Toxicology Letters 225 (2014) 413–421

Contents lists available at ScienceDirect

Toxicology Letters

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

The effect of tributyltin chloride on *Caenorhabditis elegans* germline is mediated by a conserved DNA damage checkpoint pathway☆

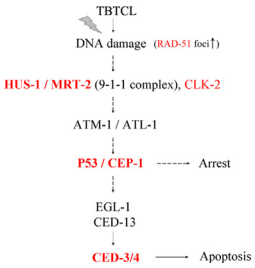
Zhe Cheng[a,1], Huimin Tian[b,1], Hongran Chu[a], Jianjian Wu[a], Yingying Li[a], Yanhai Wang[a,*]

[a] State Key Laboratory of Cellular Stress Biology, School of Life Sciences, Xiamen University, Xiamen, Fujian, China
[b] Medical College, Xiamen University, Xiamen, Fujian, China

HIGHLIGHTS

- We used *Caenorhabditis elegans* to investigate how TBTCL exhibits its effect on animal germline.
- TBTCL exposure resulted in increased sterility and embryonic lethality.
- TBTCL exposure induced increased DSBs and checkpoint activation in germline.
- Germ cell apoptosis and proliferation arrest were induced by TBTCL exposure.
- Checkpoint and p53 signaling pathways were found essential in response to TBTCL.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 December 2013
Received in revised form 3 January 2014
Accepted 7 January 2014
Available online 18 January 2014

Keywords:
Tributyltin
Germline
DNA damage
Checkpoint pathway
Apoptosis
Cell cycle arrest

ABSTRACT

Tributyltin (TBT), one of the environmental pollutants, has been shown to impact the reproduction of animals. However, due to the lack of appropriate animal model, analysis of the affected molecular pathways in germ cells is lagging and has been particularly challenging. In the present study, we investigated the effects of tributyltin chloride (TBTCL) on the nematode *Caenorhabditis elegans* germline. We show that exposure of *C. elegans* to TBTCL causes significantly elevated level of sterility and embryonic lethality. TBTCL exposure results in an increased number of meiotic DNA double-strand breaks in germ cells, subsequently leading to activated DNA damage checkpoint. Exposing *C. elegans* to TBTCL causes dose- and time-dependent germline apoptosis. This apoptotic response was blocked in loss-of-function mutants of hus-1 (op241), mrt-2 (e2663) and p53/cep-1 (gk138), indicating that checkpoints and p53 are essential for mediating TBTCL-induced germ cell apoptosis. Moreover, TBTCL exposure can inhibit germ cell proliferation, which is also mediated by the conserved checkpoint pathway. We thereby propose that TBT exhibits its effects on the germline by inducing DNA damage and impaired maintenance of genomic integrity.

Index Descriptors and Abbreviations: TBTCL, tributyltin chloride; *C. elegans*, *Caenorhabditis elegans*; NGM, nematode growth medium; DMSO, dimethyl sulfoxide; DAPI, 4′, 6-diamidino-2-phenylindole; DSBs, DNA double-strand breaks.

* Corresponding author. Tel.: +86 592 218 4559; fax: +86 592 218 3040.
E-mail address: wangyh@xmu.edu.cn (Y. Wang).

[1] These authors contributed equally to this work.

0378-4274/$ – see front matter © 2014 The Authors. Published by Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxlet.2014.01.010

1. Introduction

Tributyltin (TBT) is a widespread environmental toxicant commonly used in anti-fouling agents for boats, as well as a by-product from several industrial processes (Antizar-Ladislao, 2008). Although the use and production of TBT have been strictly regulated since the 1980s, it continues to be detected in water, sediment, and aquatic organisms (Champ, 2000; Environmental Protection Agency, 2003; Rüdel et al., 2007; Choi et al., 2009). Consumption of contaminated drinking water, beverages, and, in particular, marine food has been considered as the important route of human exposure to TBT. As a well-known endocrine disruptor, TBT can cause "imposex" (development of male characteristics in females) in gastropods and impairments in growth, development, survival and reproduction of many aquatic organisms (Haggera et al., 2005; Hano et al., 2007; Leung et al., 2007; Lau et al., 2007). In mammals, TBT induces immunosuppressive, metabolic, developmental or reproductive effects (Ogata et al., 2001; Antizar-Ladislao, 2008; Nakanishi, 2008). Rodent models of TBT exposure have revealed multiple levels of reproductive impairments, including reduction in fertility, increased fetal lethality and decreased number of gonocytes and germ cells (Ogata et al., 2001; Kishta et al., 2007; Chen et al., 2008). Although TBT has been suggested to impact the mammalian reproductive process, we still lag in our understanding of the affected genes and pathways, as well as its mechanism of action in the germline.

To investigate how TBT exerts its effect on the reproductive system, we explored the use of the genetically tractable nematode *Caenorhabditis elegans*. Owing to its easy maintenance, cellular simplicity, genetic manipulability and evolutionarily conserved biology, *C. elegans* has been proved to be an excellent model organism for studying many biological processes, as well as for both fundamental toxicity assessments and further mechanistic studies in the fields of environmental and biomedical toxicology (Ankeny, 2001; Leung et al., 2008). *C. elegans* has a short reproduction cycle and large numbers of germline nuclei which are displayed throughout the gonad in a defined order, correlating with the sequential stages of meiosis (Colaiácovo, 2006). Therefore, *C.elegans* has been used as a powerful model of remarkable relevance to mammals for meiosis studies, including the investigations into environmental effects on the germline function (Allard and Colaiácovo, 2010; Allard et al., 2013).

Tominaga et al. (2002) reported the effects of TBT on *C.elegans* for the first time. They found that TBT exposure decreased the numbers of worms in the first and second generations. A TBT-induced reduction of total number of germ cells was reported by Hoshi et al. (2003). Wang et al. (2012) indicated that TBT can induce oxidative stress-response protein expression and activate a checkpoint protein HUS-1 in pachytene. Though these studies imply that TBT impacts the reproduction of *C.elegans*, how TBT exerts its effect on the germ cells and which signaling pathways and genes are involved have not been elucidated. In this study, we show that *C.elegans* oogenesis was significantly impaired by tributyltin chloride (TBTCL) exposure, leading to an increase of sterility and embryonic lethality. TBTCL exposure results in increased meiotic DNA double-strand breaks (DSBs) and activation of DNA damage checkpoint in germline. TBTCL induces germ cell apoptosis and proliferation arrest, which is mediated by a conserved checkpoint- and p53-dependent signaling pathway. Taken together, we propose that TBT exhibits its effects on the germline by inducing DNA damage and thus leading to failed maintenance of genomic integrity during oogenesis.

2. Material and methods

2.1. Worms and reagents

Strains used in this study were wild type *N2* Bristol, *ced-3 (n717)*, *ced-4 (n1162)*, *cep-1(gk138)*, *hus-1(op241)*, *mrt-2(e2663)*, *clk-2(mn159)* and *ced-1::gfp (MD701)*, which were originally obtained from the Caenorhabditis Genetics Center (funded by the NIH National Center for Research Resource, USA). *Hus-1::gfp (opIs34)* is a gift from Dr. Yun Wang. All strains were maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli* OP50 at 20 °C as described (Brenner, 1974). Gravid nematodes were lysed in an alkaline hypochlorite solution for the synchronized cultures (Sulston and Hodgkin, 1988). Tributyltin chloride (TBTCL) was purchased from Sigma (purity ≥96%) and dissolved in dimethyl sulfoxide (DMSO, Sigma, purity ≥99.7%) prior to the exposure.

2.2. Reproduction and embryonic lethality assay

Tests were conducted in 24-well cell culture plates as described previously (Williams and Dusenberg, 1990). Briefly, TBTCL was diluted to concentrations of 0.003–1.5 μM in K-medium (52 mM NaCl and 32 mM KCl) containing OP50 as a food source. Synchronized middle-L4 hermaphrodites were picked and transferred into a well containing 1 ml test solution of TBTCL or solvent control (DMSO, <0.1%) and incubated at 20 °C in dark. Worms were transferred daily to new wells containing the same ingredients. The eggs remaining on the old wells were counted daily within 4 days of exposure. The embryonic lethality was calculated by dividing the number of dead (un-hatched) eggs by the total number of eggs. 15 worms were used in every treatment and control group for each experiment.

2.3. Germ cell apoptosis assay

Synchronized middle-L4 worms were exposed to TBTCL in 24-well cell culture plates. TBTCL were diluted to concentrations of 0.003–1.5 μM in K-medium containing OP50 as a food source. Corpses were counted under Nomarski optics as described by Gartner et al. (2000). Alternatively, we used a transgenic strain *ced-1:gfp(MD701)* which carries fluorescent reporter to allow visualization of the apoptotic corpses (Zhou et al., 2001) in the test. Stage-matched *ced-1:gfp* animals were exposed to TBTCL and observed under a fluorescence microscope to detect transgenic green fluorescent protein (GFP) expression.

2.4. Mitotic germ cell proliferation arrest assay

TBTCL exposure for germ cell proliferation assay was conducted as described in apoptosis analysis. Mitotic germ cell proliferation arrest was assessed as described by Gartner et al. (2000) and Stergiou et al. (2007). Fifteen worms were picked out from test wells at indicated time points and stained with 4′,6-diamidino-2-phenylindole (DAPI, Sigma). The mitotic nuclei presented at the distal end of the germ line were counted by a blinded observer under a Nikon 80i fluorescence microscope.

2.5. RNAi studies

To facilitate visualization of germ cell apoptosis in animals with loss of gene function, we took advantage of RNA interference (RNAi) for gene knockout using the *ced-1:gfp(MD701)* strain as the background. RNAi by feeding was performed as previously described (Kamath et al., 2003; Salinas et al., 2006). TBTCL exposures were performed as described in Section 2.3. Briefly, synchronized L1