Original Research Paper

Gadolinium at Low Concentration Suppresses both Osteoclastic and Osteoblastic Activities in the Scales of Goldfish

[1]Nobuo Suzuki, [2]Kazuki Watanabe, [1]Aika Sekimoto, [1,3]Makoto Urata, [1,4]Mohamed Ibrahim Zanaty, [1]Toshio Sekiguchi, [1]Yoichiro Kitani, [5]Hajime Matsubara, [6]Ajai Kumar Srivastav and [2]Atsuhiko Hattori

[1]*Noto Marine Laboratory, Institute of Nature and Environmental Technology,*
Kanazawa University, Ogi, Noto-cho, Ishikawa 927-0553, Japan

[2]*Department of Biology, College of Liberal Arts and Sciences, Tokyo Medical and Dental University,*
Ichikawa, Chiba 272-0827, Japan

[3]*Institute of Noto SATOUMI Education Research, Ogi, Noto-cho, Ishikawa 927-0553, Japan*

[4]*Biotechnology and Life Sciences Department, Faculty of Postgraduate Studies for Advanced Sciences,*
Beni-Suef University, Beni-Suef, Egypt

[5]*Noto Center for Fisheries Science and Technology, Kanazawa University, Ossaka, Noto-cho,*
Ishikawa 927-0552, Japan

[6]*Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur 273-009, India*

Abstract: For determining the effect of environmental pollutants on fish bone metabolism, we have developed an *in vitro* bioassay system using teleost scales that has osteoclasts, osteoblasts and bone matrix as markers: alkaline phosphatase (ALP) for osteoblasts and tartrate-resistant acid phosphatase (TRAP) for osteoclasts. Using this bioassay, the influence of gadolinium (Gd) on osteoclasts and osteoblasts of goldfish scales was examined in the present study. Gd sensitively inhibited TRAP activity. Even Gd at 10^{-13} M suppressed TRAP activity at 6 hours of incubation. At 18 hours of incubation, this inhibition occurred only at 10^{-7} and 10^{-6} M. After 36 hours of incubation, Gd did not influence TRAP activity. In osteoblasts, ALP activity was also suppressed by Gd in the range of 10^{-10} to 10^{-6} M for 6 to 18 hours of incubation. At 36 and 64 hours of incubation, ALP activity was significantly suppressed by Gd (36 hours: 10^{-9} to 10^{-6} M; 64 hours: 10^{-7} and 10^{-6} M). At 96 hours of incubation, however, Gd did not influence ALP activity. This is the first report to indicate the toxicity of Gd on fish bone metabolism using TRAP and ALP enzyme activities. The toxicity of Gd to osteoblasts is comparable to that of tributyltin, an aquatic environmental pollutant used as a biocide in anti-fouling paint. Gd is used in Magnetic Resonance Imaging (MRI) for clinical diagnoses. To avoid the toxicity of Gd ions, chelated forms, known as Gd-based contrast agents, are used for MRI diagnosis. Without a specific recycling process, these compounds are quickly released by urinary excretion and released into environmental waters. Therefore, it is possible that anthropogenic Gd influences aquatic animals. Considering our present data together with that of anthropogenic Gd pollution, we should conduct a Gd risk assessment to protect the ecosystem in the aquatic environment.

Keywords: Gadolinium, Osteoclasts, Osteoblasts, Fish Scales, Goldfish

Introduction

Gadolinium (Gd) is a ductile rare-earth metal. Gd^{3+} is currently used in magnetic resonance imaging (MRI) for clinical diagnoses because Gd^{3+} has paramagnetic properties (Möller *et al.*, 2002; Birka *et al.*, 2016). 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; Braun *et al.*, 2018). Therefore, a significant amount of anthropogenic Gd-concentration in surface waters has been reported worldwide (Möller *et al.*, 2002; Telgmann *et al.*, 2013; Birka *et al.*, 2016; Braun *et al.*, 2018). 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). Actually, Gd influences calcified tissue such as sea urchin spicules (Saitoh *et al.*, 2010; Martino *et al.*, 2017; 2018). As Gd affects the skeletal formation of sea urchins, we presumed that Gd affects bone metabolism in fish.

We have developed an original *in vitro* bioassay system using goldfish scales that has osteoclasts, osteoblasts and bone matrix as markers: alkaline phosphatase (ALP) for osteoblasts and tartrate-resistant acid phosphatase (TRAP) for osteoclasts (Suzuki *et al.*, 2000; Suzuki and Hattori, 2002; 2003; Suzuki *et al.*, 2004). Using this *in vitro* bioassay, we have reported the toxicity of heavy metals such as cadmium (Cd) and mercury (Hg) on fish bone metabolism. We found that inorganic mercury (InHg) (10^{-5} to 10^{-3} M) (Suzuki *et al.*, 2011) and methylmercury (MeHg) (10^{-8} to 10^{-6} M) (Suzuki *et al.*, 2004) significantly inhibited the osteoclastic activity of goldfish scales. Cd responded very sensitively to osteoclasts. We demonstrate that even at 10^{-13} M, Cd suppressed osteoclastic activity in goldfish scales at 6 hours of incubation (Suzuki *et al.*, 2004). Thus, in the present study, we examined the effect of Gd on osteoclastic and osteoblastic activities with this goldfish scale *in vitro* bioassay.

Materials and Methods

Animals

In goldfish (*Carassius auratus auratus*), we have reported that sensitivity of osteoblastic and osteoclastic activities of scales to calcemic hormones (estrogen and calcitonin) was higher in mature females than in mature males (Suzuki *et al.*, 2000; Yoshikubo *et al.*, 2005). Therefore, female goldfish (n = 9, bw 30.33±1.71 g) 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.

Effects of Gd on Osteoclastic Activity in the Cultured Scales of Goldfish at 6 hours of Incubation

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$_3$CO)$_3$4H$_2$O) (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. We examined the effects of Gd on osteoclasts using TRAP enzyme activity because, in mammals, the effects of hormones and some bioactive substances on osteoclasts have been investigated using TRAP (Vaes, 1988; Suda *et al.*, 1999). Therefore, we used TRAP activity as an indicator of osteoclasts.

TRAP activity was measured as follows. Each scale was transferred to its own well in a 96-well microplate. An aliquot of 200 μL of 10 mM para-nitrophenyl-phosphate and 20 mM tartrate in a 0.1 M sodium acetate buffer (pH 5.3) was added to each well. This plate was then incubated at 20°C for 30 min while being shaken. After incubation, the reaction was stopped by adding 50 μL of 2 N NaOH. The 150 μL of colored solution was transferred to a new plate and the absorbance was measured at 405 nm. The absorbance was converted to the amount of para-nitrophenol (pNP) produced using a standard curve for pNP (Suzuki and Hattori, 2002).

Effects of Gd on Scale Osteoclastic Activity in goldfish Scales after Long-Term Culture

To examine the influence of Gd after long-term incubation, scales extracted from goldfish under anesthesia with ethyl 3-aminobenzoate, methanesulfonic acid salt (Sigma-Aldrich) were incubated in MEM with or without Gd for 18, 36, 64 and 96 h. After incubation, the

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, TRAP activity was measured as described above.

Effects of Gd on Osteoblastic Activity in the Cultured Scales of Goldfish at 6 Hours of Incubation

Goldfish scales were removed under anesthesia with ethyl 3-aminobenzoate, methanesulfonic acid salt (Sigma-Aldrich). Using these collected scales, we examined the effects of Gd on the osteoblasts with ALP as an indicator of osteoblasts (Dimai *et al.*, 1998; Suda *et al.*, 1999). The removed scales were incubated in MEM with or without Gd for 6 h. 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. The ALP activity was measured as follows.

Each scale was transferred to its own well in a 96-well microplate. An aliquot of 200 μL of 10 mM para-nitrophenyl-phosphate in an alkaline buffer (100 mM Tris-HCl, pH 9.5; 1 mM $MgCl_2$) was added to each well. Then, this plate was incubated at 20°C for 30 min while being shaken. After incubation, the reaction was stopped by adding 50 μL of 2 N NaOH. The 150 μL of colored solution was transferred to a new plate and the absorbance was measured at 405 nm. The absorbance was converted to the amount of pNP produced using a standard curve for pNP (Suzuki and Hattori, 2002).

Effects of Gd on Scale Osteoblastic Activity in goldfish Scales after Long-Term Culture

Scales were extracted from goldfish under anesthesia with ethyl 3-aminobenzoate, methanesulfonic acid salt (Sigma-Aldrich). Using these scales, we examined the effect of Gd during long-term incubation. The extracted scales were incubated in MEM with or without Gd for 18, 36, 64, and 96 hours. After incubation, the incubated scales were fixed in 10% formalin in a 0.05 M cacodylate buffer (pH 7.4) and then rinsed with distilled water. These scales were kept in a 0.05 M cacodylate buffer at 4°C until analysis. Then, ALP activity was measured as above described.

Statistical Analyses

All results are expressed as the means ± SE. The statistical significance between the control and experimental groups was assessed with one-way analysis of variance followed by Bonferroni's test. In all cases, the selected significance level was p<0.05.

Results

Effect of Gd on Osteoclastic Activity as Seen in the Cultured Scales of Goldfish at 6 Hours of Incubation

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} (Fig. 1a), 10^{-11} (Fig. 1b) and 10^{-11} M (Fig. 1c).
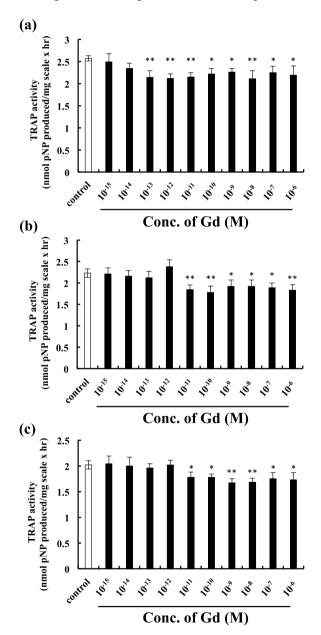

Fig. 1: Effects of Gd on osteoclastic activity in the cultured scales of goldfish at 6 h of incubation. Results are shown for 3 individual goldfish. * and ** indicate statistically significant differences at p<0.05 and p<0.01, respectively, from the values in the control scales.
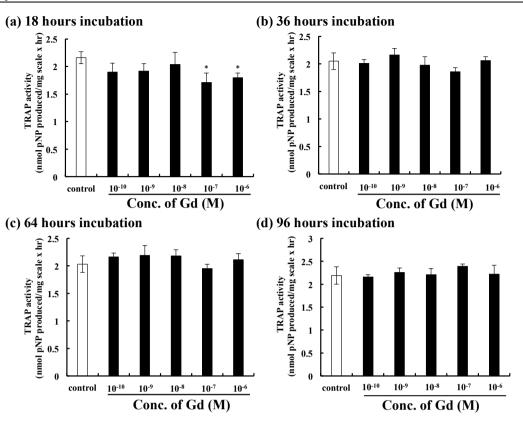
(a) 18 hours incubation   **(b) 36 hours incubation**

(c) 64 hours incubation   **(d) 96 hours incubation**

Fig. 2: Effect of Gd on osteoclastic activity after the long-term culture of goldfish scales. * indicates a statistically significant difference at p<0.05 from the values in the control scales.
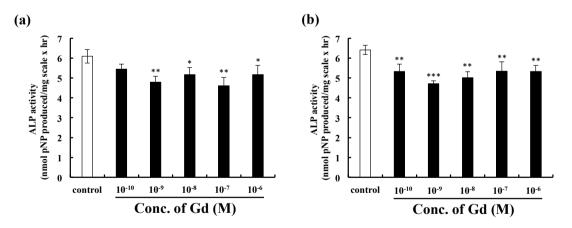
(a)   **(b)**

Fig. 3: Effects of Gd on osteoblastic activity in the cultured scales of goldfish at 6 hours of incubation. Results are shown for 2 individual goldfish. *, ** and *** indicate statistically significant differences at p<0.05, p<0.01 and p<0.001, respectively, from the values in the control scales.

Effect of Gd on Osteoclastic Activity in the Long-Term Culture of Goldfish Scales

At 18 hours of incubation, TRAP activity was significantly inhibited only at 10^{-7} and 10^{-6} M (Fig. 2a). After 36, 64 and 96 hours of incubation, Gd did not influence TRAP activity (Fig. 2b–d).

Effect of Gd on Osteoblastic Activity as Seen in the Cultured Scales of Goldfish at 6 Hours of Incubation

ALP activity was significantly suppressed by Gd (Fig. 3a and b), 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} M (Fig. 3a) and 10^{-10} M (Fig. 3b).
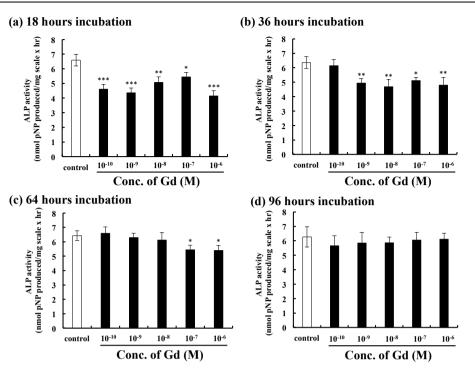
Fig. 4: Effect of Gd on the osteoblastic activity after the long-term culture of goldfish scales. *, ** and *** indicate statistically significant differences at p<0.05, p<0.01 and p<0.001, respectively, from the values in the control scales.

Effect of Gd on Osteoblastic Activity in the Long-Term Culture of Goldfish Scales

At 18 hours of incubation, Gd (10^{-10} to 10^{-6} M) significantly inhibited ALP activity (Fig. 4a). At 36 hours of incubation, ALP activity was significantly suppressed by Gd (10^{-9} to 10^{-6} M) (Fig. 4b). This inhibition was maintained at 64 hours of incubation. Gd (10^{-7} and 10^{-6} M) significantly suppressed ALP activity at 64 hours of incubation (Fig. 4c). However, at 96 h of incubation, Gd did not influence ALP activity (Fig. 4d).

Discussion

This is the first report to indicate the toxicity of Gd on fish bone metabolism using TRAP and ALP enzyme activities. In scales as well as mammalian bone, bone γ-carboxyglutamic acid protein (Nishimoto *et al.*, 1992), osteonectin (Lehane *et al.*, 1999) and osteocalcin (Thamamongood *et al.*, 2012) are present. Thus, the scales of some teleosts are a better potential internal calcium reservoir than vertebral bone during periods of increased calcium demand, such as sexual maturation and starvation (Mugiya and Watabe, 1977; 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. In the case of the sea urchin, Gd in a range of 10^{-7} to 10^{-5} M influenced skeletogenesis (Saitoh *et al.*, 2010; Martino *et al.*, 2017; 2018). We found that the detection limit of Gd in our bioassay was 10^{-13} M. 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; 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; 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; 2011). This phenomenon is also observed in the scales of nibbler fish, *Girella punctata* (marine fish) (Yachiguchi *et al.*, 2014). ALP activity in the scales of nibbler fish decreased after exposure to InHg (10^{-5} and 10^{-4} M) and MeHg (10^{-6} to 10^{-4} M) for 18 and 36 hours, although ALP activity did not change after 6 hours of incubation (Yachiguchi *et al.*, 2014). Furthermore, we previously analyzed the toxicity

Table 1: Toxicity of environmental pollutants on osteoclasts (OC) and osteoblasts (OB) in the cultured scales of teleosts

Pollutants	Detection Limit	Target	Name of Teteost	References
Cd	10^{-13} M	OC	goldfish	Suzuki *et al.* (2004)
Cd	10^{-7} M	OB	goldfish	Suzuki *et al.* (2004)
MeHg	10^{-8} M	OC	goldfish	Suzuki *et al.* (2004)
MeHg	10^{-7} M	OB	goldfish	Suzuki *et al.* (2004)
InHg	10^{-5} M	OC	goldfish	Suzuki *et al.* (2011)
InHg	10^{-6} M	OB	goldfish	Suzuki *et al.* (2011)
MeHg	10^{-6} M	OC	nibbler fish	Yachiguchi *et al.* (2014)
MeHg	10^{-6} M	OB	nibbler fish	Yachiguchi *et al.* (2014)
InHg	10^{-5} M	OC	nibbler fish	Yachiguchi *et al.* (2014)
InHg	10^{-5} M	OB	nibbler fish	Yachiguchi *et al.* (2014)
TBT	10^{-10} M	OB	goldfish	Suzuki *et al.* (2006)
TBT	10^{-8} M	OB	nibbler fish	Suzuki *et al.* (2006)
TBT	10^{-8} M	OB	wasse	Suzuki *et al.* (2006)

Cd: cadmium; MeHg: methylmercury; InHg: inorganic mercury; TBT: tributyltin.

of tributyltin (TBT), an aquatic environmental pollutant used as a biocide in anti-fouling paint (Suzuki *et al.*, 2006). Osteoblastic activity significantly decreased from the control values as a result of TBT treatment (10^{-10} to 10^{-5} M) at 6 hours of incubation (Suzuki *et al.*, 2006). Judging from the comparison of heavy metals (Cd and MeHg) and TBT, the inhibitory effect on osteoblasts at 6 hours of incubation appears to have lower expression of MT mRNA in TBT-treated scales than in Cd- or MeHg-treated scales (Suzuki *et al.*, 2006). Therefore, the toxicity of Gd to osteoblasts may be related to MT expression, as with TBT.

In Table 1, we summarized the comparison of toxicity to osteoclasts and osteoblasts of fish scales. Toxicity of Gd to osteoclasts was higher than those of MeHg and InHg. The toxicity of Gd was comparable to that of Cd. Gd inhibited osteoblastic activity and was toxic to osteoblasts. This inhibition of Gd was almost equal to that of TBT. In addition, the sensitivity of Gd in osteoclasts and osteoblasts was varied among respective goldfish. In the present study, female goldfish were used because sensitivity of osteoblastic and osteoclastic activities of scales to calcemic hormones was higher in mature females than in mature males (Suzuki *et al.*, 2000; Yoshikubo *et al.*, 2005). It seems that the sensitivity of Gd is related to the maturation of female goldfish. In the future, we are planning to study the correlation between sex hormones and Gd toxicity.

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 a level of Gd that is quite low (10^{-13} to 10^{-11} M) has 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.

Conclusion

We are the first to indicate the toxicity of Gd on fish bone metabolism using TRAP and ALP enzyme activities. We found that the detection limit of Gd in our bioassay was 10^{-13} M. Therefore, our assay system is quite effective as a biosensor of Gd. In addition, Gd toxicity to osteoblasts appears to be higher than that to Cd, MeHg and InHg and equal to TBT. The toxicity of Gd in osteoclasts was comparable to that of Cd. Gd used for clinical diagnoses in MRI is released into environmental waters without a specific recycling process. Gd was found in tap water samples in the area of Berlin, Germany. Up to 18 ng/L of anthropogenic Gd on top of the geogenic background of 0.54 ng/L could be determined (Kulaksiz and Bau, 2011). Recently, the need for Gd risk assessments for human health has been emphasized (Fraum *et al.*, 2017; Gwenzi *et al.*, 2018). In addition to the risk to human, we should also emphasize Gd risk assessment to protect the ecosystem in the aquatic environment.

Funding Information

Authors' Contributions

N. Suzuki and A. Hattori: Designed this study.

N. Suzuki, K. Watanabe, A. Sekimoto, M.I. Zanaty, T. Sekiguchi, Y. Kitani, H. Matsubara and M. Urata: Analyzed the data and wrote the paper including graph and table preparation.

A. Hattori and A.K. Srivastav: Supervised this work.

Conflict of Interest

The authors have no conflicts of interest to declare.

Ethics

All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of Kanazawa University.

References

Bereiter-Hahn, J. and L. Zylberberg, 1993. Regeneration of teleost fish scale. Comp. Biochem. Physiol. Part A, 105: 625-641.
DOI: 10.1016/0300-9629(93)90262-3

Birka, M., C.A. Wehe, O. Hachmöller, M. Sperling and U. Karst, 2016. Tracing gadolinium-based contrast agents from surface water to drinking water by means of speciation analysis. J. Chromatogr. A, 1440: 105-111. DOI: 10.1016/j.chroma.2016.02.050

Braun, M., G. Zavanyi, A. Laczovics, E. Berényi and S. Szabó, 2018. Can aquatic macrophytes be biofilters for gadolinium based contrasting agents? Water Res., 135: 104-111.
DOI: 10.1016/j.watres.2017.12.074

Dimai, H.P., T.A. Linkhart, S.G. Linkhart, L.R. Donahue and W.G. Beamer *et al*., 1998. Alkaline phosphatase levels and osteoprogenitor cell numbers suggest bone formation may contribute to peak bone density differences between two inbred strains of mice. Bone, 22: 211-216.
DOI: 10.1016/S8756-3282(97)00268-8

Fraum, T.J., D.R. Ludwig, M.R. Bashir and K.J. Fowler, 2017. Gadolinium-based contrast agents: A comprehensive risk assessment. J. Magn. Reson. Imaging, 46: 338-353. DOI: 10.1002/jmri.25625

Gwenzi, W., L. Mangori, C. Danha, N. Chaukura and N. Dunjana *et al*., 2018. Sources, behaviour, and environmental and human health risks of high-technology rare earth elements as emerging contaminants. Sci. Total Environ., 636: 299-313. DOI: 10.1016/j.scitotenv.2018.04.235

Knappe, A., P. Möller, P. Dulski and A. Pekdeger, 2005. Positive gadolinium anomaly in surface water and ground water of the urban area Berlin, Germany. Chemie der Erde – Geochemistry, 65: 167-189. DOI: 10.1016/j.chemer.2004.08.004

Kulaksız, S. and M. Bau, 2011. Anthropogenic gadolinium as a microcontaminant in tap water used as drinking water in urban areas and megacities. Appl. Geochem., 26: 1877-1885.
DOI: 10.1016/j.apgeochem.2011.06.011

Lehane, D.B., N. Mckie, R.G.G. Russell and I.W. Henderson, 1999. Cloning of a fragment of the osteonectin gene from goldfish, *Carassius auratus*: Its expression and potential regulation by estrogen. Gen. Comp. Endocrinol., 114: 80-87.
DOI: 10.1006/gcen.1998.7237

Martino, C., C. Costa, M.C. Roccheri, D. Koop and R. Scudiero *et al*., 2018. Gadolinium perturbs expression of skeletogenic genes, calcium uptake and larval development in phylogenetically distant sea urchin species. Aquat. Toxicol., 194: 57-66. DOI: 10.1016/j.aquatox.2017.11.004

Martino, C., R. Chiarelli, L. Bosco and M.C. Roccheri, 2017. Induction of skeletal abnormalities and autophagy in *Paracentrotus lividus* sea urchin embryos exposed to gadolinium. Mar. Environ. Res., 130: 12-20.
DOI: 10.1016/j.marenvres.2017.07.007

Möller, P., T. Paces, P. Dulski and G. Morteani, 2002. Anthropogenic Gd in surface water, drainage system and the water supply of the city of Prague, Czech Republic. Environ. Sci. Technol., 36: 2387-2394.
DOI: 10.1021/es010235q

Mugiya, Y. and N. Watabe, 1977. Studies on fish scale formation and resorption II: Effect of estradiol on calcium homeostasis and skeletal tissue resorption in the goldfish, *Carassius auratus* and the killifish, *Fundulus heteroclitus*. Comp. Biochem. Physiol. Part A, 57: 197-202.
DOI: 10.1016/0300-9629(77)90455-8

Nishimoto, S.K., N. Araki, F.D. Robinson and J.H. Waite, 1992. Discovery of bone γ-carboxyglutamic acid protein in mineralized scales. J. Biol. Chem., 267: 11600-11605.
DOI: 10.1111/j.2040-1124.2010.00046.x

Rogowska, J., E. Olkowska, W. Ratajczyk and L. Wolska, 2018. Gadolinium as a new emerging contaminant of aquatic environments. Environ. Toxicol. Chem., 37: 1523-1534.
DOI: 10.1002/etc.4116

Saitoh, M., R. Kuroda, Y. Muranaka, N. Uto and J. Murai *et al*., 2010. Asymmetric inhibition of spicule formation in sea urchin embryos with low concentrations of gadolinium ion. Develop. Growth Differ., 52: 735-746.
DOI: 10.1111/j.1440-169X.2010.01210.x

Sherry, A.D., P. Caravan and R.E. Lenkinski, 2009. Primer on gadolinium chemistry. J. Mag. Reson. Imaging 30: 1240-1248. DOI: 10.1002/jmri.21966

Suda. T., N. Takahashi, N. Udagawa, E. Jimi and M.T. Gillespie *et al.*, 1999. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocrine Rev., 20: 345-357. DOI: 10.1210/edrv.20.3.0367

Suzuki, N. and A. Hattori, 2002. Melatonin suppresses osteoclastic and osteoblastic activities in the scales of goldfish. J. Pineal Res., 33: 253-258. DOI: 10.1034/j.1600-079X.2002.02953.x

Suzuki, N. and A. Hattori, 2003. Bisphenol A suppresses osteoclastic and osteoblastic activities in the cultured scales of goldfish. Life Sci., 73: 2237-2247. DOI: 10.1016/S0024-3205(03)00603-9

Suzuki, N., K. Yachiguchi, K. Hayakawa, K. Omori and K. Takada *et al.*, 2011. Effects of inorganic mercury on osteoclasts and osteoblasts of the goldfish scales *in vitro*. J. Fac. Agr. Kyushu Univ., 56: 47-51.

Suzuki, N., M. Yamamoto, K. Watanabe, A. Kambegawa and A. Hattori, 2004. Both mercury and cadmium directly influence calcium homeostasis resulting from the suppression of scale bone cells: The scale is a good model for the evaluation of heavy metals on bone metabolism. J. Bone Miner. Metab., 22: 439-446. DOI: 10.1007/s00774-004-0505-3

Suzuki, N., T. Suzuki and T. Kurokawa, 2000. Suppression of osteoclastic activities by calcitonin in the scales of goldfish (freshwater teleost) and nibbler fish (seawater teleost). Peptides, 21: 115-124. DOI: 10.1016/S0196-9781(99)00181-3

Suzuki, N., T.M. Tabata, M.A. Kambegawa, A.K. Srivastav and A. Shimada *et al.*, 2006. Tributyltin inhibits osteoblastic activity and disrupts calcium metabolism through an increase in plasma calcium and calcitonin levels in teleosts. Life Sci., 78: 2533-2541. DOI: 10.1016/j.lfs.2005.10.004

Telgmann, L., M. Sperling and U. Karst, 2013. Determination of gadolinium-based MRI contrast agents in biological and environmental samples: A review. Anal. Chim. Acta, 764: 1-16. DOI: 10.1016/j.aca.2012.12.007

Thamamongood, T.A., R. Furuya, S. Fukuba, M. Nakamura and N. Suzuki *et al.*, 2012. Expression of osteoblastic and osteoclastic genes during spontaneous regeneration and autotransplantation of goldfish scale: A new tool to study intramembranous bone regeneration. Bone, 50: 1240-1249. DOI: 10.1016/j.bone.2012.03.021

Vaes, G., 1988. Cellular biology and biochemical mechanism of bone resorption: A review of recent developments on the formation, activation and mode of action of osteoclasts. Clin. Orthop. Relat. Res., 231: 239-271.

Yachiguchi, K., T. Sekiguchi, M. Nakano, A. Hattori and M. Yamamoto *et al.*, 2014. Effects of Inorganic mercury and methylmercury on osteoclasts and osteoblasts in the scales of the marine teleost as a model system of bone. Zool. Sci., 31: 330-337. DOI: 10.2108/zs130265

Yoshikubo, H., N. Suzuki, K. Takemura, M. Hoso and S. Yashima *et al.*, 2005. Osteoblastic activity and estrogenic response in the regenerating scale of goldfish, a good model of osteogenesis. Life Sci., 76: 2699-2709. DOI: 10.1016/j.lfs.2004.10.063