EFFECT OF ENDOCRINE DISRUPTING CHEMICALS ON LYMPHOCYTE RESPONSES

Tomomi Yamazaki[1], Yumiko Okada[2], Yoshiharu Hisamatsu[3], Shunichiro Kubota[4] and Fujio Kayama[5]

National Institute of Public Health, Minato-ku, Tokyo 108-8638, Japan
[1]Department of Nutrition and Biochemistry, National Institute of Public Health, 4-6-1 Shirokanedai, Minato, Tokyo 108-8638, Japan
[2]Department of Veterinary Public Health, National Institute of Public Health, 4-6-1 Shirokanedai, Minato, Tokyo 108-8638, Japan
[3]Department of Community Environmental Sciences, National Institute of Public Health, 4-6-1 Shirokanedai, Minato, Tokyo 108-8638, Japan
[4]Department of Physiological Chemistry and Metabolism, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo 113-0033, Japan
[5]Division of Environmental Immunology and Toxicology, Department of Health Science, Jichi Medical School, 3311-1 Yakushiji, Kawachi, Tochigi 329-1100, Japan

Introduction

Endocrine disrupting chemicals (EDC), compounds that modify natural endocrine function, have emerged as a major public health issue due to their potentially disruptive effects on physiological processes through interaction with steroid hormone receptors[1]. They are also reported to disrupt the immune system in the wildlife[2-7]. T-cell activation is a key step in the initiation of an immunological response. Stimulation of T cells by specific antigens, mitogenic lectins, or monoclonal antibody directed against the TCR-CD3 complex activates a cascade of signaling events and the combination of these events lead to the initiation of IL-2 transcription[8-12]. The rise and fall in IL-2 gene expression is paralleled by changes in T-cell proliferation. Thus, the regulation of the IL-2 gene is critically involved in the control of T-cell growth and normal immune response[11, 13].

In the present study, we investigated the effect of bisphenol A (BPA), 4-nonylphenol (NP), benzyl *n*-butyl phthalate (BBP), di-*n*-butyl phthalate (DBP), dicyclohexyl phthalate (DCHP), di (2-ethylhexyl) phthalate (DEHP) and diethyl phthalate (DEP) on human lymphocyte responses to Concanavalin A (Con A).

Materials and Methods

Human lymphocytes were prepared from the venous blood of healthy volunteers by centrifugation on Ficoll-conray. Monocytes were depleted by Sephadex G-10 column. The lymphocyte fraction contained less than 1 % monocytes. Lymphocytes were suspended in serum replacement solution-supplemented phenol red-free RPMI 1640 at a density of 1 x 10^6 cells / ml and incubated with various concentrations of tested chemicals in a round bottom, 96 - well microplate, each in triplicate, in an humidified atmosphere of 5 % CO_2 for 4 h. After the incubation, Con A was added to a concentration of 10 %. Then the cells were incubated for 3 days under the same conditions. At 16 h before the end of the incubation, [^{3}H]thymidine was added at a concentration of 1 μCi / ml. After this period, the cells were harvested on a glass fiber filter, and its radioactivity was counted in scintillation fluid. The stimulation index is the ratio of the average cpm of each case with Con A to that with tested chemicals alone.

For the lactate dehydrogenase (LDH) assay, lymphocytes were incubated with various concentration of EDC for 3 days and medium was centrifuged. The activity of LDH of the supernatants was determined using Cyto Tox 96 Non-Radioactive Cytotoxicity Assay (Promega, Madison, WI) according to the manufacturer's instruction. The percentage LDH activity was calculated as the percentage of activity in the supernatant relative to that obtained from lysed lymphocytes.
The IL-2 levels in the cell culture supernatants obtained 24h after stimulation were determined using Human Interleukin-2 ELISA kit (Endogen, Inc. Woburn, MA) according to the manufacturer's instruction.
Total RNA was isolated from cells by the AGPC method[14]. The RNA pellets were dissolved in sterile water, and quantified by optical density at 260 nm. cDNAs were synthesized from 1 μg of total RNA using Thermoscript RT(Life Technologies, Inc. Rockville, MD) and PCR was performed using TaKaRa Taq (Takara, Japan) according to the manufacturer's instruction. The forward PCR primers were: 5'-TTCAGATAATCGACGCCAGG-3' for estrogen receptor α and 5'-CCAAGAGAAGTGGCGGCCACG-3' for estrogen receptor β. The reverse PCR primers were: 5'-TCATCTCTCTGGCGCTTGTG-3' for estrogen receptor α and 5'-TGATTTTGGAGGGATCTCGC-3' for estrogen receptor β.

Results and Discussion

BPA, NP, BBP, DBP, DCHP and DEHP decreased lymphocyte proliferative responses to Con A (Table 1). DEP (up to 10^{-4} M) had no effect on lymphocyte proliferative activity. LDH activity was measured and shown in Table 1. At 10^{-4} M of BPA, NP, BBP, DBP, DCHP, and DEHP, lymphocytes did not show response to Con A but LDH activities increased only about 10 %. To assess the effects of EDC on cytokine production, human lymphocytes were incubated with Con A and EDC, and the resulting supernatants were collected and analyzed for IL-2. Negligible levels of IL-2 were found in culture supernatants without Con A stimulation, and these were not affected by the presence of EDC. IL-2 levels were significantly decreased in cultures incubated with Con A and 10^{-4} M of BPA, NP, BBP, DBP, DCHP, DEHP and DEP (Table 1). Next, to determine whether the effect of these chemicals on lymphocytes could be mediated by the estrogen receptor, 10^{-6} M of ICI 182, 780, a high affinity estrogen receptor antagonist, was added in the experiment of lymphocyte prolirferative responses to Con A. Interestingly, ICI 182, 780 did not restore the lymphocyte proliferative activity. The mRNAs for estrogen receptor α and estrogen receptor β were detected in lymphocytes by RT-PCR analysis. Taken together, EDC may affect lymphocyte functions by estrogen receptor and/or other pathways.

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Table 1 The effect of EDC on lymphocyte functions

	Concentration (M)	SI	IL-2 production (pg / ml)	LDH (%)
Control (+DMSO)		3 . 99 ± 0 . 95	271 ± 39	0 . 00
BPA	10^{-7}	3 . 89 ± 0 . 66	324 ± 14	0 . 00
	10^{-6}	3 . 66 ± 0 . 68	413 ± 43	0 . 35 ± 0 . 03
	10^{-5}	0 . 89 ± 0 . 52	226 ± 9	0 . 75 ± 0 . 03
	10^{-4}	0 . 86 ± 0 . 41	17 ± 4	6 . 57 ± 0 . 86
NP	10^{-8}	3 . 12 ± 0 . 53	287 ± 22	0 . 18 ± 0 . 01
	10^{-7}	2 . 68 ± 0 . 83	317 ± 30	0 . 17 ± 0 . 03
	10^{-6}	0 . 95 ± 0 . 42	322 ± 63	0 . 35 ± 0 . 02
	10^{-5}	0 . 94 ± 0 . 35	8 ± 4	6 . 77 ± 0 . 95
	10^{-4}	0 . 91 ± 0 . 64	0	11 . 94 ± 1 . 08
BBP	10^{-7}	3 . 76 ± 0 . 87	322 ± 34	0 . 00
	10^{-6}	2 . 81 ± 0 . 85	305 ± 10	0 . 00
	10^{-5}	2 . 69 ± 0 . 63	227 ± 32	0 . 52 ± 0 . 44
	10^{-4}	1 . 12 ± 0 . 50	26 ± 9	3 . 98 ± 0 . 07
DBP	10^{-7}	4 . 19 ± 1 . 00	358 ± 51	0 . 38 ± 0 . 33
	10^{-6}	4 . 06 ± 0 . 81	407 ± 63	0 . 35 ± 0 . 0 5
	10^{-5}	2 . 96 ± 0 . 88	276 ± 4	0 . 17 ± 0 . 02
	10^{-4}	1 . 01 ± 0 . 45	3 ± 1	10 . 21 ± 0 . 99
DCHP	10^{-7}	4 . 43 ± 0 . 87	247 ± 45	0 . 00
	10^{-6}	4 . 11 ± 0 . 85	301 ± 47	0 . 87 ± 0 . 06
	10^{-5}	1 . 80 ± 0 . 51	184 ± 20	3 . 81 ± 0 . 24
	10^{-4}	1 . 04 ± 0 . 42	36 ± 8	10 . 23 ± 1 . 02
DEHP	10^{-7}	4 . 64 ± 0 . 98	248 ± 12	0 . 35 ± 0 . 04
	10^{-6}	4 . 03 ± 0 . 66	319 ± 35	1 . 21 ± 0 . 08
	10^{-5}	0 . 84 ± 0 . 52	106 ± 6	6 . 22 ± 0 . 53
	10^{-4}	0 . 73 ± 0 . 41	51 ± 10	13 . 67 ± 1 . 13
DEP	10^{-7}	4 . 29 ± 0 . 69	251 ± 22	0 . 69 ± 0 . 06
	10^{-6}	4 . 38 ± 0 . 52	294 ± 48	0 . 52 ± 0 . 06
	10^{-5}	4 . 34 ± 0 . 64	265 ± 26	0 . 52 ± 0 . 04
	10^{-4}	4 . 01 ± 1 . 05	139 ± 20	0 . 87 ± 0 . 07