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A comparison of the effects of tributyltin chloride and triphenyltin chloride on cell proliferation, proapoptotic p53, Bax, and antiapoptotic Bcl-2 protein levels in human breast cancer MCF-7 cell line

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ABSTRACT

In recent years it was disclosed, that numerous organotin(IV) derivatives have remarkable cytotoxicity against several types of cancer cells. The property to inhibit cell growth makes these compounds promising for antitumor therapy, as the clinical effectiveness of cisplatin is limited by drug resistance and significant side effects.

Tributyltin and triphenyltin are known as endocrine disruptors. Moreover, the compounds exert their toxicity in mammals predominantly through nuclear receptor signaling. Here we present the effects of tributyltin chloride (TBT-Cl) and triphenyltin chloride (TPT-Cl) on cell proliferation, expression of proapoptotic p53, Bax, and antiapoptotic Bcl-2 proteins in human breast cancer MCF-7 cell line. Dose and time dependent (24, 48 and 72 h) cell expositions have demonstrated TBT-Cl as more effective in inhibiting MCF-7 cell proliferation than TPT-Cl. Short time treatment with TBT-Cl displayed marked stimulation of p53 protein expression when compared to TPT-Cl. Both organotin compounds displayed similar mild enhancement of Bax protein expression. The 24 h exposition of TPT-Cl induced substantial diminution of Bcl-2 protein expression in comparison with both, untreated cells and TBT-Cl treated cells. Our observations indicate that TBT-Cl and TPT-Cl have different antiproliferative potency and distinct impact on expression of apoptosis marker proteins.

1. Introduction

Organotins represent a group of organic pollutants with potent endocrine-disrupting properties in both invertebrates and vertebrates. Several organotin compounds have found their exploitation as biocides, agricultural fungicides, wood preservatives, effective disinfecting agents in circulating industrial cooling waters, and special paints for marine vessels (Delgado Filho et al., 2011). Organotins have been largely released into water, resulting in bizarre effects on aquatic organisms (Sousa et al., 2014). It was described, that tributyltin (TBT) and triphenyltin (TPT) at very low (nanomolar) concentrations induce an irreversible sexual abnormality in females mollusks, a phenomenon known as "imposex", i.e. irreversible sex-organ alterations as a result of masculinization process (Matthiessen and Gibbs, 1998; Nakanishi, 2008).

Bettin et al. (1996) considered those organotin compounds as potential competitive inhibitors of aromatase enzyme activity, involved in conversion of androgen to estrogen. Organotins inhibit the catalytic activity of human 5-alpha reductases, enzymes responsible for conversion of testosterone into the more potent dihydrotestosterone (Doering et al., 2002). Moreover, the compounds are potent stimulators of human placental estrogen biosynthesis and hCG production (Nakanishi et al., 2005; Nakanishi, 2008). From the very recent data, Si et al. (2013) has shown that exposure to low doses of tributyltin chloride reduces sperm count and quality in mice. With an estimated annual production of about 62,000 tons, organotins belong to the most widely used organometallic compounds family (Nath, 2008).

Both TBT and TPT were identified as nanomolar agonists for all types of retinoid X receptors and peroxisome proliferator-activated receptor gamma, which are members of the nuclear receptor superfamily (le Maire et al., 2009). It was observed, that organotin

Abbreviations: TBT-Cl, tributyltin chloride; TPT-Cl, triphenyltin chloride; MCF-7, human breast cancer cell line; ATRA, all-*trans* retinoic acid; FBS, fetal bovine serum; RAR, nuclear all-*trans* retinoic acid receptor; RXR, nuclear 9-*cis* retinoic acid receptor; ERK, extracellular-signal-regulated kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase.

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compound(s) exposure of higher vertebrates is linked also to adipocyte differentiation, and thus promotion of obesity by organotin compounds may represent another adverse effects on human health and diseases (Grün and Blumberg, 2006). Recently, Cho et al. (2012) have reported that selected organotin compounds may act as inhibitor of transcriptional activation of human estrogen receptor. The remarkable cytotoxicity of organotin derivatives were explained by both, oxidative damage and increased concentration of intracellular calcium as the major factors contributing to triorganotin-induced apoptosis in many cell lines (Alama et al., 2009). Moreover, activation of retinoid X receptor and PPARγ may play additional roles in the antiproliferative activity of organotins (Nakanishi, 2008).

To our knowledge, there are no literary data concerning the relation of triorganotin compounds and apoptosis in human breast cancer cell line MCF-7. Thus, the aim of this study was to evaluate and compare the effects of two organotin compounds differing in chemical structure, tributyltin chloride (TBT-Cl) and triphenyltin chloride (TPT-Cl), on cell proliferation, proapoptotic P53, Bax, and antiapoptotic Bcl-2 proteins in human breast cancer MCF-7 cell line.

2. Material and methods

Tributyltin chloride (TBT-Cl) and triphenyltin chloride (TPT-Cl), all-*trans* retinoic acid (ATRA) and other conventional chemicals were obtained from Sigma, USA.

2.1. Cell culture and treatment

Human breast cancer epithelial cell line MCF-7 was obtained from INSERM U682, Strasbourg, France (Prof. G. Cremel). The cells were grown and passaged routinely as monolayer culture in 75 cm^2 flasks (Sarstedt, Germany). In our experiments, the cells were used at passage 10–25. In proliferation studies MCF-7 cells were seeded into 96-well plates at a density 1×10^4/well in DMEM medium supplemented with 10% fetal bovine serum (FBS), both from Gibco BRL, Parsley, Scotland, antibiotics (penicillin 20,000 U/l, streptomycin 20,000 U/l, and gentamycin 80 mg/l, Cambrex, USA) and cultivated in a humified atmosphere of 5% CO_2 and 95% air at 37 °C. After 24 h of attachment, the medium was changed for the fresh one and the tested compounds were added in various concentrations for construction of dose response curves. Each dose was tested in triplicates. TBT-Cl concentrations were in range from 1×10^{-12} to 1×10^6 M; TPT-Cl starting at 1×10^{-10} to 1×10^6 M; and the reference compound – all-*trans* retinoic acid (ATRA) was used in the same concentration range 1×10^{-12} to 1×10^6 M. TBT-Cl, TPT-Cl and ATRA were dissolved in 1% (vol/vol) ethanol/medium mixture (0.1% final ethanol concentration in cell culture). Reference cells (control values) were cultivated either in culture medium alone or in the presence medium + ethanol mixture (0.1% final concentration). This concentration of solvent had no effect on examined end points of the present study. To avoid the effects of compound metabolites, the incubation medium and tested compounds were changed for fresh ones every 24 h. Time dependent effects were examined after 24, 48 and 72 h cell treatments; all compounds investigated were tested simultaneously. For cell cultivations and cell treatments appropriate plastic ware (75 cm^2, 25 cm^2 flasks, 96 microplates and Petri dishes) of Sarstedt (Germany) origin were used.

2.2. Cell proliferation test

MTT assay expressing cellular metabolic activity was employed to assess cell proliferation/cytotoxicity of tested compounds. MTT one Solution Cells proliferation Assay, CellTiter 96® AQ_{ueous} (Promega, USA) was used according the producer instruction. The colorimetric measurement of the violet product was red at 490 nm in Universal plate reader ELx800 (Bio-Tek Instruments, Inc., USA).

2.3. Apoptotic proteins studies

MCF7 cells (250,000) were seeded on Petri dishes (d = 5 cm) and identically treated as for proliferation studies. TBT-Cl, TPT-Cl and ATRA were used in concentrations equal to 0.1, 1.0 and 10-fold of the IC_{50} values calculated from dose response curves for individual compound. The cell treatment was finished by placing the Petri dish on ice, washing the cells with cold PBS, scraped off in the presence of 500 μl ice cold lysate buffer. Composition of lysate buffer: 50 mM Tris–HCl, 150 mM NaCl, 10 mM NaF, 100 mM Na_2VO_4, 1% Triton X-100 plus 1 tablet Complete (protease inhibitor cocktail, Roche, Switzerland)/50 ml of lysate buffer. The cell lysates were intensively vortexed and centrifuged at 14,000 rpm at 4 °C for 25 min. The protein content in supernatant was analyzed by bicinchinolic acid (BCA™ Protein assay kit, Thermo Scientific, USA). The lysates were normalized for protein content then combined with SDS Laemli sample buffer and denaturated at 100 °C for 5 min. The samples were aliquoted and frozen at −70 °C.

2.4. Western blot analysis

25 μg solubilized proteins were separated by 5% concentration and 12% separation SDS–PAGE and then transferred on PVDF membrane (Hybond P, Amersham Biosciences, England). Membranes were blocked with 5% nonfat milk (Blotting-Grade Blocker, BIO-RAD, USA) and blotted with specific primary antibodies for Bax, Bcl-2 and p53 (Santa Cruz Biotechnology, Inc., USA; dilution 1:500) overnight at 8 °C. The target proteins were recognized by secondary antibody Anti-rabbit IgG (H + L) (DyLight® 800 Conjugate) in dilution 1:10,000 (Cell Signaling Technology, Danvers, MA). The intensities of fluorescent signals obtained were measured using LI-COR Biosciences Odyssey® System (Lincoln, NE). Quantification of protein level was performed using Odyssey IR Imaging System Software version 2.0. The stained band(s) was compared to molecular weight marker (Odyssey® Molecular Weight Marker, ladder 250–10 kDa, LI-COR, Lincoln, NE) loaded on the same gel as the analyzed samples. The amount of target proteins were normalized to the structural protein, β-actin.

2.5. Calculations and statistical analysis

The effects of ATRA, TBT-Cl and TPT-Cl on cell proliferation were calculated as the effect (%) of individual dose vs value for control cells (0.1% ethanol) at each time point. The data are presented as the means ± SE. Presenting value is the result of three individual experiments performed in triplicate for each dose. The effects for each compound are expressed by IC_{50} (e.g., the lowest concentration where the effect is inhibited by 50%) and by the magnitude of maximal effect. The IC_{50} values were calculated from dose response curves by computer program GraphPad Prism. Statistical differences between time intervals and individual compounds were evaluated by one-way ANOVA and Bonferroni *post hoc* test.

3. Results

3.1. Antiproliferative effects

As shown in Fig. 1, ATRA independently of dose and time of the treatment did not affect MCF-7 cell proliferation. Dose dependent