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Early cellular responses against tributyltin chloride exposure in primary cultures derived from various brain regions

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ABSTRACT

Tributyltin (TBT) is a potent biocide and commonly used in various industrial sectors. Humans are mainly exposed through the food chain. We have previously demonstrated tin accumulation in brain following TBT-chloride (TBTC) exposure. In this study, effect of TBTC on dissociated cells from different brain regions was evaluated. Cytotoxicity assay (MTT), mode of cell death (Annexin V/PI assay), oxidative stress parameters (ROS and lipid peroxidation), reducing power of the cell (GSH), mitochondrial membrane potential (MMP) and intracellular Ca^{2+} were evaluated to ascertain the effect of TBTC. Expression of glial fibrillary acidic protein (GFAP) was measured to understand the effect on astroglial cells. TBTC as low as 30 nM was found to reduce GSH levels, whereas higher doses of 300 and 3000 nM induced ROS generation and marked loss in cell viability mainly through apoptosis. Striatum showed higher susceptibility than other regions, which may have further implications on various neurological aspects.

1. Introduction

Tributyltin (TBT) belongs to the organotin class of compounds which are extensively used in several industrial applications like heat stabilizers, catalyst, biocide, etc. It is being used in various industries including glass & chemicals, wood & pulp, paints, food, and several consumer good sectors for a long time now (Piver, 1973). For the last four decades, TBT pollution has spread tremendously resulting in significant human exposure risks (RPA, 2005). Among all the organotin compounds, use of TBT especially has been restricted or regulated in most countries all over the world (Champ, 2000). Due to its long half life, adsorbing efficacy on sediments and lipophilic nature, the levels of TBT was found to be considerably higher in marine sediments and fishes (EFSA, 2004). Humans primarily get exposed to TBT through the food chain by consuming contaminated fish, water from PVC pipes, general food (vegetables & dairy products) and several others (WWF factsheet). An evaluation of human samples demonstrated presence of butyltin compounds in blood in the range of 50–400 nM (Whalen et al., 1999) and also in the liver tissue (Kannan and Falandysz, 1997; Takahashi et al., 1999).

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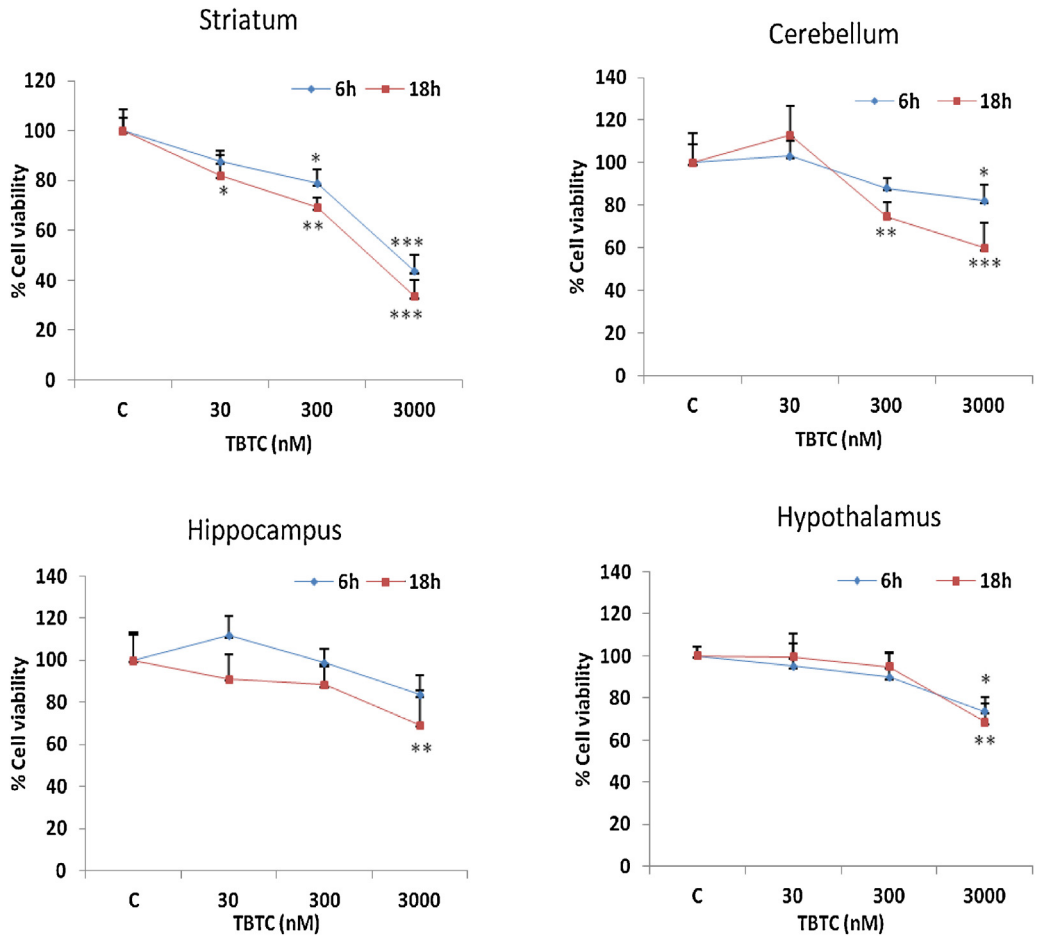

Fig. 1 – TBTC exposure leads to loss of cell viability at 6 and 18 h in dissociated cells isolated from various brain parts. 4 week old male Wistar rats were sacrificed and dissociated mixed cell cultures were isolated from different brain parts. Cells were maintained in DMEM with 10% FBS for 24 h and then exposed to various TBTC concentrations (30, 300 and 3000 nM) for indicated time points. Cell viability was assessed by MTT assay. Data represents mean ± S.E. of four independent experiments performed in quadruplets. $^{***}p < 0.001$, $^{**}p < 0.01$ and $^{*}p < 0.05$, when compared to control using one-way ANOVA.

Initially reported as an endocrine disruptor and then established as an immunotoxic agent, TBT has been estimated to affect a large number of organs including reproductive organs (Omura et al., 2001), liver (Liu et al., 2006) and kidney (Kobayashi-Hattori et al., 2006). The neurotoxic outcome of TBT exposure has been evaluated by a number of studies (Ema et al., 1991; Ocallaghan and Miller, 1988; Tsunoda et al., 2004) but the mechanism involved is largely elusive. Previous reports highlighted the involvement of various mechanisms (Nakatsu et al., 2006a, 2007) to describe the effect of TBTC exposure on neuronal cells, but these studies were limited only to a specific cell population or were derived from a single brain region (Konno et al., 2005; Mizuhashi et al., 2000; Yamada et al., 2010). We previously demonstrated that TBT-chloride (TBTC) can disrupt the blood brain barrier (BBB) even after 7 days post single oral dose of 10, 20, 30 mg/kg, resulting in oxidative stress and cell death (Mitra et al., 2013). However, since different brain regions are comprised of diverse microenvironments including non-identical cell populations, the influence of TBT may differ. Since oxidative stress acts as a trigger for the development of various neuropathies, it is pertinent to ascertain the role of TBT in this regard. In line with our previous findings, we analyzed the influence of TBTC under ex vivo conditions on dissociated mixed cell cultures derived from different brain parts: cerebellum (CB), hippocampus (HI), hypothalamus (HY) and striatum (ST). To ascertain the sensitivity of each brain part to TBTC exposure, cellular biochemical and cell death parameters were analyzed. This study highlights the differential influence of TBTC and assists in identifying the susceptible brain regions.

2. Materials and methods

2.1. Chemicals

Tributyltin chloride (TBTC) was purchased from Merck, Germany (purity > 99%); Dulbecco's modified Eagle's medium (DMEM-high glucose) was from Invitrogen, Life Technologies, USA; Dulbecco's phosphate buffered saline (PBS), 3-(4,5-dimethyl-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), bovine serum albumin (BSA), acrylamide, N,N′-methylene