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Nobuo SUZUKI: Gadolinium suppresses both osteoclastic and osteoblastic activities in the scales of goldfish

Background

Gadolinium (Gd) is a ductile rare-earth metal. Gd^{3+} is currently used in magnetic resonance imaging for clinical diagnoses because Gd^{3+} has paramagnetic properties (Möller *et al*., 2002). To avoid the toxicity of Gd^{3+}, chelated forms, known as Gd-based contrast agents (Gd-CAs), have been used (Möller *et al*., 2002; Telgmann *et al*., 2013). In general, Gd-CAs are stable complexes. The agents are rapidly eliminated from a patient's body. After excretion, they enter the public sewer and, subsequently, the wastewater treatment plant. Because of their polar or anionic nature, however, the Gd complexes most likely are neither adsorbed onto surfaces nor by particulate organic matter (Knappe *et al*., 2005) but are released into environmental water without a specific recycling process (Telgmann *et al*., 2013). Therefore, a significant amount of anthropogenic Gd-concentration in surface waters has been reported worldwide (Möller *et al*., 2002; Telgmann *et al*., 2013). It is possible that the anthropogenic Gd impacts aquatic animals. Gd appears toxic in animals because Gd functions as a blocker of Ca channels, causing its ionic radius to be nearly equal to that of Ca (Sherry *et al*., 2009). Therefore, we examined the effects of Gd in fish bone metabolism.

Methods

Female goldfish (*Carassius auratus*) were purchased from a commercial source (Higashikawa Fish Farm, Yamatokoriyama, Japan) and used in the scale in vitro bioassay. In addition, all experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of Kanazawa University.

The culture medium was prepared. First, we added 1% penicillin-streptomycin mixture (ICN Biomedicals Inc., Aurora, OH, USA) and HEPES (Research Organics, Inc., Cleveland, OH, USA) (20 mM) to Earle's Minimum Essential Medium (MEM; ICN Biomedicals Inc.). After filtration, the MEM was used in this experiment.

After preparation of the culture medium, goldfish were anesthetized with ethyl 3-aminobenzoate, methanesulfonic acid salt (Sigma-Aldrich, Inc., St. Louis, MO, USA) and the scales on both sides of the body were then removed. The collected scales were incubated in MEM supplemented with gadolinium acetate ($Gd(OCH_3CO)_3 4H_2O$) (Wako Pure Chemicals, Osaka, Japan) (10^{-15} to 10^{-6} M) and compared with Gd-free medium as a control. The incubation time and temperature were 6 hours and 15°C, respectively. We have reported the toxicity of Cd at 15°C at 6 hours of incubation (Suzuki et al., 2004). Therefore, these culture conditions were adopted in the present study. After incubation, scales were fixed in 10% formalin in a 0.05 M cacodylate buffer (pH 7.4) and then rinsed in distilled water. These scales were kept in a 0.05 M cacodylate buffer at 4°C until analysis. Then, tartrate-resistant acid phosphatase (TRAP) for osteoclasts and alkaline phosphatase (ALP) for osteoblasts were measured by the methods of Suzuki et al. (2004).

Results

Gd inhibited TRAP activity at 6 hours of incubation. Gd significantly suppressed TRAP activity. In 3 goldfish used in the present study, the detection limit of Gd was 10^{-13}, 10^{-11}, and 10^{-11} M, respectively.

ALP activity was significantly suppressed by Gd, although the Gd sensitivity was lower than in TRAP. In the 2 goldfish used in the present study, the detection limit of Gd was 10^{-9} and 10^{-10} M.

Discussion

This is the first report to indicate the toxicity of Gd on fish bone metabolism using TRAP and ALP enzyme activities. The scales of some teleosts are a better potential internal calcium reservoir than vertebral bone during periods of increased calcium demand (Bereiter-Hahn and Zylberberg, 1993). Therefore, we believe that fish scales are a suitable bone model for the analysis of environmental pollutants. Furthermore, we demonstrated that Gd quite sensitively inhibited TRAP activity. Even Gd of 10^{-13} M suppressed TRAP activity at 6 hours of incubation. Therefore, our assay system is quite effective as a biosensor for Gd.

At 6 hours of incubation, very low concentrations of Gd (10^{-10} and 10^{-9} M) influenced osteoblasts and suppressed osteoblastic activity. Therefore, the toxicity of Gd to osteoblasts appears to be higher than that of Cd, MeHg and InHg (Suzuki et al., 2004; Suzuki et al., 2011). Heavy metals such as Cd, MeHg and InHg were resistant to each metal as a result of the production of metallothionein (MT), which is a metal-binding protein that protects an organism from heavy metals (Suzuki et al., 2004; Suzuki et al., 2011). Because the mRNA expression of MT in Cd-, MeHg- and InHg-treated goldfish scales was increased, osteoblastic activity did not change at 6 hours of incubation (Suzuki et al., 2004; Suzuki et al., 2011). Thereafter, osteoblastic activity was inhibited by Cd (10^{-7} M), MeHg (10^{-7} M) and InHg (10^{-6} to 10^{-4} M) at 36 or 64 hours of incubation (Suzuki et al., 2004; Suzuki et al., 2011). Therefore, the toxicity of Gd to osteoblasts may be related to MT expression.

Gd was present in recycled water in which the stable organic Gd complexes pass through several sewage treatment plants without being significantly decomposed (Knappe et al., 2005). Therefore, anomalously high concentrations of Gd in surface waters are of anthropogenic origin (Rogowska et al., 2018). Specifically, anthropogenic Gd was detected in surface waters (up to 1,100 ng/L) and sediments (up to 90.5 μg/g) (Rogowska et al., 2018). Therefore, aquatic plants, fungi, small planktonic crustaceans, and freshwater fish (*Cyprinus carpio*) were able to take up anthropogenic Gd from the polluted water and it accumulated in their bodies (Rogowska et al., 2018). Furthermore, in the present study, we demonstrated that low levels of Gd (10^{-13} to 10^{-11} M) have toxicity for bone metabolism in goldfish. Thus, we strongly believe that anthropogenic Gd has toxicity for aquatic animals and we must consider a Gd risk assessment to protect the polluted aquatic environment.

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