プラスチック由来の有害化学物質(スチレン)の魚類に対する影響評価

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Umi KAWAGO, Nobuo SUZUKI: Evaluation of the plastic-derived toxic chemicals (styrene) on fish

BACKGROUNDS

About 9 billion tons of plastic products are produced worldwide. However, only 9% of plastic waste is recycled; the rest is thrown away and becomes marine waste. As a result of plastic being broken into small fragments by ultraviolet rays and waves, it becomes microplastic particles in the marine environment. It has been believed that microplastics cannot decompose in the low-temperature environment of the ocean. However, it has been reported that microplastics can degrade at low temperatures and actually present in the ocean as styrene oligomers (especially a styrene trimer) (Fig. 1) (Amemiya et al., 2020). It is highly likely that these styrene oligomers affect marine organisms.

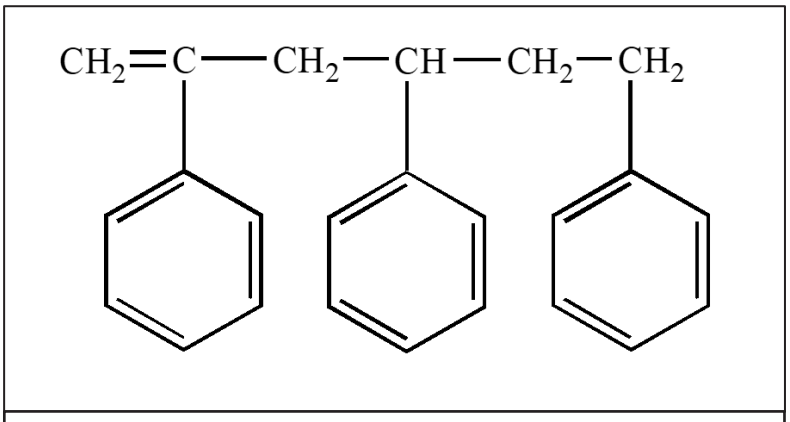

Fig. 1: Chemical structure of 2,4,6-triphenyl-1-hexene used in the present study as a styrene trimer.

On the other hand, it is well-known that fish scales have osteoblasts (bone-formation cells) and osteoclasts (bone-resorption cells) (Suzuki et al., 2008). The coexistence of osteoblasts and osteoclasts in a calcified bone matrix makes the scale a suitable model for analyzing the response of bone cells to environmental pollutants (Suzuki et al., 2008). It has been reported that bisphenol-A suppressed osteoblastic and osteoclastic activities, although estrogen enhanced both osteoblastic and osteoclastic activities (Suzuki and Hattori, 2003). Therefore, it is possible that styrene oligomers disrupt bone metabolism in fish as bisphenol-A did. In the present study, the endocrine-disruptive effects of styrene oligomers on bone metabolism in fish were examined and compared with those of estrogen.

METHODS

*Analyses of osteoblastic and osteoclastic marker enzyme activity and mRNA expression (*in vitro*)*

Regenerating scales were taken from goldfish (*Carassius auratus*) under anesthesia. The scales were incubated for 6 h at 15°C in Leibovitz's L-15 medium containing 100 μg/L styrene oligomers. After incubation, osteoclastic and osteoblastic marker enzyme activities (osteoclasts: tartrate-resistant acid phosphatase activity; osteoblasts: alkaline phosphatase activity) in the styrene oligomer–treated scales were measured as described in

Suzuki and Hattori (2003).

In the case of mRNA expression analysis, regenerating scales were also extracted from goldfish under anesthesia. After incubation of regenerating scales under the above conditions, the styrene oligomer–treated scales were immediately frozen and kept at -80°C. Thereafter, total RNAs were prepared from goldfish scales using a total RNA isolation kit. Then complementary DNA was synthesized with a kit. Using these cDNAs, the osteoblastic and osteoclastic marker mRNA expression was analyzed using a real-time PCR apparatus.

Oral injection of a styrene oligomer into goldfish (in vivo)

Goldfish identified individually by fin clipping were anesthetized and their weights measured. A styrene oligomer was orally administered to each goldfish (1 μg/g body weight) under anesthesia. Blood samples from the caudal vein were taken after 12 and 24 hours. The collected blood was centrifuged, and the calcium concentration in the separated plasma was measured using a kit (FUJIFILM Corporation, Osaka, Japan).

RESULTS AND DISCUSSION

In the scale *in vitro* bioassay, the action of the styrene trimer was found to be stronger than that of other oligomers. It was found that the activities of osteoblasts and osteoclasts significantly increased at concentrations of 10 μg/L and 100 μg/L of the styrene trimer, as with estrogen (Suzuki and Hattori, 2003). Particularly, a significant difference was obtained in the activity of osteoclasts exposed to 100 μg/L of styrene trimer. Therefore, our experiment focused on the action of the styrene trimer.

The results of qPCR for OPG and DKK1, markers of osteoblasts, showed significant upregulation by styrene trimer (100 μg/L) treatments. NFATc-1 and CathK, markers of osteoclasts, were significantly increased by styrene trimer (100 μg/L) treatments. Therefore, the styrene trimer activated both osteoblasts and osteoclasts in the scales of goldfish.

In an *in vivo* experiment, furthermore, the plasma calcium concentration was measured after a styrene trimer was orally administered to goldfish (each 1 μg/g body weight). As a result, the plasma calcium concentrations were significantly increased at 12 and 24 hours after injection.

Taking these results into consideration, the osteoclasts were activated by the styrene trimer using the fish-scale assay system. This activation of osteoclasts seems to induce an elevation of plasma calcium levels and disrupt calcium metabolism in goldfish. To elucidate the detailed mechanism of the styrene trimer in osteoclasts, we are planning to examine a comprehensive analysis using RNA sequencing.

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