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Tributyltin and triphenyltin inhibit osteoclast differentiation through a retinoic acid receptor-dependent signaling pathway

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Abstract

Organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), have been widely used in agriculture and industry. Although these compounds are known to have many toxic effects, including endocrine-disrupting effects, their effects on bone resorption are unknown. In this study, we investigated the effects of organotin compounds, such as monobutyltin (MBT), dibutyltin (DBT), TBT, and TPT, on osteoclast differentiation using mouse monocytic RAW264.7 cells. MBT and DBT had no effects, whereas TBT and TPT dose-dependently inhibited osteoclast differentiation at concentrations of 3–30 nM. Treatment with a retinoic acid receptor (RAR)-specific antagonist, Ro41-5253, restored the inhibition of osteoclastogenesis by TBT and TPT. TBT and TPT reduced receptor activator of nuclear factor-κB ligand (RANKL) induced nuclear factor of activated T cells (NFAT) c1 expression, and the reduction in NFATc1 expression was recovered by Ro41-5253. Our results suggest that TBT and TPT suppress osteoclastogenesis by inhibiting RANKL-induced NFATc1 expression via an RAR-dependent signaling pathway.

Keywords: Organotin compound; Tributyltin; Triphenyltin; Osteoclast; Bone resorption

Organotin compounds have been widely used in agriculture and industry as biocides, wood preservatives, and stabilizers for polyvinylchloride polymers. Notably, tributyltin (TBT) and triphenyltin (TPT) have been widely used in antifouling paint for ships and fishing nets, and have contaminated marine areas. Accumulation of organotin compounds has been reported in marine fish and mammals [1,2]. TBT and TPT are well-known as endocrine disruptors that induce masculinization of mollusks [3,4]. In mammals, organotin compounds are toxic toward a number of organs [5]. Recently, organotin compounds were reported to modulate the differentiation of several cell types, such as neutrophils [6], adipocytes [7–9], and osteoblasts [10].

Bone metabolism is regulated by the balance between bone resorption by osteoclasts and bone formation by osteoblasts. Osteoclasts are multinucleated cells formed from hematopoietic precursors of the monocyte/macrophage lineage. Increased bone resorption by osteoclasts is involved in the pathogenesis of several lytic bone diseases, such as osteoporosis, hypercalcemia, rheumatoid arthritis, tumor metastasis into bone, periodontitis, and Paget's disease [11]. Osteoclast differentiation consists of multiple steps, including differentiation of osteoclast precursors into mononuclear osteoclasts, fusion of mononuclear osteoclasts to form multinucleated osteoclasts, and activation of osteoclasts to resorb bone [12]. Receptor activator of nuclear factor-κB (RANK) ligand (RANKL), a member of the tumor necrosis factor family, is expressed in osteoblasts and plays crucial roles in osteoclast differentiation and activation [13,14]. Binding of RANKL to its receptor (RANK) expressed in

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osteoclast precursors induces the recruitment of tumor necrosis factor receptor-associated factors (TRAFs) and activates nuclear factor-κB (NF-κB) via the inhibitor of NF-κB (IκB) kinase (IKK)-IκB pathway and mitogen-activated protein kinases (MAPKs) such as extracellular signal-regulated kinase (ERK), p38 MAPK, and c-Jun N-terminal kinase (JNK) [13,14]. These signaling pathways take part in osteoclast differentiation and activation [13,14]. Recently, it was reported that nuclear factor of activated T cells (NFAT) c1 is induced by RANKL and acts as a master regulator of osteoclastogenesis [13,14].

In the present study, we investigated the effects of organotin compounds on osteoclast differentiation using the RAW264.7 macrophage cell line. RAW264.7 cells are induced to differentiate into osteoclasts following treatment with RANKL alone and can therefore be used to determine the direct effects of organotin compounds on osteoclastogenesis.

Materials and methods

Reagents. Monobutyltin trichloride (MBT), dibutyltin dichloride (DBT), TBT, TPT, and methoprene acid (MTA) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). All-trans retinoic acid (ATRA) and 4-[(*E*)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (TTNPB) were purchased from Sigma Chemical Co. (St. Louis, MO). Ro41-5253 was purchased from Biomol (Plymouth Meeting, PA). PD98059 was obtained from Calbiochem (La Jolla, CA). All these reagents were dissolved in DMSO or ethanol and used at concentrations of less than 0.1% in culture medium. Recombinant human soluble RANKL (sRANKL) was purchased from PeproTech EC Ltd. (London, England). Anti-phospho-IκB-α, anti-IκB-α, anti-phospho-p38 MAPK, anti-p38 MAPK, anti-phospho-ERK, and anti-ERK antibodies were obtained from Cell Signaling Technology (Beverly, MA), while anti-NFATc1 and anti-GAPDH antibodies were obtained from BD Pharmingen (San Diego, CA) and Advanced Immunochemical Inc. (Long Beach, CA), respectively. All other reagents were obtained from Sigma or Wako Pure Chemical Industries Ltd.

Cell culture. The murine macrophage cell line RAW264.7 was obtained from the RIKEN Cell Bank (Tsukuba, Japan). The cells were maintained in α-MEM containing 10% FBS.

Osteoclast differentiation. For osteoclastogenesis experiments, RAW264.7 cells were seeded in 96-well plates (3×10^3 cells/well) and cultured in the presence of RANKL (50 ng/ml) for 3 days. Mature osteoclasts were formed from RAW264.7 cell cultures in the presence of RANKL and PD98059 (20 μM) for 4 days. Since multinucleated osteoclasts were strongly attached to the dish, whereas mononuclear osteoclasts did not, the mononuclear osteoclasts could be harvested by gentle pipetting. Using this property for fusion assays, mononuclear osteoclasts were prepared from differentiated mature osteoclast culture plates and cultured in 96-well plates (2×10^4 cells/well) in the presence of RANKL for 2 days. The cells were then fixed with 10% formalin for 10 min and ethanol for 1 min, and dried. Measurement of tartrate-resistant acid phosphatase (TRAP) activity and staining for TRAP ware performed as described previously [15]. TRAP-positive multinucleated cells ($TRAP^+$MNCs) with three or more nuclei were counted.

Viability assay. Cell viability was measured by the MTT assay. RAW264.7 cells were cultured under the same conditions used for osteoclastogenesis experiments, and MTT was added 1 h before the end of the culture. The supernatants were carefully removed and dissolved in DMSO, before their absorbances at 570 nm were measured using a microplate reader.

Resorption pit formation. To evaluate bone resorption pit formation, RAW264.7 cells were cultured on BD BioCoat Osteologic plates (BD Biosciences, Ontario, Canada) in the presence of RANKL for 4 days. The cells were then removed with bleach solution and images of the resorbed pits were obtained under light microscopy.

Western blotting. Cells were lysed with lysis buffer (50 mM Tris–HCl, pH 7.4, 1% Triton X-100, 270 mM sucrose, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, 1 mM Na_3VO_4, 50 mM NaF, 0.5 mM Pefabloc SC), and centrifuged at 12,000 rpm for 10 min. The supernatants were collected and their protein concentrations were measured using the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules, CA). Equal amounts of protein were separated by 10% SDS–PAGE and transferred to a PVDF membrane (GE Healthcare, Piscataway, NJ). The membrane was then blocked with 5% skim-milk in TBS-T (20 mM Tris–HCl, pH 7.4, 137 mM NaCl, 0.1% Tween 20) for 1 h and sequentially incubated with primary antibodies followed by a horseradish peroxidase-conjugated secondary antibody (GE Healthcare). Subsequently, the membranes were washed and developed using an enhanced chemiluminescence (ECL) detection system (GE Healthcare).

Results

Effects of organotin compounds on osteoclast differentiation

To investigate the effects of organotin compounds on osteoclast differentiation, RANKL-stimulated RAW264.7 cells were treated with MBT, DBT, TBT or TPT. MBT and DBT had no effects at nontoxic concentrations, whereas TBT and TPT significantly inhibited $TRAP^+$ osteoclast formation at very low concentrations (Fig. 1A). TBT and TPT dose-dependently inhibited the increase in RANKL-induced TRAP activity (Fig. 1B), although no cytotoxicity was observed (Fig. 1B). The IC_{50} values for the effects of TBT and TPT on the TRAP activity were approximately 2.2 and 6.6 nM, respectively. TBT and TPT also significantly suppressed $TRAP^+$MNC formation (Fig. 1C). Next, in order to evaluate the effects of TBT and TPT on resorption pit formation by osteoclasts, RAW264.7 cells were cultured on calcium phosphate apatite-coated plates in the presence of RANKL for 4 days. RANKL induced resorption pit formation and this effect was significantly suppressed by TBT and TPT (Fig. 1D).

Effects of organotin compounds on the fusion of mononuclear osteoclasts and survival of mature osteoclasts

Mononuclear osteoclasts were cultured with or without TBT and TPT in the presence of RANKL for 2 days. The results revealed that TBT and TPT slightly inhibited the TRAP activity (Fig. 1E) as well as the $TRAP^+$MNC formation resulting from mononuclear osteoclast fusion (Fig. 1F). Next, we examined the effects of organotin compounds on mature osteoclasts formed in the presence of RANKL and PD98059. TBT and TPT did not have any effects on the morphology or survival of mature osteoclasts during a 24-h treatment, even at concentrations as high as 100 nM (data not shown). These results indicate that TBT and TPT selectively affect the early differentiation from precursors to mononuclear osteoclasts, and not the late

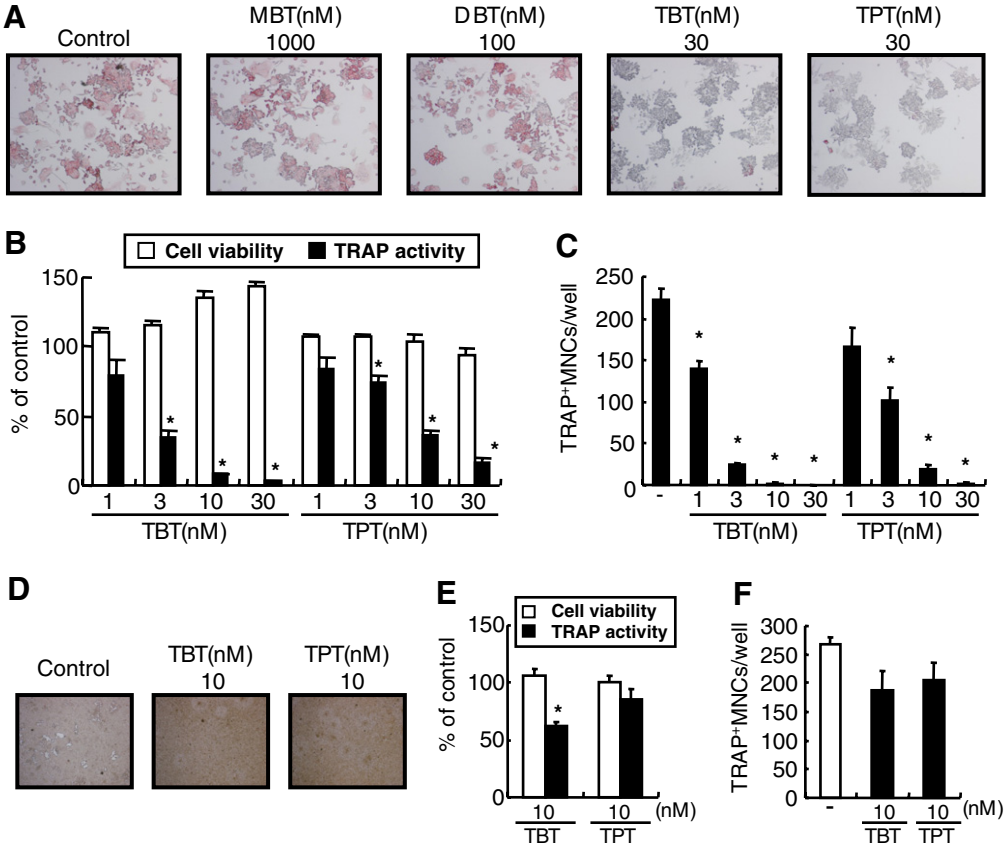

Fig. 1. Effects of organotin compounds on osteoclast differentiation. RAW264.7 cells were cultured in the presence of RANKL (50 ng/ml) with or without organotin compounds at the indicated concentrations for 3 days. The cells were stained for TRAP and photographed (A). TRAP activity and cell viability was determined (B). The numbers of $TRAP^+$MNCs containing three or more nuclei were counted (C). Data represent means ± SE of the results from four determinations. $^*P < 0.001$ vs. control cells. RAW264.7 cells were cultured on calcium phosphate apatite-coated plates in the presence of RANKL (50 ng/ml) with or without TBT and TPT for 4 days. Following removal of the cells, the resorbed pits were photographed (D). Mononuclear osteoclasts were collected as described in the Materials and methods and cultured with or without organotin compounds in the presence of RANKL (50 ng/ml) for 2 days. Cell viability and TRAP activity were measured (E). The numbers of $TRAP^+$MNCs containing three or more nuclei were counted (F). Data represent means ± SE of the results from four determinations. $^*P < 0.001$ vs. control cells.

differentiation from mononuclear osteoclasts to MNCs and mature osteoclasts.

Effects of aromatase inhibitors on osteoclast differentiation

TBT and TPT have been reported to inhibit the activity of aromatase, which catalyzes the conversion of androgen to estrogen [16,17]. To determine whether the effects of TBT and TPT on osteoclastogenesis are related to the suppression of aromatase activity, we examined the effects of aromatase inhibitors (aminoglutethimide and letrozole) on osteoclast differentiation in cultures of RAW264.7 cells. Aminoglutethimide and letrozole showed no inhibitory effects on RANKL-induced TRAP activity at concentrations as high as 100 μM (data not shown).

Effects of retinoic acid receptor (RAR) and/or retinoid X receptor (RXR) agonists on osteoclast differentiation

Retinoic acid (RA) is a ligand for two types of nuclear receptors, namely RAR and RXR. Recently, TBT and TPT were reported to act as ligands of RXR [18,19]. In addition, RA was reported to inhibit osteoclast differentiation [20,21], although it remains unknown whether the effects of RA on osteoclastogenesis are mediated by either RAR or RXR. Hence, we examined the effects of RAR and/or RXR agonists on osteoclast differentiation. When RAW264.7 cells were treated with RANKL in the presence of ATRA (RAR and RXR agonist), TTNPB (RAR-specific agonist) [22] or MTA (RXR-specific agonist) [23], osteoclast differentiation was significantly inhibited by ATRA and TTNPB at concentrations of less than 1 nM, but not by MTA, even at 1 μM (Fig. 2A). These results indicate that RAR negatively regulates osteoclast differentiation.

Effects of an RAR antagonist on the suppression of osteoclastogenesis by organotin compounds and retinoid receptor agonists

To determine whether the suppression of osteoclast differentiation by TBT and TPT is mediated via RAR, RAW264.7 cells were treated with RANKL in the presence

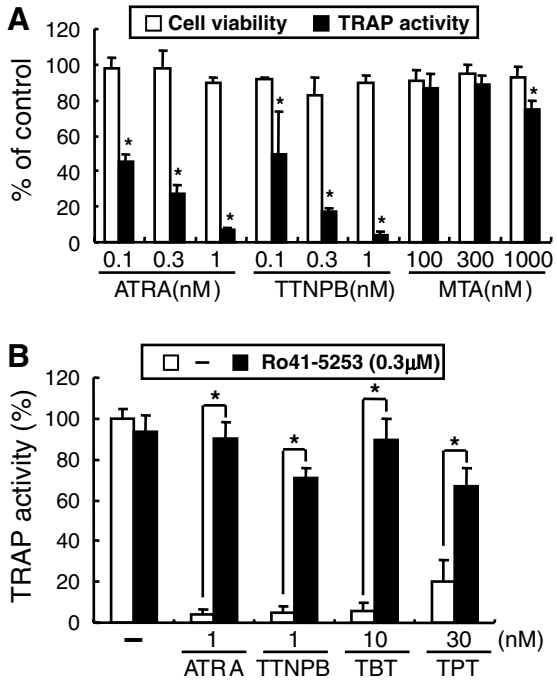

Fig. 2. Effects of RAR and/or RXR agonists on osteoclast differentiation, and effects of an RAR antagonist on the inhibitory ability of retinoids and organotin compounds. (A) RAW264.7 cells were cultured in the presence of RANKL (50 ng/ml) with or without RAR and/or RXR agonists at the indicated concentrations for 3 days. Cell viability and TRAP activity was measured. Data represent means ± SE of the results from four determinations. $^{*}P < 0.001$ vs. control cells. (B) RAW264.7 cells were cultured in the presence of RANKL (50 ng/ml) and ATRA, TTNPB, TBT or TPT with or without Ro41-5253 for 3 days. TRAP activity was measured (B). Data represent means ± SE of the results from four determinations. $^{*}P < 0.001$, without Ro41-5253 vs. with Ro41-5253.

of organotin compounds and Ro41-5253, an RAR-specific antagonist [24]. Ro41-5253 was able to recover the ability of both TBT and TPT to decrease the TRAP activity (Fig. 2B). Similar results were obtained when RAW264.7 cells were treated with ATRA or TTNPB in the presence of Ro41-5253 (Fig. 2B). These results suggest that not only ATRA and TTNPB but also TBT and TPT inhibit osteoclast differentiation via an RAR-dependent pathway.

Effects of TBT and TPT on RANKL-induced signaling pathways

To define the molecular mechanisms of the effects of TBT and TPT on osteoclastogenesis, we examined each of the major RANKL-induced signaling pathways. When RAW264.7 cells were stimulated with RANKL, phosphorylation of ERK, p38 and JNK was increased (Fig. 3A). TBT and ATRA did not affect the phosphorylation of these MAPKs (Fig. 3A). Similar results were obtained for TPT (data not shown). Therefore, it appears that the MAPK pathways are not related to the actions of TBT, TPT and ATRA on osteoclastogenesis. RANKL-induced NF-κB activation in osteoclast precursor cells is mediated by the activation of IKK and subsequent IκBα phosphorylation and degradation [16,17]. Therefore, we next determined whether TBT inhibits IκBα phosphorylation and degradation. The results revealed that TBT had no effect on the phosphorylation and degradation of IκBα, similar to the case for ATRA treatment (Fig. 3A). RANKL also induces expression of the transcription factor NFATc1, which is a key regulator of osteoclastogenesis [13,14]. When RAW264.7 cells were treated with TBT or ATRA in the presence of RANKL, RANKL-induced expression of NFATc1 was suppressed (Fig. 3B). Furthermore, the suppression of NFATc1 expression induced by TBT or ATRA was restored by the RAR-antagonist Ro41-5253 (Fig. 3B). Similar results were also observed

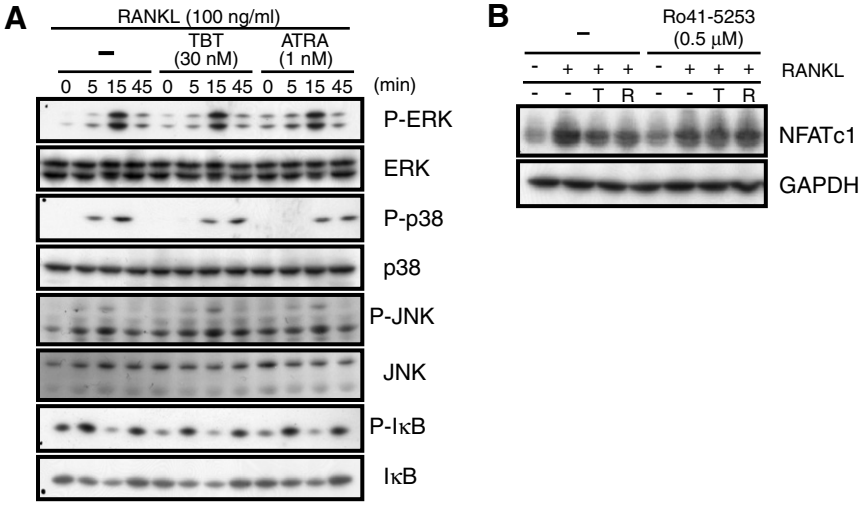

Fig. 3. Effects of TBT and retinoids on RANKL-induced signaling pathways. RAW264.7 cells were preincubated in the presence of TBT or ATRA for 30 min and then treated with RANKL (100 ng/ml) for the indicated times. Cell lysates were collected and separated by 10% SDS–PAGE. The levels of phosphorylated (P) or nonphosphorylated ERK, p38 MAPK, JNK, and IκB were determined by Western blotting (A). RAW264.7 cells were cultured in the presence of RANKL (50 ng/ml) and TBT (T, 30 nM) or ATRA (R, 1 nM) with or without Ro41-5253 for 24 h. Cell lysates were separated by 10% SDS–PAGE and analyzed by Western blotting with anti-NFATc1 and anti-GAPDH antibodies (B). The results are representative of three independent experiments.

for TPT (data not shown). These results suggest that TBT and TPT as well as ATRA inhibit RANKL-induced expression of NFATc1 through an RAR-dependent signaling pathway.

Discussion

In the present study, we found that TBT and TPT inhibited osteoclast differentiation at very low concentrations (3–30 nM). TBT and TPT were found to have little or no effects on the late steps in osteoclastogenesis such as the fusion of mononuclear osteoclasts and survival of mature osteoclasts.

TBT and TPT cause imposex in mollusks [3,4] and inhibit the activity of aromatase [16,17]. Since the aromatase inhibitors aminoglutethimide and letrozole did not inhibit osteoclastogenesis, the suppression of osteoclastogenesis by TBT and TPT is unlikely to be mediated via inhibition of aromatase. Recently, TBT and TPT were reported to induce the transactivation of RXR [18,19]. Therefore, we examined the effects of retinoid receptor agonists on osteoclast differentiation. RANKL-induced osteoclastogenesis was inhibited by the RAR and RXR agonist ATRA and RAR-selective agonist TTNPB, but not by the RXR-selective agonist MTA, and the suppression was recovered by the RAR-specific antagonist Ro41-5253. These data suggest that osteoclast differentiation is negatively regulated by an RAR signaling pathway. The suppression of osteoclastogenesis by TBT and TPT was also restored following treatment with Ro41-5253. Further investigation of the effects of TBT and TPT on RANKL-induced signaling pathways revealed that early signaling pathways, such as MAPKs (ERK, p38 MAPK, and JNK) and IκB, were not affected, whereas the expression of NFATc1 was decreased by TBT and TPT. This reduction in NFATc1 was recovered by the RAR-selective antagonist Ro41-5253. Consequently, TBT and TPT appear to inhibit osteoclast differentiation by suppressing NFATc1 expression via an RAR-dependent pathway.

ATRA acts as an agonist of both RAR and RXR and can activate RAR/RXR heterodimers and RXR/RXR homodimers. RXR-specific agonists can activate not only RXR/RXR but also permissive heterodimers, such as peroxisome proliferator-activated receptor (PPAR)/RXR and farnesoid X-activated receptor (FXR)/RXR, by the RXR ligand alone, but not RAR/RXR [25,26]. Nakanishi et al. reported that TBT and TPT are RXR-specific agonists, and therefore only activate RXR/RXR, and not RAR/RXR [18]. However, our present results suggest that TBT and TPT activate an RAR/RXR pathway, since the effects of TBT and TPT were abolished by the RAR-selective antagonist Ro41-5253. This discrepancy can probably be explained by the fact that an RXR ligand can activate RAR/RXR in the presence of an RAR agonist [25,26]. Indeed, the cells in the present study were cultured in medium containing 10% FBS, which includes some retinoids, such as vitamin A. In addition, $TRAP^{+}MNC$ formation was enhanced by treatment with the RAR antagonist Ro41-5253 alone (data not shown). Thus, TBT and TPT possibly activate RAR/RXR and inhibit osteoclast differentiation. It remains unclear why the RXR agonist MTA did not affect osteoclastogenesis. RAR and RXR each have three subtypes (α, β, and γ). The expressions of these subtypes are dependent on the cell type, tissue and developmental stage, and furthermore, the receptor subtype-specificity differs among ligands. These differences regulate multiple target gene expressions. Hence, the effects of TBT and TPT on osteoclastogenesis are probably different from the effect of MTA.

Retinoids are known to inhibit the activation of activating protein-1 (AP-1) through RAR or RXR [27]. The AP-1 components c-Fos and c-Jun play crucial roles in the induction of NFATc1 expression by RANKL [13,14]. Therefore, it is possible that TBT and TPT as well as retinoids inhibit osteoclast differentiation by repression of RANKL-induced AP-1 activation via RAR/RXR.

In the present study, we have demonstrated that TBT and TPT inhibit osteoclastogenesis *in vitro* for the first time. Previous studies have reported the detection of organotin compounds in human blood [28] and liver [29]. Accordingly, further *in vivo* studies are necessary to further our understanding of the effects of organotin compounds.

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References

[1] H. Harino, M. Fukushima, S. Kawai, Accumulation of butyltin and phenyltin compounds in various fish species, Arch. Environ. Contam. Toxicol. 39 (2000) 13–19.
[2] K. Kannan, S. Corsolini, S. Focardi, S. Tanabe, R. Tatsukawa, Accumulation pattern of butyltin compounds in dolphin, tuna, and shark collected from Italian coastal waters, Arch. Environ. Contam. Toxicol. 31 (1996) 19–23.
[3] T. Horiguchi, H. Shiraishi, M. Shimizu, M. Morita, Effects of triphenyltin chloride and five other organotin compounds on the development of imposex in the rock shell, *Thais clavigera*, Environ. Pollut. 95 (1997) 85–91.
[4] P. Matthiessen, P.E. Gibbs, Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in mollusks, Environ. Toxicol. Chem. 17 (1998) 37–43.
[5] N.J. Snoeij, A.H. Penninks, W. Seinen, Biological activity of organotin compounds—an overview, Environ. Res. 44 (1987) 335–353.
[6] H. Watanabe, R. Adachi, A. Hirayama, T. Kasahara, K. Suzuki, Triphenyltin enhances the neutrophilic differentiation of promyelocytic HL-60 cells, Biochem. Biophys. Res. Commun. 306 (2003) 26–31.
[7] F. Grun, H. Watanabe, Z. Zamanian, L. Maeda, K. Arima, R. Cubacha, D.M. Gardiner, J. Kanno, T. Iguchi, B. Blumberg, Endocrine-disrupting organotin compounds are potent inducers of adipogenesis in vertebrates, Mol. Endocrinol. 20 (2006) 2141–2155.
[8] H. Inadera, A. Shimomura, Environmental chemical tributyltin augments adipocyte differentiation, Toxicol. Lett. 159 (2005) 226–234.
[9] T. Kanayama, N. Kobayashi, S. Mamiya, T. Nakanishi, J. Nishikawa, Organotin compounds promote adipocyte differentiation as

agonists of the peroxisome proliferator-activated receptor gamma/retinoid X receptor pathway, Mol. Pharmacol. 67 (2005) 766–774.
[10] Y. Tsukamoto, Y. Ishihara, S. Miyagawa-Tomita, H. Hagiwara, Inhibition of ossification in vivo and differentiation of osteoblasts in vitro by tributyltin, Biochem. Pharmacol. 68 (2004) 739–746.
[11] G.A. Rodan, T.J. Martin, Therapeutic approaches to bone diseases, Science 289 (2000) 1508–1514.
[12] T. Suda, N. Takahashi, N. Udagawa, E. Jimi, M.T. Gillespie, T.J. Martin, Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families, Endocr. Rev. 20 (1999) 345–357.
[13] W.J. Boyle, W.S. Simonet, D.L. Lacey, Osteoclast differentiation and activation, Nature 423 (2003) 337–342.
[14] Z.H. Lee, H.H. Kim, Signal transduction by receptor activator of nuclear factor κB in osteoclasts, Biochem. Biophys. Res. Commun. 305 (2003) 211–214.
[15] J.T. Woo, H. Nakagawa, M. Notoya, T. Yonezawa, N. Udagawa, I.S. Lee, M. Ohnishi, H. Hagiwara, K. Nagai, Quercetin suppresses bone resorption by inhibiting the differentiation and activation of osteoclasts, Biol. Pharm. Bull. 27 (2004) 504–509.
[16] G.M. Cooke, Effect of organotins on human aromatase activity in vitro, Toxicol. Lett. 126 (2002) 121–130.
[17] M. Saitoh, T. Yanase, H. Morinaga, M. Tanabe, Y.M. Mu, Y. Nishi, M. Nomura, T. Okabe, K. Goto, R. Takayanagi, H. Nawata, Tributyltin or triphenyltin inhibits aromatase activity in the human granulosa-like tumor cell line KGN, Biochem. Biophys. Res. Commun. 289 (2001) 198–204.
[18] T. Nakanishi, J. Nishikawa, Y. Hiromori, H. Yokoyama, M. Koyanagi, S. Takasuga, J. Ishizaki, M. Watanabe, S. Isa, N. Utoguchi, N. Itoh, Y. Kohno, T. Nishihara, K. Tanaka, Trialkyltin compounds bind retinoid X receptor to alter human placental endocrine functions, Mol. Endocrinol. 19 (2005) 2502–2516.
[19] J. Nishikawa, S. Mamiya, T. Kanayama, T. Nishikawa, F. Shiraishi, T. Horiguchi, Involvement of the retinoid X receptor in the development of imposex caused by organotins in gastropods, Environ. Sci. Technol. 38 (2004) 6271–6276.
[20] Y. Ishimi, M. Ohumura, X. Wang, M. Yamaguchi, S. Ikegami, Inhibition by carotenoids and retinoic acid of osteoclast-like cell formation induced by bone-resorbing agents in vitro, J. Clin. Biochem. Nutr. 27 (1999) 113–122.
[21] X. Wang, J. Wu, Y. Shidoji, Y. Muto, N. Ohishi, K. Yagi, S. Ikegami, T. Shinki, N. Udagawa, T. Suda, Y. Ishimi, Effects of geranylgeranoic acid in bone: induction of osteoblast differentiation and inhibition of osteoclast formation, J. Bone Miner. Res. 17 (2002) 91–100.
[22] A. Astrom, U. Pettersson, A. Krust, P. Chambon, J.J. Voorhees, Retinoic acid and synthetic analogs differentially activate retinoic acid receptor dependent transcription, Biochem. Biophys. Res. Commun. 173 (1990) 339–345.
[23] M.A. Harmon, M.F. Boehm, R.A. Heyman, D.J. Mangelsdorf, Activation of mammalian retinoid X receptors by the insect growth regulator methoprene, Proc. Natl. Acad. Sci. USA. 92 (1995) 6157–6160.
[24] C. Apfel, F. Bauer, M. Crettaz, L. Forni, M. Kamber, F. Kaufmann, P. LeMotte, W. Pirson, M. Klaus, A retinoic acid receptor alpha antagonist selectively counteracts retinoic acid effects, Proc. Natl. Acad. Sci. USA. 89 (1992) 7129–7133.
[25] B. Blumberg, R.M. Evans, Orphan nuclear receptors—new ligands and new possibilities, Genes Dev. 12 (1998) 3149–3155.
[26] A. Aranda, A. Pascual, Nuclear hormone receptors and gene expression, Physiol. Rev. 81 (2001) 1269–1304.
[27] M. Pfahl, Nuclear receptor/AP-1 interaction, Endocr. Rev. 14 (1993) 651–658.
[28] K. Kannan, K. Senthilkumar, J.P. Giesy, Occurrence of butyltin compounds in human blood, Environ. Sci. Technol. 33 (1999) 1776–1779.
[29] S. Takahashi, H. Mukai, S. Tanabe, K. Sakayama, T. Miyazaki, H. Masuno, Butyltin residues in livers of humans and wild terrestrial mammals and in plastic products, Environ. Pollut. 106 (1999) 213–218.