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Short communication

THE EFFECT OF TRIBUTYLTIN ON HUMAN EOSINOPHYLIC LEUKEMIA EoL-1 CELLS [#]

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Abstract: Organotin compounds are chemicals that are widely used in industry and agriculture as plastic stabilizers, catalysts and biocides. Many of them, including tributyltin (TBT), have been detected in human food and, as a consequence, detectable levels have been found in human blood. As organotin compounds were shown to possess immunotoxic activity, we focused our attention on the effect of TBT on the basic determinants of the function of eosinophils, i.e. cell adhesiveness and motility. We used human eosinophylic leukemia EoL-1 cells, a common *in vitro* cellular model of human eosinophils. Here, we demonstrate that TBT causes a dose-dependent decrease in the viability of EoL-1 cells. When administered at sub-lethal concentrations, TBT significantly decreases the adhesion of EoL-1 cells to human fibroblasts (HSFs) and inhibits their migration on fibroblast surfaces. Since the basic function of eosinophils is to invade inflamed tissues, our results indicate that TBT, and possibly other organotin compounds, may affect major cellular properties involved in the determination of *in vivo* eosinophil function.

Key words: Tributyltin, Cytotoxicity, Cell adhesion, Cell migration, EoL-1 cells

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Abbreviations used: HSFs – human skin fibroblasts; SEM- standard error of the mean; TBT – tributyltin

INTRODUCTION

Butyltins have a broad range of applications. They are often used in industry as plastic stabilizers, catalysts and antifouling keel paints, and in agriculture as biocides. Humans are exposed to these chemicals primarily through the intake of food. Butyltins, including tributyltin (TBT), have been detected in human blood at concentrations ranging between 50 and 400 nM [1]. With the increased usage of these compounds, considerable attention has been focused on their potential toxicity. It has been shown that butyltins are highly hepatotoxic, neurotoxic and/or immunotoxic [2-4]. However, the specific mechanisms responsible for the immunotoxic effects of TBT are still unclear. For instance, to the best of our knowledge, the role of TBT in the regulation of eosinophil function has not been elucidated.

Eosinophils are multi-functional cells that play a crucial role in various pathological events. Their activation is mainly associated with parasitic infections or allergic manifestations, and it depends on cell extravasation, which is a multi-step process that includes endothelial layer transmigration and the migration of eosinophils towards locally inflamed tissue [5]. The immunotoxicity of TBT has been demonstrated [1-4]. In this study, we investigated the effect of tributyltin on the basic cellular functions involved in eosinophil activity *in vivo*, namely the adhesion and motile activity of human eosinophylic leukemia EoL-1 cells. This cell line has the cytological features of myeoloblasts and can differentiate phenotypically and functionally into eosinophils, thus providing an *in vitro* model for studying human eosinophil function and their regulation [6-7].

MATERIALS AND METHODS

Cell culture

A human eosinophilic leukemia (EoL-1) cell line was established from the peripheral blood of a patient with acute myeloid leukemia. Cells were cultured in RPMI-1640 medium (Sigma, St. Louis, MO/USA) supplemented with 10% FCS (Gibco, Lab., New York, USA), 100 IU/ml penicillin and 10 μg/ml streptomycin (Polfa, Tarchomin, Poland) in a humidified atmosphere with 5% CO_2 at 37ºC.

Human skin fibroblasts (HSFs) were cultured in MEM (Sigma, St. Louis, MO/USA) supplemented with 10% FCS, 100 IU/ml penicillin and 10 μg/ml streptomycin in a humidified atmosphere with 5% CO_2 at 37ºC.

Cell viability

To determine the effect of TBT (Sigma, St. Louis, MO/USA) on the cell viability, EoL-1 cells were plated to 6-well plates at a density of 160,000/cm^2 in the culture medium without TBT or with different concentrations of TBT in the range between 6.25 and 100 nM. They were cultured for 24 hours in a humidified atmosphere with 5% CO_2 at 37ºC. Then the fluoresceine diacetate and ethidium bromide tests were applied [8].

Adhesion assay
EoL-1 cells suspended in RPMI-1640 medium with 10% FCS without TBT or with 6.25 or 12.5 nM TBT were seeded at a density of 160,000/cm^2 onto plastic or the surface of a monolayer of HSFs. They were cultured for 24 hours in a humidified atmosphere with 5% CO_2 at 37ºC. Then the percentage of cells attached to the substrata was calculated as described previously [9].

Time lapse-monitoring of cell movement
Time-lapse monitoring of cell movement was carried out as described previously [10]. 24 hours before the experiment, EoL-1 cells were seeded onto the surface of a monolayer of HSFs at a density of 125,000/cm^2 and cultured in RPMI-1640 medium supplemented with 10% FCS in a humidified atmosphere with 5% CO_2 at 37ºC. Immediately before the experiment, coverslips were placed into Sykes-Moore chambers. Cell movement in the culture medium was recorded at 37ºC for 5 hours with a 5 minute time-lapse interval under an inverted Olympus IMT-2 microscope. Afterwards, the culture medium containing TBT (at a final concentration of 6.25 nM) was added, and recording was continued for a further 5 hours. Individual cell tracks were determined by changes in the cell centroid position, and plotted in circular diagrams. Parameters characterizing cell locomotion were computed for each cell or cell population as described previously [11-12].

Statistical analysis
Statistical significance was determined using the non-parametric Mann-Whitney U-test with $p < 0.05$ considered to indicate significant differences. The value of each of the variables characterising the motility was calculated for 50 cells. Each variable was expressed as the mean of 3-6 independent experiments.

RESULTS AND DISCUSSION

Several studies demonstrated that TBT affects the viability of a wide range of normal and tumour cells [13-16]. To examine the effect of TBT on EoL-1 cell viability, in the first series of experiments, the cells were incubated with TBT in concentrations ranging from 6.25 to 100 nM for 24 hours, and the percentage of viable cells was calculated. As shown in Fig. 1, TBT at concentrations at and above 25 nM exerted a potent cytotoxic effect on EoL-1 cells.
EoL-1 cells cultured *in vitro* are anchorage-independent; they do not adhere to glass or plastic, but grow in suspension (data not shown). This corresponds to the behaviour of eosinophils *in vivo*, where they persist in the circulation with a half-life of 6-12 hours. The recruitment of eosinophils into sites of inflammation includes adhesion, endothelial cell layer transmigration and migration of eosinophils towards locally inflamed tissue. Tissue infiltration by eosinophils provides an opportunity for direct interactions with resident fibroblasts, which may in turn serve to support eosinophil survival, adhesion, activation and secretion [4, 17-20].

Therefore, in further experiments we investigated the effect of TBT on the adhesion of EoL-1 cells to HSFs at non-toxic concentrations, i.e. those not affecting cell viability (6.25 and 12.5 nM).

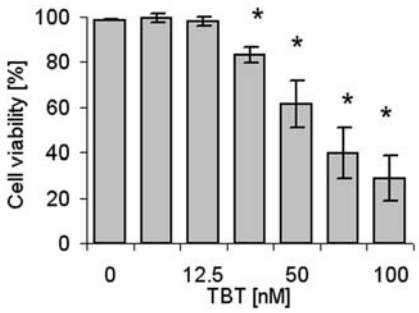

Fig. 1. The effect of TBT on the viability of EoL-1 cells. Cells were treated with TBT for 24 hours and then the percentage of viable cells was calculated. Error bars represent SD, * indicates statistical significance ($p < 0.05$).

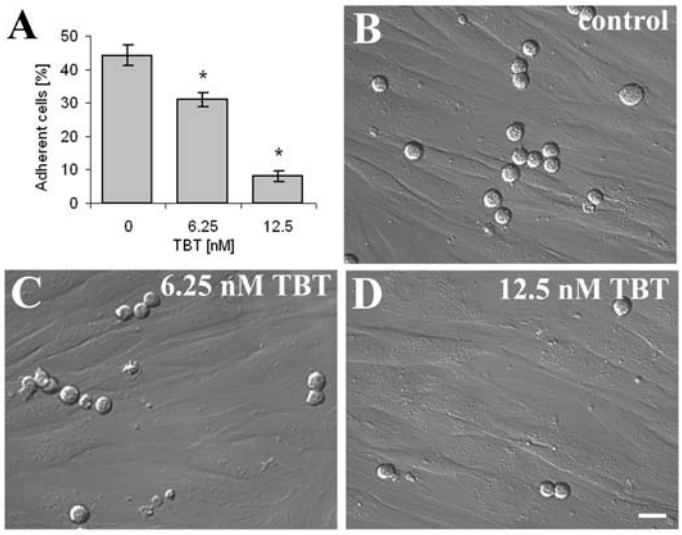

Fig. 2. The effect of TBT on EoL-1 cell attachment to the surfaces of the underlying HSFs. A – Cells were incubated without TBT or with 6.25 or 12.5 nM TBT for 24 hours, and the percentage of cells attached to the substrata was calculated. B, C, D – Nomarski`s differential interference contrast. Error bars represent SD, *indicates statistical significance ($p < 0.05$). Scale bar = 20 μm.

As shown in Fig. 2, over 45% of EoL-1 cells adhered to the surface of HSFs after 24 hours of incubation in the control medium. The treatment of cells with 6.25 or 12.5 nM TBT for 24 hours caused a significant and concentration-dependent decrease in the fraction of adherent EoL-1 cells. In the presence of 12.5 nM TBT, only 8.1 ± 1.6% adhered to the surface of HSFs; therefore, we subsequently concentrated on the effect of 6.25 nM TBT on the motile activity of EoL-1 cells. The trajectories of the investigated cells moving on the surfaces of the underlying HSFs in control conditions and in the presence of TBT are shown in Fig. 3A, B, and the quantitative data is summarized in Tab. 1.

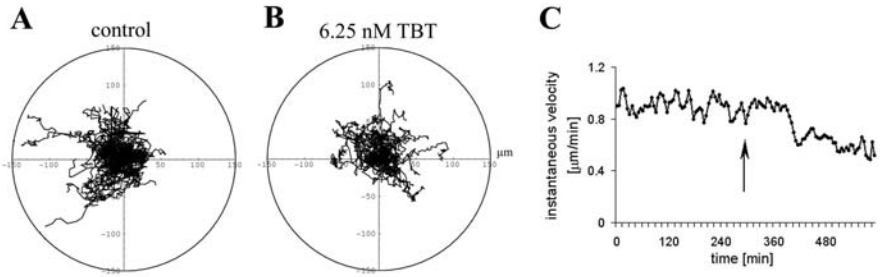

Fig. 3. The effect of TBT on the migration of EoL-1 cells moving on the surfaces of the underlying HSFs. Cell movement was recorded for 5 hours at 5-minute intervals. Afterwards, 6.25 nM TBT was added and the recording of cell locomotion was continued for a further 5 hours. The composite trajectories of EoL-1 cells migrating in (A) the control medium, and (B) the medium containing 6.25 nM TBT are shown in circular diagrams. C – A plot of the average instantaneous velocity of the EoL-1 cells. The arrow shows the point at which 6.25 nM TBT was added.

Tab. 1. A summary of the quantitative data showing the effect of TBT on the migration of EoL-1 cells on the surfaces of the underlying HSFs.

Parameters (± SEM)	Control	6.25 nM TBT
Average speed of cell movement [µm/min]	0.9 ± 0.03	0.7 ± 0.02*
Total cell displacement [µm]	46.4 ± 4.7	37.8 ± 3.8*

*significant at $p < 0.05$ compared to the control

It was found that 6.25 nM TBT caused a significant decrease in the average speed of cell locomotion (to 77% of the control) and in the total length of cell displacement (to 81% of the control). Moreover, analysis of the instantaneous velocity of EoL-1 revealed that under control conditions, the values of this parameter remained unchanged over the entire duration of the experiment. By contrast, upon administration of 6.25 nM TBT, a gradual decrease in the instantaneous velocity was observed (Fig. 3C).

In summary, these results demonstrate that TBT is cytotoxic to EoL-1 cells at concentrations of 25 nM and above. Moreover, at non-toxic concentrations, this

compound significantly inhibits the adhesion of EoL-1 cells to HSFs, and their motile activity (< 25 nM). The accumulation of eosinophils in tissues is one of the most important aspects in non-allergic and allergic inflammatory reactions, and eosinophil recruitment is regulated by several processes including cell adhesion and migration. Thus, our results indicate that TBT, commonly used in agriculture and industry, can significantly affect the biological functions of EoL-1 cells *in vitro* at very low concentrations, suggesting that TBT can interfere with the vital physiological functions of eosinophils.

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REFERENCES

1. Whalen, M.M., Logananthan, B.G. and Kannan, K. Immunotoxicity of environmentally relevant concentrations of butyltins on human natural killer cells *in vitro*. **Env. Res.** 81 (1999) 108-116.
2. Fent, K. Ecotoxicology of organotin compounds. **Crit. Rev. Toxicol.** 26 (1996) 1-117.
3. Hoch, M. Organotin compounds in the environment. **Appl. Geochem.** 16 (2001) 719-743.
4. Appel, K.E. Organotin compounds: Toxicokinetic aspects. **Drug. Met. Rev.** 36 (2004) 763-786.
5. Silberstein D.A. Eosinophil function in health and disease. **Crit. Rev. Oncol. Hematol.** 19 (1995) 47-77.
6. Mayumi, M. EoL-1, a human eosinophilic cell line. **Leuk. Lymphoma** 7 (1992) 243-250.
7. Wong, C.K., Ho, C.Y., Lam, C.W., Zhang, J.P. and Hjelm, N.M. Differentiation of a human eosinophilic leukemic cell line, EoL-1: characterization by the expression of cytokine receptors, adhesion molecules, CD95 and eosinophilic cationic protein (ECP). **Immunol. Lett.** 68 (1999) 317-323.
8. Szydłowska, H., Zaporowska, E., Kuszlik-Jochym, K., Korohoda, W. and Branny, J. Membranolytic activity of detergents as studied with cell viability tests. **Folia Histochem. Cytochem.** 16 (1978) 69-78.
9. Wójciak, B. and Korohoda, W. Ehrlich ascites tumour cells show tissue-specific adherence and modify their shape upon contact with embryonic fibroblasts and myotubes. **J. Cell Sci.** 97 (1990) 433-438.
10. Korohoda, W., Golda, J., Sroka, J., Wojnarowicz, A., Jochym, P. and Madeja, Z. Chemotaxis of *Amoeba proteus* in the developing pH gradient within a pocket-like chamber studied with the computer assisted method. **Cell Motil. Cytoskel.** 38 (1997) 38-53.

11. Sroka, J., Kamiński, R., Michalik, M., Madeja, M., Przestalski, S. and Korohoda, W. The effect of triethyllead on the motile activity of Walker 256 carcinosarcoma cells. **Cell. Mol. Biol. Lett.** 9 (2004) 15-30.
12. Miękus, K. and Madeja, Z. Genistein inhibits the contact-stimulated migration of prostate cancer cells. **Cell. Mol. Biol. Lett.** 12 (2007) 348-361.
13. Reader, S., Moutardier, V. and Denizeau, F. Tributyltin triggers apoptosis in trout hepatocytes: the role of Ca2+, protein kinase C and proteases. **Biochim. Biophys. Acta.** 1448 (1999) 473-485.
14. Umebayashi, C., Oyama, Y., Chikahisa-Muramastu, L., Nakao, H., Nishizaki, Y., Nakata, M., Soeda, F., Takahama, K., Seibert, H., Morchel, S. and Gulden, M. Tri-n-butyltin-induced cytotoxicity on rat thymocytes in presence and absence of serum. **Toxicol. In Vitro** 18 (2004) 55-61.
15. Zucker, R.M., Elstein, K.H., Easterling, R.E. and Massaro, E.J. Flow cytometric comparison of the effects of trialkyltins on the murine erythroleukemic cell. **Toxicology** 58 (1989) 107-119.
16. Seibert, H., Morchel, S. and Gulden, M. Cytotoxic potency of trialkyltins to C6 glioma cells *in vitro*: impact of exposure conditions. **Cell. Biol. Toxicol.** 20 (2004) 273-283.
17. Owen, W.F. Jr, Rothenberg, M.E., Silberstein, D.S., Gasson, J.C., Stevens, R.L., Austen, K.F. and Soberman, R.J. Regulation of human eosinophil viability, density, and function by granulocyte/macrophage colony-stimulating factor in the presence of 3T3 fibroblasts. **J. Exp. Med.** 166 (1987) 129-141.
18. Rothenberg, M.E., Owen, W.F. Jr, Silberstein, D.S., Woods, J., Soberman, R.J., Austen, K.F. and Stevens, R.L. Human eosinophils have prolonged survival, enhanced functional properties, and become hypodense when exposed to human interleukin 3. **J. Clin. Invest.** 81 (1988) 1986-1992.
19. Solomon, A., Shmilowich, R., Shasha, D., Frucht-Pery, J,, Pe'er, J., Bonini, S. and Levi-Schaffer, F. Conjunctival fibroblasts enhance the survival and functional activity of peripheral blood eosinophils *in vitro*. **Invest. Ophthalmol. Vis. Sci.** 41 (2000) 1038-1044.
20. Gomes, I., Mathur, S.K., Espenshade, B.M., Mori, Y., Varga, J. and Ackerman, S.J. Eosinophil-fibroblast interactions induce fibroblast IL-6 secretion and extracellular matrix gene expression: implications in fibrogenesis. **J. Allergy Clin. Immunol.** 116 (2005) 796-804.