirculating perfusion of the rat liver [2)]. The isolated primary hepatocytes are comparable to normal hepatocytes in appearance. The system allows us to perform reproducible experiments, such as mercury resistance test and mercury metabolism test *in vitro*.

Major organs (liver, lung, spleen, kidney, adrenal gland and salivary gland) in Javan mongoose were histologically investigated by hematoxylin and eosin staining, von Kossa staining, Berlin-Blue staining, and periodic acid-Schiff staining. Focal inflammatory lesions with mononuclear cell infiltration were identified in the liver and kidney, where large amount of mercury accumulation was determined with a cold vapor system [1), 3)]. No other particular changes were found.

We could successfully apply autometallographic (AMG) technique [4)] to histological detection of mercury. Mercury was localized at centrilobular region in the liver and at proximal tubule in the

kidney. The results suggest that mercury is metabolized at some localized regions in the liver and kidney in Javan mongoose.

To elucidate the resistance mechanism to mercury and other heavy metals in Javan mongooses, we determined IC_{50} values (the dose which kills 50% cells) for the hepatocytes from Javan mongooses and a rat in the response to various heavy metals. These hepatocytes were exposed to MeHg (1, 5, 10, 15, 20, 30, 50 μM), $HgCl_2$ (5, 10, 40, 100, 200, 300, 500 μM), and Se (5, 10, 40, 100, 200, 300, 500 μM) for 24 hours, and then these of IC_{50} values were determined by MTT assay. IC_{50}s of Javan mongooses and rat hepatocytes for MeHg, $HgCl_2$ and Se were 5.04 μM and 9.02 μM, 22.1 μM and 61.6 μM, and 67.2 μM and 153 μM respectively. Unexpectedly, hepatocytes from the Javan mongooses were more sensitive to Hg and Se than that from the rat. We also estimated the heavy metal concentration in the livers and found that Hg and Se accumulations were higher in the livers of Javan mongooses than in that of the rat. However, as the Hg concentrations in the livers of Javan mongooses in this experiment were lower than those in the previous experiments, we need assess the tocixity of Hg to hepatocytes from high Hg accumulated Javan mongooses.

We explored key molecules of Hg metabolism and detoxication in Javan mongooses. The genes which respond to metal exposure, have potential roles in the protection of cell against the metal toxicity [3]. We therefore made primer sets for detection to Javan mongoose Thioredoxin reductase 2 (TrxR2). TrxR2 gene and mRNA were detected from some organs and cells (fibroblast, testis and ovary) in Javan mongoose with these primers by PCR analysis.

For understanding the Hg concentration process in the liver of Javan mongoose, excrete routes were also determined and evaluated. One excrete rout is a molting. Mercury concentrations in the hair from Javan mongoose are relatively lower when compared with those from Japanese weasel inhabiting Zamami island near Okinawa Island. Japanese weasel is one of top predators in this island as same as Javan mongoose in the Yambaru area. Mercury levels in its organs and tissue such as liver, kidney and muscle of Japanese weasel were lower than those of Javan mongoose. Therefore, it is concluded that the amount of excreted Hg from Javan mongoose is small. Another route of Hg excretion was assumed as delivering. Only one pair (mother and fetus) of Javan mongoose was analyzed. Relatively high concentrations of Hg were observed in the organs and tissues of fetus. At this case, mercury levels of mother also high when compared with other female Javan mongoose. This phenomenon suggested that old female accumulating high levels of Hg deliver the neonatal infant who also accumulates Hg with high level and fetus of this female experience quite high risk of Hg in mother's uterus.

Approach 2) Using mercury analysis of the animals distributing Yambaru area

Relatively high levels of Hg were determined in the bodies of low tropic animals inhabiting Yambaru area when compared with unpolluted ecosystems [5]. Observed value of Yambaru was comparable to polluted area by Hg [5]. Especially, large centipede species which are the strong carnivore of invertebrates accumulated Hg with high concentration due to their high tropic in food web. In the same way, some species of raptors and carnivorous snake inhabiting forest accumulated Hg in their bodies. These facts supported that biomagnification of Hg exists in the ecosystem of

Yambaru area. Interestingly, rare animals in the Yambaru area such as Anderson's crocodile newt as amphibians, Okinawa woodpecker, Ryukyu robin and Okinawa rail as birds accumulated Hg with higher levels when compared with other animals niching at the same tropic levels. It is known that they prey certain invertebrate such as snail and earthworms. In this study, detail investigation about snail and earthworms of this area is not yet done. However, to elucidate the dynamics of Hg in the ecosystem of Yambaru area, these types of analyses are required.

From analysis of inter-elemental relationships using concdentration data of 25 trace elements in the animal bodies collected from Yambaru area, some three elements such as lead (Pb), cadmium (Cd) and silver (Ag) and five essential elements (however, they are used as various industrial materials) such as selenium (Se), chromium (Cr), vanadium (V), nickel (Ni) and cobalt (Co) and one trace element, strontium (Sr) were correlated with Hg in the various animal group such as invertebrate, amphibians, reptilian, omnivorous birds, carnivorous birds, omnivorous mammals, and carnivorous mammals. Especially, Se that acts important role for detoxification of Hg, Cd and Cr as pollutants and Sr showed strong correlation with Hg in the higher tropic groups such as carnivorous birds and mammals. In contrast, Pb, Ag, Co and Ni showed clear correlation in the lower tropic animal groups such as invertebrate, amphibians, reptilian, omnivorous birds and mammals. Only V (vanadium) showed clear correlation with Hg throughout all tropic levels. These inter-elemental relationships suggested that these toxic and industrial elements might have the same source as Hg; however dynamics in the ecosystem and food web is different due to their characteristics.

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Major Publications

None.