EFFECT OF *IN VITRO* EXPOSURE TO SELECTED ENDOCRINE DISRUPTING CHEMICALS ON HUMAN NATURAL KILLER (NK) CELL FUNCTION

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Introduction

In recent years, there has been increasing concern, worldwide, over compounds in the environment, which may adversely affect reproduction, immune function, and cancer formation in humans and wildlife[1-3]. Alkylphenols, such as nonylphenol (NP) and octylphenol (OP), and Bisphenol A are described as "endocrine disruptors" and have been shown to mimic or antagonize the actions of steroid hormones and cause serious health problems in fish and mammals. Nonyl and octylphenols are degradation products of alkylphenol ethoxylates (APEs) that are widely used surfactants in detergents, paints, emulsifying agents, pesticides, herbicides, and dispersing agents for agricultural and industrial applications[4, 5]. Bisphenol A (BPA) is used in polycarbonate plastics, epoxy resins, and phenoxy resins, which are utilized in food storage containers and in dental sealants[6]. These compounds enter the environment via industrial and municipal wastewater effluents[1-6]. Recently, these products are described as ubiquitous contaminants in the aquatic environment and are detected in surface waters, sediments and fish at concentrations ranging from nanogram to milligram per liter[2]. These compounds are reported to elicit estrogenic responses leading to irreversible abnormalities including impaired reproductive functions, immunotoxicity or cause cancer in aquatic organisms[4, 5,7]. Human exposure to these compounds may come from consumption of fish contaminated with NP, OP and BPA and occupational exposure during the manufacture and formulation of the compounds. However, little is known about their effect on immune function such as human natural killer (NK) cell function.

Human NK cell are lymphocytes are capable of killing tumor cells, virally infected cells and antibody-coated cells. NK cells limit the spread of blood-borne metastases as well as limit the development of primary tumors[8, 9]. The present study describes the effect of *in vitro* exposure to individual and mixtures of the estrogenic compounds on human NK cell function.

Materials and Methods

Peripheral blood from healthy adult (male and female), volunteer donors was used for this study. Methods used for blood sampling, NK cell isolation, chemical preparations were described elsewhere[9]. 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. Neat standards were resuspended in dimethylsulfoxide (DMSO). The chemicals were diluted in gelatin media (0.5% gelatin replaced the calf serum in complete medium) and serial dilutions were prepared to achieve a range of concentrations, so that the final concentration of DMSO did not exceed 0.01%. The ranges of concentrations prepared were 0.1, 0.5, 1.0, 5.0, 10μM, 1mM and 10mM. Assays were conducted at 24 h and 96 h. Cell numbers and viability

were assessed at the beginning and end of the assays. NK cytotoxicity was measured using ^{51}Cr release assay[9]. The target cell in all cytotoxicity assays was the NK-susceptible K562 (human chronic mylegenous leukemia) cells. 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 alkylphenols and bisphenol A and duration of exposure were determined based on NK cell viability tests conducted prior to cytotoxicity assays. Individual and combined *in vitro* effects of alkyl phenols and bisphenol A on human NK cell function are presented in Table 1.

Table 1. Effect of individual and mixtures of nonylphenol, octylphenol and bisphenol A on human NK cells *in vitro.* (*: indicate different donor).

Compound	Treatment Concentration	Exposure period	Result
Nonylphenol (NP)	1µM	24 h	No Inhibition
NP	10µM	24 h	32 % Inhibition
Bisphenol A (BPA)*	10µM	24 h	No Inhibition
NP + BPA	10µM + 1mM	24 h	100% Inhibition
NP + BPA	1µM + 1mM	24 h	88% Inhibition
Octylphenol (OP)	10µM	24 h	No Inhibition
OP + BPA	50µM + 1mM	24 h	90% Inhibition
NP	1µM	4 days	No Inhibition
NP	10µM	4 days	42% Inhibition
OP*	10µM	4 days	No Inhibition
NP + OP	1µM + 1µM	4 days	No Inhibition
NP + BPA	1µM + 10µM	4 days	No Inhibition

The results suggested that OP and BPA individually did not suppress NK cell function at 10µM exposure concentration for 24 h. However, 24 h exposure of 10µM NP inhibited NK cell function by about 30%. However, mixtures of NP+BPA (10µM + 1mM) remarkably inhibited (100%)NK cell function. Similarly, OP + BPA (at 50µM + 1mM exposure concentrations) also suppressed the cancer killing potential by about 90%. It appears that the BPA at 1mM level is very inhibitory and there may be some synergy with NP and OP.

The results of this laboratory *in vitro* study provide evidence that nonylphenol, octylphenol and bisphenol-A negatively affect human natural killer cell function and possible NK cell mediated immunotoxic potential in humans. This study emphasizes the need for further investigations dealing with alkylphenols monitoring in human blood and mechanism of action.

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