
Monitoring of the sea (Tonhe) pollution with the use of biological samples from the stranded cetaceans and crab

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【ABSTRACT】

There have been few reports on the survey of pathological findings of cetaceans stranded on the coast of Japan. This is partly because of lack of the procedures and/or network of systemic sampling of fresh tissues for the pathological study of stranded marine mammals. In contrast, there are a numbers of reports on the cause of illness and death in wild, free-living cetaceans examined in other countries; the commonest cause of death was parasitic and bacterial pneumonia except for entanglement in fishing gear.

Anthracosis, lung and hilar lymph nodes polluted by suspended particulate matter in the air, has been recently found in some cetaceans stranded on the coast of Japan. In addition to the data from the chemical analysis of tissues, scientific data obtained from pathological study of stranded marine mammals would be also one of the useful base for the assessment of global environment. Usefulness of metallothionein in the hepatopancreas of crabs as a biomarker of marine pollution monitoring was also discussed in this study.

【Introduction】

There is a variety of potential biomarkers in marine pollution monitoring; special attention has been given to metallothionein (MT) induction, acetylcholinesterase inhibition, cytochrome P450 system induction, imposex, lysosomal enlargement and lysosomal membrane destabilisation, and peroxisome proliferation. These biomarkers can be used to evaluate exposure to and effect of different contaminants (metals, organic xenobiotics and organometallic compounds). It is necessary to include analysis of standard reference materials and to obtain detailed knowledge of basal values and seasonal variations of the biomarkers in various species, as well as to integrate the information obtained with the different biomarkers.

The number of the reports on the pathological study of marine mammals living in the heavily polluted marine environment are limited; most of which are from Europe and North America^{1,2}. This is partly because of the difficulty in getting fresh samples suitable to the pathological examination from the field where the animals are found dead (Fig. 1); cetacean stranding network for the scientific purpose is yet to be established in the world including Asia. The network is now being organized in Japan. We found anthracosis in the lung and hilar lymph node of two cetaceans stranded at the coast of Japan Sea in 1999. Additional 5 cases with a sign of anthracosis were found stranded at the Japanese sea coast. The findings may be a possible biomarker of the transboundary air pollution.

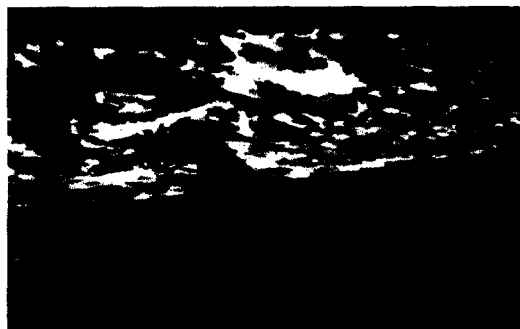


Fig. 1. Stranded dolphin

【Materials and Methods】

1. Study of lung and hilar lymph nodes from mammals

Lung and hilar lymph nodes from 7 cetaceans (5 *Mesoplodon stejnegeri*, *Lagenorhynchus obliquidens*, *Kogia breviceps*) stranded at the coast of Japan Sea. Controls (comparison): lung and hilar lymph nodes from dogs, cattle and raccoon dogs. Histological examination of the tissues: HE, Autometallography for Mercury, metallothionein immunohistochemistry.

Metal analysis: scanning electron microscopy-x line microanalyzer, inductively coupled plasma (ICP) atomic emission spectrometry, frameless atomic absorption spectrophotometry (AAS).

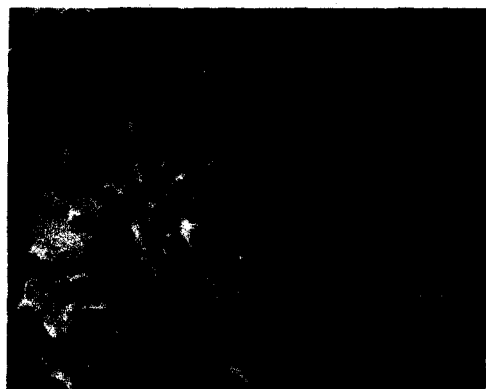


Fig. 2. Macrophages in the hilar lymph node showing ingested air born particulate matter. H.E. stain.
x 800.

2. Study of hepatopancreas from crab (*Portunus pelagicus*)

Mercury (HgCl_2) injection to crab. Pathological examination. Metal analysis: inductively coupled plasma (ICP) atomic emission spectrometry, frameless atomic absorption spectrophotometry (AAS). MT analysis: Cd^{2+} - Hg^{2+} saturation method

【Results】

Study 1

On necropsy, moderate degree of anthracosis was observed in the hilar lymph nodes of both cetaceans. Histological examination demonstrated mild to moderate accumulation of black particles in the lung and hilar lymph nodes. Higher magnification clearly showed a large amount of black particles in the cytoplasm of macrophages (Fig. 2).

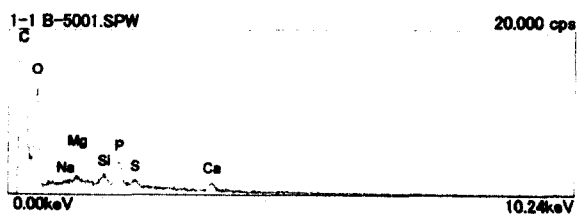


Fig. 3. Elements in a macrophage in the hilar lymph node from a stranded dolphin (case 1). Scanning electron microscopy-x line microanalyzer analysis.

Substantial number of mercury-positive granules were also demonstrated by the autometallography. Mercury and cadmium were detected in the hilar lymph nodes from the two cetaceans; aged dogs also showed similar changes. A variety of elements including P and Ca were demonstrated by the scanning electron microscopy-x line microanalyzer analysis (Fig. 3).

Study 2

Exposure of coastal crab to mercury led to an increase of MT in the hepatopancreas; amount of MT induced in the hepatopancreas differed among the animals. Pathological study revealed degenerative and necrotic changes in a variety of organs including hepatopancreas, gill and ovary. Mercury granules were demonstrated in the cytoplasm of epithelial cells of hepatopancreas from both captured and experimentally exposed animals with the use of autometallography method.

【Discussion】

Suspended particulate matter (SPM, particles smaller than 10 μm in diameter) in the air can be easily inhaled by way of the air pathway of mammals. Particles get to the alveoli are trapped by alveolar macrophages.

The macrophages containing a large amount of SPM accumulate in the hilar lymph nodes with time (age). Data from the chemical analysis of these accumulated SPM in the hilar lymph node, thus, may reflect the history of air pollution of the environment where the animal lived³.

The amount of toxic metals (mercury, lead and cadmium) released to the air is increasing with human activities, especially as a result of energy consumption (coal, gasoline)³. In China, more than 70 % of energy source for both industry and domestic use is crude coal, which is known to contain high level of mercury. SPM containing these pollutants may move from the continent to the other continent by seasonal wind (transboundary air pollution).

In this study, anthracosis with accumulation of toxic metal was observed in the hilar lymph nodes of the wild cetaceans migrating in the Japan Sea, where air pollution has not been concerned. Examination (pathology, biochemistry) of lung and hilar lymph nodes from the cetacean and domestic animals including dogs may be a suitable method for the monitoring the world wide air pollution⁴.

As to the experimental study with the use of crab, natural factors influencing MT synthesis have to be taken into account

when hepatopancreatic MT of crabs are used aiming a potential bioindicator of the metallic contamination of the marine environment. We have recently started international cooperative research for this purpose.

References

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