IMMUNOSUPPRESSIVE EFFECTS OF PHENYLTIN COMPOUNDS ON HUMAN NATURAL KILLER (NK) CELL FUNCTION *IN VITRO*

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Introduction

Organotin compounds are widely used as heat stabilizers in polyvinyl chloride polymers, industrial and agricultural biocides and as industrial catalysts in variety of chemical reactions [1]. Triphenyltin (TPT) is an organotin, which is primarily used in agriculture as a contact fungicide to treat a variety of crops including potatoes, peanuts, carrots, soybeans, sugar beets and rice [2-5]. TPT is also used in antifouling paints to prevent growth of barnacles and other fouling organisms on aluminum hulled boats and ships over 25 m. Due to its extensive use, TPT contamination has been reported in water, sediment and fish from both freshwater and marine environments in the United States, Europe and Japan [6-9]. Laboratory TPT exposure studies has revealed a wide spectrum of toxic effects in aquatic and terrestrial organisms. Immune function studies with rats fed TPT revealed that the thymus dependent immune responses were suppressed including delayed-type hypersensitivity reactions. TPT also produced tumors in rats and mice [5,10].

Human exposure to PTs might come from occupational exposure and consumption of contaminated food. Very limited information is available on the toxic effects of TPT in humans. Because of the deleterious effect of this compound, the Occupational Safety and Health Administration (OSHA) has established workplace exposure limits of 0.1 mg m^3 of air for organotin compounds [5]. The Food and Agricultural Organization of the United Nations/ World Health Organization's (FAO/WHO) proposed a provisional tolerable daily intake (TDI) of 0.5 µg TPT/kg body wt/day [11]. Despite widespread occurrence in the environment and toxic health effects reported in animals and humans [5-9,12], information pertaining to PTs affect on human immune function mediated through natural killer (NK) lymphocytes has not been addressed. Human NK cells play a central role in immune defense against virus infection and formation of primary tumors [13, 14]. In this study, we used NK cells obtained from peripheral blood of adult male and female volunteer donors and measured the ability of PT treated versus control NK cells to kill K562 (human chronic myelogenous leukemia) tumor cell line. The effect of *in vitro* exposure to TPT and its metabolites/degradation products (monophenyltin (MPT), diphenyltin (DPT)) on the ability of NK cells to lyse tumor targets was measured using a ^{51}Cr release assay[4].

Material and Methods

Peripheral blood samples from healthy adult (male and female), volunteer donors were used for this study. Methods used for blood sampling, NK cell isolation and chemical preparations were described elsewhere [4, 7, 8]. Cell viability was determined by trypan blue exclusion. Prior to assay, the NK cells were separated by centrifugation from complete medium (RPMI 1640) and transferred to gelatin media. Assays were conducted at 1 hr, 24 h and 48 h. Cell numbers and viability were assessed at the beginning and end of the assays. Cell numbers and their viability did not vary among experimental conditions. NK cytotoxicity was measured using a ^{51}Cr release assay [15]. The target cell in all cytotoxicity assay was the NK-susceptible K562 cell line. Specific lysis was calculated as follows: 100x[(test c.p.m - spontaneous c.p.m.)/maximum c.p.m.-spontaneous c.p.m.)]. Maximum release was produced by adding 100 µL of 10% Triton X-100.

Results and Discussion

The concentrations of PTs (MPT, DPT and TPT) and duration of exposure were determined based on NK cell viability tests conducted prior to cytotoxicity assays. Treatment concentrations of 3, 5 and 10 µM MPT did not affect NK cell function after a 1-h exposure period. However, a 24-h preincubation of NK cells with the same concentrations significantly decreased the percent specific lysis at 5 µM and 10 µM compared to control. MBT suppressed NK cell function by about 6%, 32% and 98% respectively at the 50:1 lymphocyte:target (Table 1). Unlike MPT, a 1 h preincubation with 3, 5 and 10 µM DBT inhibited NK cell cytotoxic function by greater than 70%, 90% and 98% respectively (Table 1). DBT markedly reduced NK function after a 24-h exposure using lower concentrations: 1.0, 1.5 and 3.0 µM DPT showing inhibition of >85% at a 50:1 lymphocyte:target ratio. TPT appeared to be the more toxic than MPT and DPT. A one hour preincubation with 0.3, 0.5 and 1.0 µM TPT inhibited NK cell cytotoxic function by greater than 45%, 65% and 95% respectively at the 50:1 lymphocyte:target (data not shown). TPT drastically reduced NK function after a 24-h exposure using lower concentrations of 0.2, 0.5 and 1.0µM. A 24-h preincubation of NK cells at 0.2 µM TPT reduced NK cytotoxic function by over 80% at a 50:1 lymphocyte:target (Table 2). We also tested NK cell function after 48-hr exposure to TPT. NK cell tumor killing ability was greatly reduced. Percent inhibition of NK cell specific lysis of K562 tumor cells observed were 57%, 79% and 89% for exposure concentrations of 0.15 µM, 0.2 µM and 0.3 µM respectively. Both male and female donors showed similar percent inhibition of NK cells treated with PTs. The results revealed that the degree of inhibition varied with PT compound and the *in vitro* exposure concentration. The toxic potential followed the order of TPT > DPT > MPT.

The inhibitory effect of PTs on the tumorlytic function of NK cells was not a result of a toxic effect of PTs on the NK cell, resulting in NK cell death. PT concentrations used in the experiment did not kill NK cells during the period of study, as determined by the cell viability tests. Conjugation assays were performed to elucidate a possible mechanism for disruption of NK cell cytotoxic function when exposed to PTs. The ability of NK cells to

bind to tumor targets is not affected by a 1-h treatment with any of the PTs. However, a 24-h treatment with 0.5 µM TPT decreased binding of NK cells to tumor targets by about 57%. Pretreatment of NK cells with 3 µM DPT and 10 µM MPT decreased NK/target binding by about 83% and 54% respectively.

TABLE 1. Effect of varying concentrations of MPT and DPT on the ability of human NK cells to lyze K562 target cells. The percent specific lysis values for each of treatment concentrations are mean ± standard deviation of triplicate determinations.

Compound/ Exposure Time	Effector:Target	PERCENT LYSIS			
		Control	Concentrations		
			3 µM	5 µM	10 µM
MPT/24 h.	50:1	52.82 ± 4.73	46.37 ± 3.99	34.77 ± 2.69	1.32 ± 0.50
	25:1	40.45 ± 3.28	33.26 ± 3.80	19.92 ± 1.20	0.10 ± 0.05
	12.5:1	20.43 ± 3.88	15.03 ±1.04	10.62 ± 0.88	0.03 ± 0.03
DPT/1 h.	50:1	20.86 ±1.26	5.64 ± 1.78	1.56 ± 1.26	0.0
	25:1	11.52 ± 0.76	4.58 ± 0.69	0.10 ± 0.08	0.16 ± 0.07
	12.5:1	6.25 ± 1.53	2.86 ± 1.82	1.49 ± 0.51	0.10 ± 0.10

TABLE 2. Effect of varying concentrations of DPT and TPT on the ability of human NK cells to lyze K562 target cells. The percent specific lysis values for each of treatment concentrations are mean ± standard deviation of triplicate determinations.

Compound/ Exposure time	Effector:target	PERCENT LYSIS			
		Control	Concentrations		
			1.0 µM	1.5 µM	3 µM
DPT/24 h.	50:1	19.23 ± 1.11	2.67 ± 0.55	1.05 ± 0.29	1.40 ± 0.39
	25:1	7.07 ± 1.09	1.48 ± 1.18	0.98 ± 0.87	1.22 ± 0.80
	12.5:1	3.22 ± 0.64	0.98 ± 0.06	1.47 ± 0.75	0.85 ± 0.80
TPT/24 h.			0.2 µM	0.5 µM	1.0 µM
	50:1	43.10± 2.98	6.6 ± 1.43	0.11± 0.10	0.0 ± 0.0
	25:1	28.84 ± 1.24	3.87 ± 0.67	0.09 ± 0.06	0.0 ± 0.0
	12.5:1	18.63 ± 1.83	2.07 ± 0.56	0.4 ± 0.10	0.26 ± 0.05

TPT appears to have effect on immunity in both rats and mice as shown by the moderate changes in immunoglobulins IgG, IgA, IgM in both males and females[5]. Although bioassay of triphenyltin hydroxide for possible carcinogenicity studies conducted at National Cancer Research Institute using Fischer 344 rats and B6C3F1 mice concluded negative evidence for carcinogenicity [16], it is possible that phenyltin compounds suppress cancer fighting ability of NK cells and make the animal susceptible for viral infection and tumor formation. Little work is available to regarding human exposure to PTs and effect on NK lymphocytes. Reduced NK cell numbers have been reported in humans and marine mammals who consumed large quantities of fish contaminated with PCBs and DDT [17, 18]. An accidental exposure of phenyltin lead to death of a female worker due to renal failure 12 days after exposure [19]. Our recent study revealed that butyltin compounds, under in *vitro* experimental conditions, individual as well mixtures (mono-, di- and tributyltin) affected negatively on the function of human NK cells [4]. Based on inhibitory effect concentrations of butyltins and phenyltins on NK cell function *in vitro*, phenyltins appear to be approximately 5 times less toxic than butyltins. However, human exposure to PTs can occur by multiple routes (occupational exposure during manufacture, application in agricultural fields, consumption of contaminate fish from coastal and freshwater ecosystems etc.)[5,20]. The results of this study provide evidence that PTs significantly inhibit NK cell function and possible NK cell-mediated immunosuppressive potential in humans.

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