Chemosphere xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

The roles of DNA damage-dependent signals and MAPK cascades in tributyltin-induced germline apoptosis in *Caenorhabditis elegans*

Yun Wang *, Shunchang Wang, Xun Luo, Yanan Yang, Fenglei Jian, Xuemin Wang, Lucheng Xie

Department of Life Sciences, Huainan Normal University, Huainan, Anhui 232001, PR China

HIGHLIGHTS

- TBT exposure induced germline apoptosis in *Caenorhabditis elegans.*
- Elevated TBT concentrations induced cell cycle arrest in *C. elegans.*
- DNA damage response (DDR) pathway was involved in TBT-induced germline apoptosis.
- ERK, JNK, and p38 MAPK cascades were required for TBT-induced germline apoptosis.

ARTICLE INFO

Article history:
Received 18 September 2013
Received in revised form 23 December 2013
Accepted 11 January 2014
Available online xxxx

Keywords:
Tributyltin
Caenorhabditis elegans
Apoptosis
DNA damage response
MAPK

ABSTRACT

The induction of apoptosis is recognized to be a major mechanism of tributyltin (TBT) toxicity. However, the underlying signaling pathways for TBT-induced apoptosis remain unclear. In this study, using the nematode *Caenorhabditis elegans*, we examined whether DNA damage response (DDR) pathway and mitogen-activated protein kinase (MAPK) signaling cascades are involved in TBT-induced germline apoptosis and cell cycle arrest. Our results demonstrated that exposing worms to TBT at the dose of 10 nM for 6 h significantly increased germline apoptosis in N2 strain. Germline apoptosis was absent in strains that carried *ced-3* or *ced-4* loss-of-function alleles, indicating that both caspase protein CED-3 and Apaf-1 protein CED-4 were required for TBT-induced apoptosis. TBT-induced apoptosis was blocked in the Bcl-2 gain-of-function strain *ced-9(n1950)*, whereas TBT induced a minor increase in the BH3-only protein EGL-1 mutated strain *egl-1(n1084n3082)*. Checkpoint proteins HUS-1 and CLK-2 exerted proapoptotic effects, and the null mutation of *cep-1*, the homologue of tumor suppressor gene p53, significantly inhibited TBT-induced apoptosis. Apoptosis in the loss-of-function strains of ERK, JNK and p38 MAPK signaling pathways were completely or mildly suppressed under TBT stress. These results were supported by the results of mRNA expression levels of corresponding genes. The present study indicated that TBT-induced apoptosis required the core apoptotic machinery, and that DDR genes and MAPK pathways played essential roles in signaling the processes.

1. Introduction

Organotin compounds, especially tributyltin (TBT), have been extensively used as antifouling agents in paints. Because of its biocidal effects, TBT has been recognized as one of the most toxic anthropogenic chemicals released into the aquatic environment (Antizar-Ladislao, 2008). Previous studies demonstrated that TBT was detectable in food, drinking water and even in human blood and liver (Nielsen and Strand, 2002; Antizar-Ladislao, 2008; Kannan et al., 2010). In China, the concentrations of TBT in the coastal water or rivers range from below the detection limit to several nM (as tin) (Jiang et al., 2001; Gao et al., 2012). Moreover, butyltins, including TBT, have been detected even in human blood at

Abbreviations: ANOVA, analysis of variance; AO, acridine orange; Apaf-1, apoptotic protease-activating factor-1; ASK, apoptosis signal-regulating kinase; BH3, Bcl-2 homology region 3; DAPI, 4′,6-diamidino-2-phenylindole; DDR, DNA damage response; DMSO, dimethyl sulfoxide; ERK, extracellular signal-regulated protein kinase; *gf*, gain-of-function; JNK, c-Jun N-terminal kinase; *lf*, loss-of-function; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; MAPKKK, MAPKK kinase; MC-LR, microcystin-LR; NGM, nematode growth medium; ROS, reactive oxygen species; SEM, standard error of the mean; TBT, tributyltin.

* Corresponding author. Tel.: +86 554 6662817; fax: +86 554 6663083.
E-mail address: yunwang2001@163.com (Y. Wang).

http://dx.doi.org/10.1016/j.chemosphere.2014.01.045
0045-6535/

concentrations ranging from 50 and 400 nM (Whalen et al., 1999). Due to the environmental persistence and bioaccumulation properties of TBT, the toxicological effects of TBT have aroused great concerns in the last decade (Hoch, 2001; Fent, 2004; Frouin et al., 2010; Mitra et al., 2013). Although there are many reports on the potential toxicological effects of TBT, the critical pathways for the toxicity of this organotin compound remain unclear.

Apoptosis is a highly organized cellular process that occurs metabolically and genotoxically in multicellular organisms. The induction of apoptosis is believed to be a major mechanism of TBT toxicity. In fact, the immunologic, neurological, and hepatic toxicities of TBT have been correlated to apoptosis both *in vitro* and *in vivo* (Grondin et al., 2007; Nakatsu et al., 2007; Zhang et al., 2008; Gupta et al., 2011). Generally, genotoxic exposure activates DNA damage response (DDR) signals, such as checkpoint protein HUS-1 and tumor suppressor gene p53, which are essential for DNA damage-induced apoptosis (Stergiou and Hengartner, 2004). Non-genotoxic exposure always induces DNA damage-independent signals, such as mitogen-activated protein kinase (MAPK) signaling cascades, which are involved in early cellular signal transduction (Stergiou and Hengartner, 2004; Roos and Kaina, 2006). The evolutionary conserved MAPK pathways have been characterized from yeast to human: the extracellular signal-regulated protein kinase (ERK), the c-Jun N-terminal kinase (JNK), and the p38 family kinase (p38 MAPK). Each kinase is activated by its immediate upstream regulator MAPK kinase (MAPKK), which in turn is activated by a MAPKK kinase (MAPKKK) (Johnson and Lapadat, 2002). MAPKs have been implicated in the cellular responses of TBT (Nakatsu et al., 2006, 2007; Urushibara et al., 2009; Dudimah et al., 2010). The activation of ERK1/2 by TBT has been reported to be responsible for the NK cell dysfunction (Dudimah et al., 2010), whereas the activation of ERK1/2 and p38 are involved in TBT-induced neurotoxicity in cortical neurons (Nakatsu et al., 2006). JNK phosphorylation has been indicated to be involved in TBT-induced apoptosis in rainbow trout RTG-2 cells (Urushibara et al., 2009) and rat pheochromocytoma PC12 cells (Nakatsu et al., 2007). However, data concerned the roles of these conserved signaling pathways in TBT-induced apoptosis were still insufficient.

Caenorhabditis elegans, a free-living nematode, is now widely considered as an ideal model animal for the study of environmental evaluation and toxicity assessment (Leung et al., 2008; Wu et al., 2012; Rui et al., 2013). In *C. elegans*, germline keeps proliferation lifely and is subject to stress-induced apoptosis and cell cycle arrest, by contrast, adult somatic cells is almost invariant (Schedl, 1997). The *C. elegans* germline is known to undergo apoptosis initiated either by DNA damage agents or nongenotoxically. On the other hand, in the germline, DNA damage can induce both cell cycle arrest and apoptosis, two responses that are spatially separated. The latter is a tightly regulated process that is genetically indistinguishable from developmental programmed cell death. Upstream of the core apoptotic machinery, components of the DDR signaling pathway lie and act either as sensors of the lesion or as transducers of the initial signal detected (Stergiou and Hengartner, 2004). Our previous results showed that the caspase protein CED-3 and Apaf-1 protein CED-4 were absolutely required for the apoptotic processes induced by hepatotoxin microcystin-LR (MC-LR), and that the DDR and MAPKs cascades played essential roles in modulating MC-LR-induced germline apoptosis in *C. elegans* (Wang et al., 2012a). To integrate cellular signal transduction pathways in TBT-induced apoptosis and deepen the understanding of TBT toxicity in wildlife and human, we used *C. elegans* as an alternative animal model. By exposing worm mutants carrying mutated genes encoding key regulators of apoptosis to TBT, we found that TBT-induced germline apoptosis was exhibited in a genotoxic and/or nongenotoxic manner.

2. Materials and methods

2.1. Worm strains and reagents

The strains used in this study were as follows: wild type Bristol N2, *ced-3(n717)*, *ced-4(n1162)*, *egl-1(n1084n3082)*, *ced-9(n1950)*, *cep-1(gk138)*, *cep-1(lg12501)*, *clk-2(mn159)*, *hus-1(op241)*, *lin-45(n2520)*, *mek-2(n1989)*, *ksr-1(ku68)*, *mpk-1(ku1)*, *nsy-1(ag3)*, *mek-1(ks54)*, *jkk-1(km2)*, *mkk-4(ju91)*, *jnk-1(gk7)*, *sek-1(ag1)*, *pmk-1(km25)*, and *opIs34*, which were provided by the Caenorhabditis Genetics Center (Minneapolis, MN, USA). All strains were maintained on nematode growth medium (NGM) plates that were seeded with *Escherichia coli* OP50 as a food source by standard procedures (Brenner, 1974). Gravid hermaphrodites were lysed in an alkaline hypochlorite solution to obtain age-synchronized worms, and the gathered eggs were subjected to an overnight hatching at 20 °C. The new hatchers were maintained for 24 h at the L1 stage in the absence of food until inoculated onto NGM. TBT chloride (97% purity) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Acridine orange (AO) was purchased from Molecular Probes (Eugene, OR, USA). TBT was dissolved into dimethyl sulfoxide (DMSO) and then diluted with K-medium (containing 32 mM KCl and 52 mM NaCl) before use. Final DMSO concentration in treatment solutions and control was 0.5% in each assay.

2.2. Sample preparation

Animal handling and chemical exposure were performed as described previously (Williams and Dusenbery, 1990). Apoptotic germ cells, cell cycle arrest, gene expression and HUS-1::GFP foci formation were assessed in the synchronized young adult worms that were exposed to the indicated TBT concentrations. For each concentration and control, three independent assays were conducted for all of the tests.

2.3. Apoptosis assay

Apoptotic germ cells were measured by AO staining using a procedure as described previously (Kelly et al., 2000). Briefly, worms at the indicated time points were picked from test wells into 24-well tissue culture plates containing 1 mL K-medium with 25 μg/mL AO and incubated for 1 h at 20 °C. To facilitate dye uptake, a drop of OP50 was added to the buffer. Animals were allowed to recover for 1 h on bacterial lawns and then mounted onto agar pads on microscope slides with a drop of 60 μg/mL levamisole in M9 buffer (3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl, 1 mL 1 M $MgSO_4$, and H_2O to 1 L, sterilized by autoclaving.). Apoptotic cells were examined under a fluorescent microscopy. At least 20 worms were measured for each treatment. The apoptotic cells appeared yellow or yellow-orange, which represented increased DNA fragmentation, whereas intact cells were uniformly green (Wang et al., 2007). In most conditions, only the posterior arm of the gonad could be scored because the autofluorescence of the intestine always shaded the gonad arm near the pharynx.

2.4. Cell cycle arrest determination

To investigate whether TBT exposure caused germline cell cycle arrest, the number of mitotic cell nuclei was determined after exposing worms to graded doses of TBT following the method of Wang et al. (2007). In brief, at least 20 worms were picked out from test wells at indicated time points and suspended in 1 μL of distilled water, fixed with Carnoy's fixative (six parts ethanol, three parts chloroform, and one part glacial acetic acid), air-dried, and stained with a small drop of 2 μg/mL 4′,6-diamidino-2-phenylindole (DAPI)

Please cite this article in press as: Wang, Y., et al. The roles of DNA damage-dependent signals and MAPK cascades in tributyltin-induced germline apoptosis in *Caenorhabditis elegans*. Chemosphere (2014), http://dx.doi.org/10.1016/j.chemosphere.2014.01.045