—Review—

New Mechanism of Organophosphorus Pesticide-induced Immunotoxicity

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Abstract

Organophosphorus pesticides (OPs) are widely used throughout the world as insecticides in agriculture and as eradicating agents for termites around homes. The main toxicity of OPs is neurotoxicity, which is caused by the inhibition of acetylcholinesterase. OPs also affect the immune response, including effects on antibody production, interleukin-2 production, T cell proliferation, decrease of CD5 cells, and increases of CD26 cells and autoantibodies, Th1/Th2 cytokine profiles, and the inhibition of natural killer (NK) cell, lymphokine-activated killer (LAK) cell, and cytotoxic T lymphocyte (CTL) activities. However, there have been few studies of the mechanism of OP-induced immunotoxicity, especially the mechanism of OP-induced inhibition of cytolytic activity of killer cells. This study reviews new mechanisms of OP-induced inhibition of the activities of NK cells, LAK cells, and CTLs. It has been reported that NK cells, LAK cells, and CTLs induce cell death in tumors or virus-infected target cells by two main mechanisms. The first mechanism is direct release of cytolytic granules that contain the pore-forming protein perforin, several serine proteases termed granzymes, and granulysin by exocytosis to kill target cells, which is called the granule exocytosis pathway. The second mechanism is mediated by the Fas ligand (Fas-L)/Fas pathway, in which FasL (CD95 L), a surface membrane ligand of the killer cell cross links with the target cell's surface death receptor Fas (CD95) to induce apoptosis of the target cells. To date, it has been reported that OPs inhibit NK cell, LAK cell, and CTL activities by at least the following three mechanisms: 1) OPs impair the granule exocytosis pathway of NK cells, LAK cells, and CTLs by inhibiting the activity of granzymes, and by decreasing the intracellular levels of perforin, granzyme A, and granulysin, which were mediated by inducing degranulation of NK cells and by inhibiting the transcription of the mRNAs of perforin, granzyme A, and granulysin. 2) OPs impair the FasL/Fas pathway of NK cells, LAK cells, and CTLs, as investigated by using perforin-knockout mice, in which the granule exocytosis pathway of NK cells does not function and only the FasL/Fas pathway remains functional. 3) OPs induce apoptosis of immune cells.
(J Nippon Med Sch 2007; 74: 92–105)

Key words: apoptosis, granulysin, granzyme, natural killer cells, organophosphorus pesticide, perforin

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Introduction

Organophosphorus pesticides (OPs) are widely used throughout the world as insecticides in agriculture and as agents for eradicating termites around homes[1–3]. There is still a large of quantity of OPs on the market in Japan[4]. OPs are potent inhibitors of serine esterases, such as acetylcholinesterase and serum cholinesterase[1–3,5]. The main toxicity of OPs is neurotoxicity, which is caused by the inhibition of acetylcholinesterase[1–3,5,6]. It has been reported that OPs affect immune response including effects on neutrophil function[7], macrophage[8–11], antibody production[12,13], interleukin (IL)-2 production[14], serum complement[15], and T-cell proliferation induced by IL-2[16], concanavalin A (Con A), and phytohemagglutinin (PHA)[17] in animals and humans. Thrasher et al.[18,19] have reported that higher-than-usual frequencies of allergies and sensitivities to antibiotics, a decrease in CD5 cells, and increases in CD26 cells and autoantibodies were found in patients following chlorpyrifos exposure. Increased expression of CD26 cells and decreased expression of CD5 cells are associated with autoimmunity, where an individual's immune system acts against itself, rather than against infections[20]. Rodgers has also reported that oral administration of malathion increases levels of anti-dsDNA antibodies in MRL-lpr mice[21]. Exposure to chlorpyrifos is associated with multiple chemical sensitivity[22,23].

Potential Mechanism of OP-induced Immunotoxicity

There are very few papers dealing with the mechanism of OP-induced immunotoxicity. Gallowway and Habdy[24] have reviewed the potential mechanism of OP-induced immunotoxicity, including the direct and indirect effects. OPs may inhibit any of the serine hydrolase class of enzyme in the immune system, including the complement[11] and thrombin systems, which influence immune function. The OP-induced inhibition of neuropathy target esterase in lymphocytes may lead to structural or functional changes in lymphocytes[25]. Histopathological damage to lymphoid tissues resulting from phosphorylation, oxidative damage, or altered neural function induced by OPs could hinder the development and viability of lymphocytes[26]. On the other hand, Osicka-Koprowska et al.[27] have reported that chlorfenvinphos induces a significant increase in corticosterone in rat plasma, suggesting that indirect immune alterations may be mediated by cholinergic responses or by stress following neurotoxic doses. Videira et al.[28] have reported that malathion, methylparathion, and parathion can directly damage cell membranes by affecting membrane lipid physicochemical properties, which may indirectly influence the function of immune cells. We have also found that organophosphorus compounds induce an increase in sister chromatid exchanges (SCEs) in human lymphocytes, which could indirectly influence the function of lymphocytes[29,30].

Effect of OPs on Th1/Th2 Cytokine Profiles

Exposure to OP has been linked to asthma or asthma-related symptoms in a small number of epidemiologic studies. Hoppin et al.[31] have reported that OP exposure in the preceding year is related to wheezing in a study of pesticide applicators in Iowa and North Carolina. Respiratory, asthma-like symptoms were associated with exposures to OPs in occupational and environmental settings among villagers in rural China[32]. Asthma is characterized by chronic inflammation in the airways and a predominance of $CD4^+$ T-helper 2 (Th2) cells that secrete IL-4, IL-5, and IL-13 cytokines[33]. Th2 cells contribute to the immunopathogenesis of asthma by recruiting eosinophils and mast cells to the airways[33–35] and by inducing B-cells to produce immunoglobulin E antibodies[36]. Conversely, T-helper 1 (Th1) cells that secrete interferon (IFN)-γ are thought to protect against the development of asthma by regulating Th2 cytokine production, although a mixed Th1/Th2 pattern has been reported[37]. Patients with allergies and asthma are more likely to have elevated levels of the Th2 cytokines IL-4 and IL-5 and reduced levels of the Th1 cytokines IFN-γ and tumor necrosis factor

(TNF)-β[33,38,39]. These findings suggest that OPs may have effects on the Th1/Th2 cytokine profiles. Thus, Duramad et al[40] investigated the effect of chlorpyrifos on the expression of Th1/Th2 cytokines in human blood by in vitro treatment and found that although chlorpyrifos did not induce cytokine expression in vitro, it did increase lipopolysaccharide (LPS)-dependent induction of IFN-γ. Moreover, Duramad et al[41] have found that early environmental exposure to OPs may affect intracellular Th1/Th2 cytokine profiles in 24-month-old children living in an agricultural area of Salinas Valley, California.

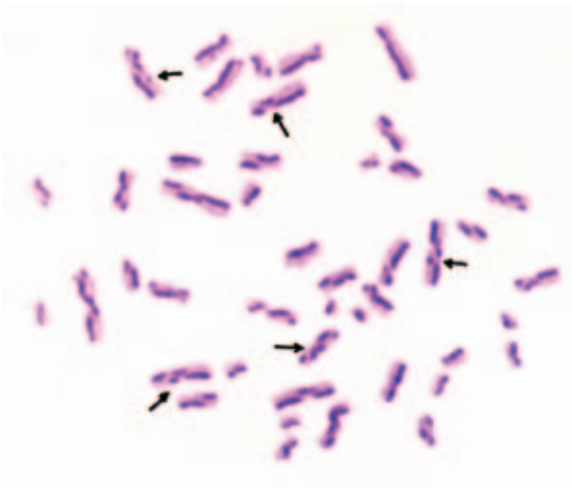


Fig. 1 A photograph of SCEs induced by N,N-diethylaniline-HCl at 500 ppm in the presence of a 20% S-9 mix by a 1-hour *in vitro* treatment (10×100). Narrows indicate SCE. Cited from Li Q and Minami M, Mutation Res 1997; 395, 151–157[45].

Organophosphorus Compounds Inhibit NK Cell, LAK Cell, and CTL Activity

On March 20, 1995, the nerve gas sarin (isopropylmethylphosphonofluoridate) was used in a terrorist attack by the members of the Aum Shinrikyo cult on subway trains in the Tokyo metropolitan area, killing 12 people and injuring more than 5,000[42]. Sarin is an organophosphorus nerve agent and a strong cholinesterase inhibitor[43]. To monitor the genetic aftereffects of sarin exposure, we measured SCEs in peripheral blood lymphocytes of the victims and found that the frequency of SCEs in lymphocytes was significantly higher in the victims than in the control group[29,44]. We also found that the by-products generated during sarin synthesis, i.e., diisopropyl methylphosphonate (DIMP), diethyl methylphosphonate (DEMP), and ethyl isopropyl methylphosphonate (EIMP), also induce SCEs in human lymphocyte *in vitro*[29]. On the other hand, in the Tokyo sarin attack, the victims were also exposed to *N,N*-diethylaniline, a stabilizing reagent of sarin synthesis. We first found that *N,N*-diethylaniline significantly increased the frequency of SCEs of human lymphocytes *in vitro*[45] **(Fig. 1)**. Because DIMP, DEMP and *N,N*-diethylaniline induce a high frequency of SCEs of lymphocytes, we have speculated that DIMP, DEMP, and *N,N*-diethylaniline also affect the function of lymphocytes. Thus, we investigated the effects of DIMP, DEMP, and *N,N*-diethylaniline on natural killer cell (NK) and cytotoxic T lymphocyte (CTL) activities both *in vitro* and *in vivo* and found that DIMP, DEMP, and *N,N*-diethylaniline significantly inhibit human and murine NK cells and murine CTL activities both *in vitro* and *in vivo*, suggesting a relationship between the increased SCEs in lymphocytes and decreased NK cell, CTL, and LAK cell activities induced by DIMP, DEMP, and *N,N*-diethylaniline[30,46]. Both DIMP and DEMP are potent inhibitors of serine esterases, such as acetylcholinesterase and serum cholinesterase, which are similar to OPs in toxicity[47]. Thus, we speculate that OPs also may inhibit NK cell and CTL activities as do DIMP and DEMP. To clarify whether OPs also affect NK cell and CTL activities, we first investigated five OPs—dimethyl 2,2-dichlorovinyl phosphate (DDVP, dichlorvos), dimethyl 2,2,2-trichloro-hydroxyethylphosphonate (DEP), dimethoate (DMTA), acephate and S-2-ethylsulfinyl-1-methylethyl O, O-dimethyl phosphorothioate (ESP)—on human NK cell activity. We found that all five OPs significantly decreased human NK cell activity in a dose-dependent manner and that the strength of inhibition differed among the five OPs in the following order: DDVP>DMTA ≥ DEP ≥ ESP > acephate[48] **(Fig. 2)**. Then we investigated the effect of DDVP on murine splenic NK cell, lymphokine-activated killer (LAK) cell, and CTL activities and human LAK cell and CTL

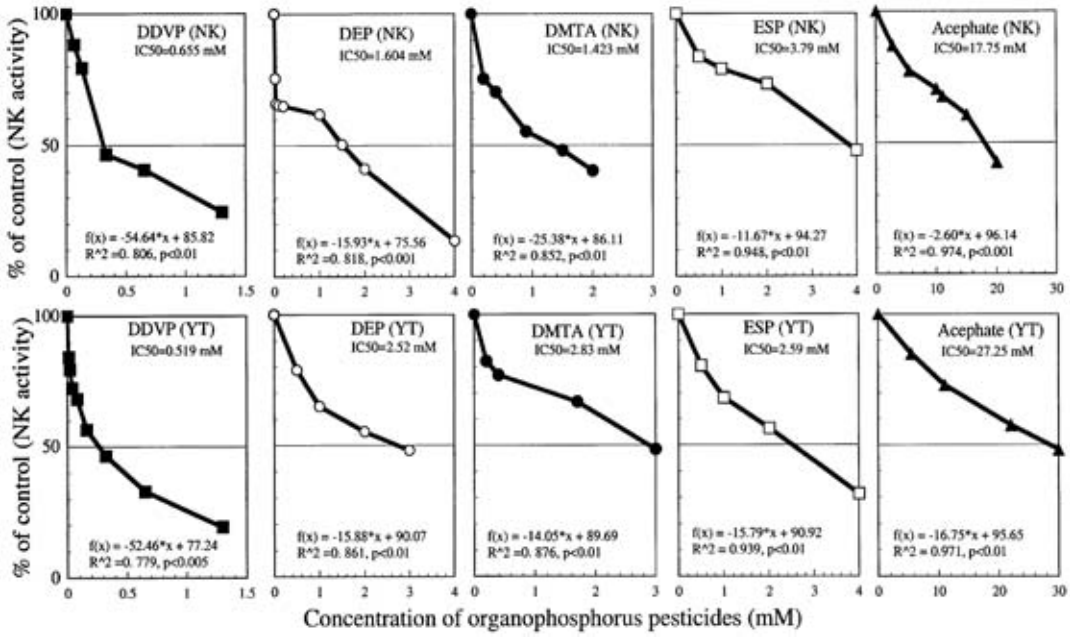


Fig. 2 Effect of OPs on human NK cell activity and YT cell activity *in vitro*. IC50: inhibitory concentration of 50% NK cell activity. The YT cell is a human NK cell line. Cited from Li Q et al., Toxicology 2002, 172: 181–190[48].

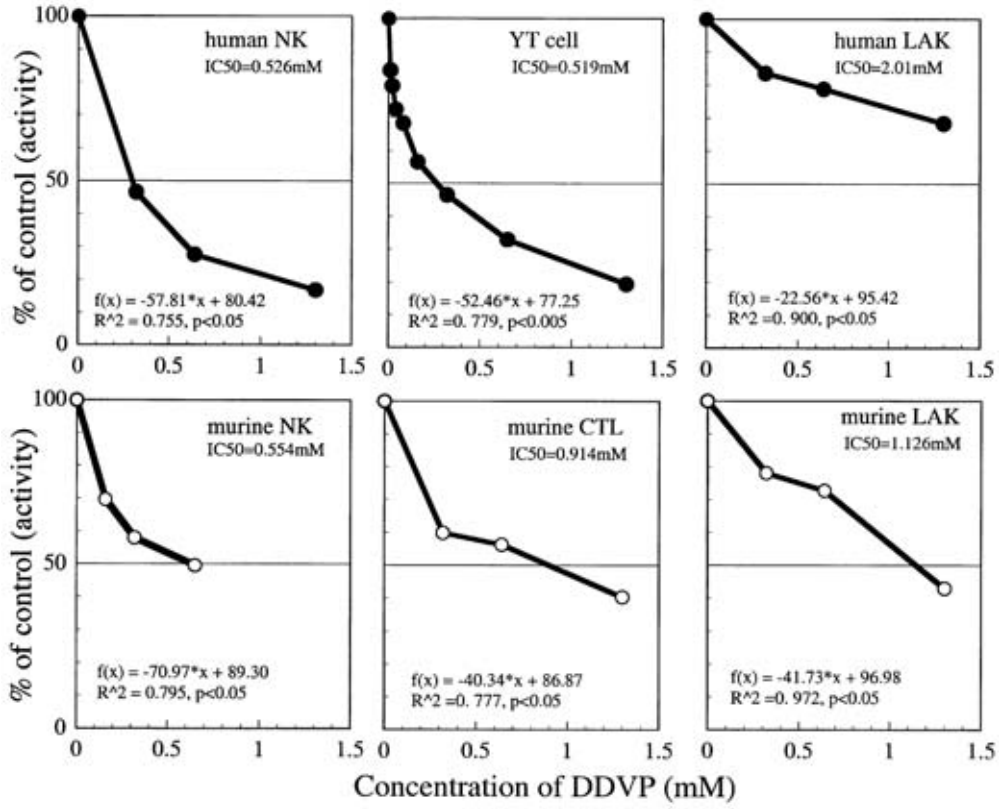


Fig. 3 Effect of DDVP on NK cell, CTL, and LAK cell activities *in vitro*. The activity values for the 40/1 (human NK cell), 10/1 (YT cell, human and murine LAK cells), 80/1 (murine NK cell), and 20/1 (murine CTL) of E/T ratios are presented, and similar results were obtained with other E/T ratios for each effector cell. Cited from Li Q et al., Toxicology 2002, 172: 181–190[48].

Table 1 Summary of organophosphorus compound-induced immunotoxicity[56]

Targets (Cells)	Parameters	Effects	Human/Animal	References
Neutrophils	Neutrophil function	↓	Human	7
Macrophages	Productions of estalase and neutral proteases, phagocytic capability and size of Mϕ	↑ ↑ ↑ ↑	Mice	8-11
B cells	Ab production (IgG/IgM)	↓ ↓	Mice	12, 13
	B cell response to LPS	→	Mice	13
	B cell population (CD19)	→→	Human	18, 19
	Autoantibodies	↑ ↑ ↑	Human/mice	18, 19, 21
T cells	IL-2 dependent proliferation	↓	Mice	16
	IL-2 production	↓	Rat	14
	Response to Con A or PHA	↓	Rat	17
	T cell subset, CD4, CD5, CD26	CD4↓→CD5↓↓CD26↑↑	Human	18, 19
	CTL activity	↓ ↓ ↓ ↓ ↓	Human/mice	48, 51, 53-55
	Th1/Th2 cytokine profiles	Th2↑, Th1↑	Human	40, 41
NK cells	NK activity	↓ ↓ ↓ ↓ ↓ ↓ ↓	Human/mice	48-54
	Granzyme activity	↓	Human	48
	Expressions of perforin, granulysin, GrA	↓ ↓	Human	49, 50
LAK cells	LAK activity	↓ ↓	Human/mice	48, 51
Others	FasL/Fas pathway	↓	Mice	51
	Apoptosis	↑ ↑ ↑ ↑ (Positive)	Human/mice	74-77
	Complement activity	↓	Human	15
	Multiple chemical sensitivity	↑ ↑	Human	22, 23
	Asthma or asthma-related symptoms	↑ ↑	Human	31, 32
	Neuropathy target esterase	↓	Human	25
	Histopathological damage in lymphoid tissues	↑ (Positive)	Mice	26
	Corticosterone in plasma (stress)	↑	Rat	27
	Sister chromatid exchanges in lymphocytes	↑ ↑ ↑	Human	29, 30, 44

↓ : Inhibition/decrease; ↑ : Increase/Activation (Induction); → : No effect. Numbers of the arrow show the number of references.

activities. DDVP significantly decreased human NK cell[48–50], LAK cell[48], and murine NK cell, LAK cell and CTL activities *in vitro*[48] and *in vivo*[51] in a dose-dependent manner, and the degree of decrease in these activities differed among the effector cells investigated. The order was as follows: human NK cells>murine NK cells>murine CTLs>murine LAK cells>human LAK cells[48] **(Fig. 3)**.

Zabrodskii and Germanchuk[52] have also reported that subcutaneous injection of DDVP at 0.2 of the median lethal dose (LD50) and 0.8 of the LD50 significantly inhibited NK cell activity and antibody-dependent cell cytotoxicity in Wistar rats. Rodgers et al. have reported that O, O, S-trimethyl phosphorothioate, an impurity in technical formulations of malathion, inhibited human NK cell activity *in vitro*[53] and murine and human CTL activity *in vivo* and *in vitro*[54,55]. **Table 1** summarizes the immunotoxicity of organophosphorus compounds in humans and animals[56].

However, there have been few studies of the mechanisms of OP-induced inhibition of NK cell, LAK cell, and CTL activities. We review the new mechanisms of OP-induced inhibition of cytolytic activity of killer cells in the present study.

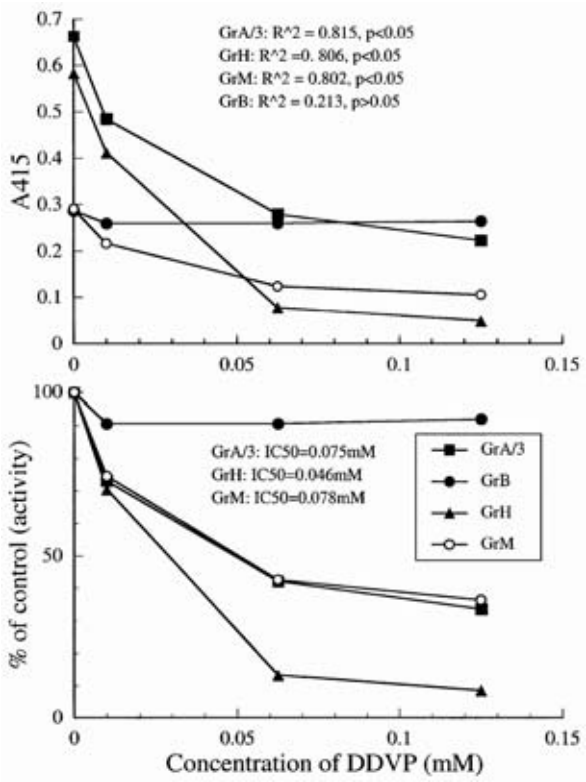


Fig. 4 Inhibitory effect of DDVP on activity of human granzymes A, B, 3, H, M. IC50: inhibitory concentration of 50% activity of granzymes. Cited from Li Q et al., Toxicology 2002, 172: 181–190[48].

OPs Impair the Granule Exocytosis Pathway of Killer Cells

It has been reported that NK cells, LAK cells, and CTLs induce tumor or virus-infected target cell death by two main mechanisms[57–59]. The first mechanism is the direct release of cytolytic granules that contain the pore-forming protein perforin, several serine proteases termed granzymes[60], and granulysin[61] by exocytosis to kill target cells. The second mechanism is mediated by the Fas ligand (FasL)/Fas pathway, in which FasL (CD95 L), a surface membrane ligand of the killer cell cross links with the target cell's surface death receptor Fas (CD95) to induce apoptosis of the target cells[51,59,62]. Human NK cells, LAK cells and CTLs have been shown to express five granzymes. Granzyme A (GrA) is expressed in NK cells, PHA-or CD3-stimulated T cells, and gamma/delta T cells and has a trypsin-like specificity, which cleaves on the carboxyl side of basic residues, such as arginine and lysine[63,64]. GrB is expressed in NK cells, PHA-or CD3-stimulated T cells, gamma/delta T cells, and cleaves on the carboxyl side of aspartic acid residues[64,65]. Gr3/K is expressed in T cells and IL-2 or ConA-stimulated T cells, NK cells and peripheral blood lymphocytes (PBLs) and is trypsin-like (cleavage after basic residues)[66,67]. GrH is expressed in IL-2- or PHA-stimulated PBLs and CTLs, and prefers cleavage after hydrophobic residues, such as phenylalanine[68]. GrM is expressed in NK cells and gamma/delta T cells, and cleaves on the carboxyl side of methionine, leucine, or norleucine[64,69].

OPs are potent inhibitors of serine esterases, such as acetylcholinesterase and serum cholinesterase[1,2], and granzymes are also serine esterases (proteases)[60,63,65–69]. Thus, we speculate that the decrease in NK cell, LAK cell, and CTL activities by OPs may be mediated by the inhibition of serine proteases (granzymes), which are released from NK cell, LAK cell, and CTL granules by exocytosis when target cells conjugate with the effector cells. To explore the underlying mechanism of the decrease in cytolytic activity in killer cells, we investigated the effects of DDVP on the enzymatic activity of human granzymes and found that DDVP significantly inhibits the enzymatic activity of human GrA, Gr3, GrH, and GrM in a dose-dependent manner. The IC50 (inhibitory concentration of 50% granzyme activity) values were 0.05 mM for GrA and Gr3, 0.03 mM for GrH and 0.05 mM for GrM (**Fig. 4**). To support our hypothesis that OP-induced inhibition of the cytolytic activity of killer cells is mediated by the inhibition of granzymes (serine proteases), we investigated the effect of 4-(2-aminoethyl) benzenesulfonyl fluoride-HCl (*p*-ABSF), an inhibitor of serine proteases, on NK cell, LAK cell, and CTL activities. We found that *p*-ABSF significantly decreased the activities of human and murine NK cells and LAK cells and of a murine CTL line in a dose-dependent manner, and that the degree of decrease in those activities also differed among the effector cells in the following order: human NK>murine NK>murine CTL line>murine

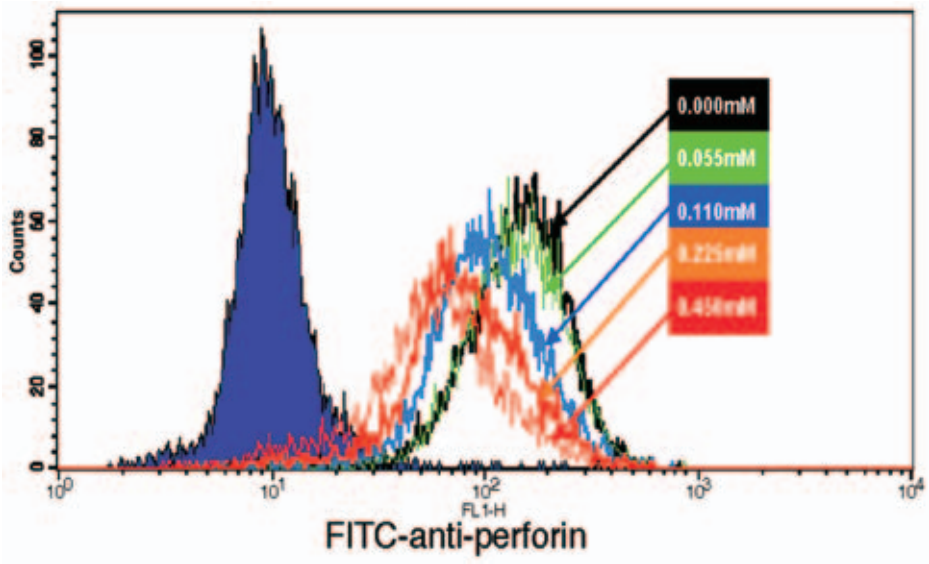


Fig. 5 Effect of DDVP on the expression of perforin in human NK-92CI after a 15-hour in vitro treatment. The X axis shows the fluorescent intensity of fluorescein isothiocyanate (FITC)-anti-perforin, which represents the intracellular level of perforin, and the Y axis shows the counts of NK cells. The solid histogram shows the control stained with FITC-mouse IgG2b (isotype control), and the blank histograms show the results of staining with FITC-mouse anti-human perforin after treatment with DDVP at 0 (black), 0.055 (green), 0.110 (blue), 0.225 (orange) and 0.452 (red) mM from the right to the left, respectively. Cited from Li Q et al., Toxicology 2005, 213: 107–116[49].

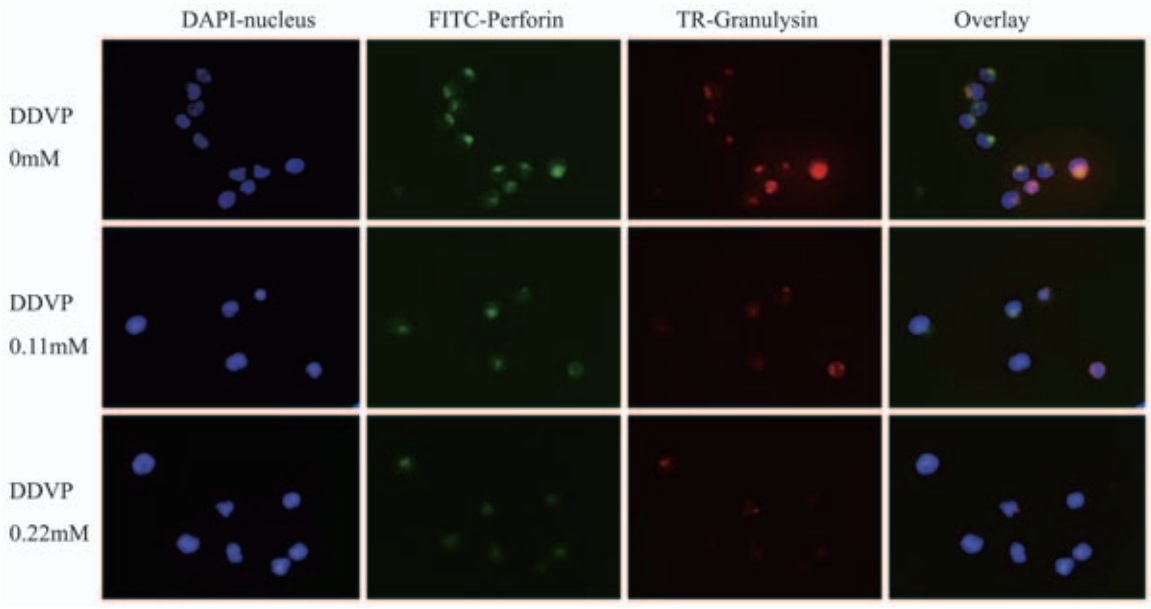
LAK>human LAK. This order coincides with the results obtained with DDVP, suggesting that DDVP and *p*-ABSF have a common inhibiting mechanism on NK cell, LAK cell and CTL activities. In addition, the decreases in NK cell, LAK cell, and CTL activities induced by *p*-ABSF + DDVP were greater than those by either *p*-ABSF alone or DDVP alone in the same concentration, suggesting that DDVP and *p*-ABSF have an additive inhibitory effect on NK cell, CTL, and LAK cell activities. Taken together, the above-mentioned findings indicate that OPs significantly decrease NK cell, LAK cell, and CTL activities *in vitro*, at least partially mediated by granzyme inhibition[48].

To investigate whether OPs also affect the expression of granzyme, granulysin, and perforin, we treated a human NK cell line, NK-92, with DDVP *in vitro* and then analyzed the expressions of granzyme, granulysin, and perforin with flow cytometry and reverse transcriptase polymerase chain reaction. We found that DDVP significantly decreased the expression of perforin **(Fig. 5)**, granzyme A, and granulysin in NK-92CI and NK-92MI cells in a dose-dependent manner[49,50]. DDVP also has a modest but significant inhibitory effect on the transcription of the mRNAs of perforin, granzyme A, and granulysin. Moreover, we found that the decreases in perforin, granzyme A, and granulysin in the granules of NK-92CI cells parallel a similar pattern determined by immunocytochemical analysis, which strongly suggests the possibility of degranulation[49].

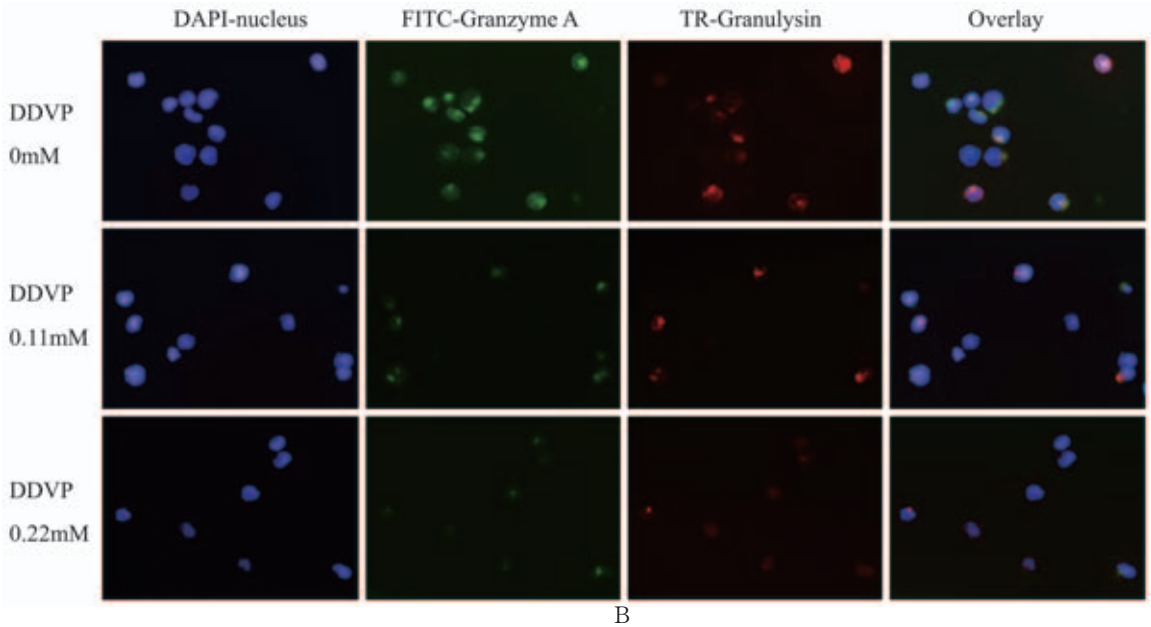
Taken together these findings indicate that DDVP inhibits the enzymatic activity of granzymes[48], the expression of granzymes, granulysin, and perforin in human NK cells, and the induction of degranulation of NK cells[49,50] **(Fig. 6)**.

OPs Impair the FasL/Fas Pathway of Killer Cells

Only one paper has investigated whether OPs affect the FasL/Fas pathway of killer cells using perforin-knockout (PKO) mice[51]. It has been reported that the granule exocytosis pathway in PKO mice does not function against Fas antigen-negative target cells[51,62,70] and that the NK cells, CTLs, and



A



B

Fig. 6 Effects of DDVP at 0.11 and 0.22 mM on intracellular perforin/granulysin (A) and granzyme A/granulysin (B) in NK-92CI cells after a 15-hour in vitro treatment. The NK-92CI cells were fixed/permeablized with Cytofix/cytoperm solution, and then double-staining of perforin/granulysin and granzyme A/granulysin were performed. The intracellular perforin and granzyme A were stained with FITC-anti-human perforin and granzyme A, respectively. Intracellular granulysin was first stained with rabbit anti-human granulusin polyclonal antibody, then stained with Texas red-goat anti-rabbit IgG. Cited from Li Q et al., Toxicology 2005, 213: 107–116[49].

LAK cells of PKO mice kill targets only by the FasL/Fas pathway[51,70]. Thus, we used PKO mice to investigate the effect of DDVP on the FasL/Fas pathway by determining the NK cell, CTL, and LAK cell activities in PKO mice.

In this study, we found that DDVP significantly decreased the NK cell, CTL, and LAK cell activities of PKO mice in a dose-dependent manner (**Fig. 7**) and that the CTL and LAK cell activities of PKO mice were significantly blocked by an anti-FasL antibody, suggesting that DDVP and the anti-FasL antibody have the same or a similar mechanism of

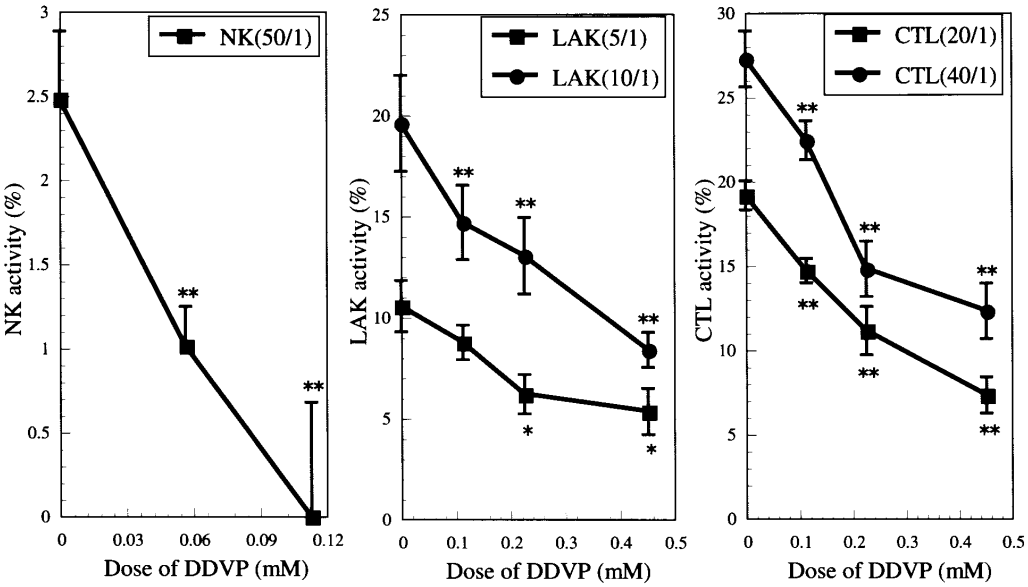


Fig. 7 Effect of DDVP on NK cell, LAK cell, and CTL activities of PKO mice *in vitro*. Cited from Li Q et al., Toxicology 2004; 204: 41–50[51].

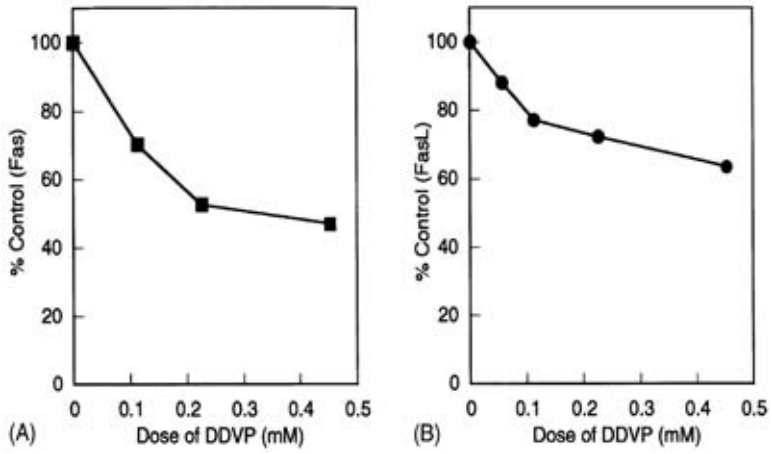


Fig. 8 Effect of DDVP on the expression of Fas antigen on the surface of YAC-1 cells (A) and on the expression of FasL on the surface of LAK cells (B). Cited from Li Q et al., Toxicology 2004; 204: 41–50[51].

inhibiting LAK cell and CTL activities. Moreover, DDVP decreases the expression of Fas antigen on YAC-1 cells (a target cell in NK cell activity assay) and the expression of FasL on LAK cells in a dose-dependent manner (**Fig. 8**). Taken together, these findings indicate that the DDVP-induced inhibition of NK cell, LAK cell, and CTL activities in PKO mice is mediated by the impairment of the FasL/Fas pathway[51].

OPs Induce Apoptosis of Immune Cells

It has been reported that OPs induced apoptosis in rat primary cortical neurons[71], in SH-SY5Y human neuroblastoma cells[72], and in murine preimplantation embryos[73]. On the other hand, 4 papers have reported that OPs induce apoptosis of immune cells[74–77]. Saleh et al[74,75] have shown that paraoxon (the bioactive metabolite of parathion) and parathion cause apoptotic cell death in a murine EL4 T-

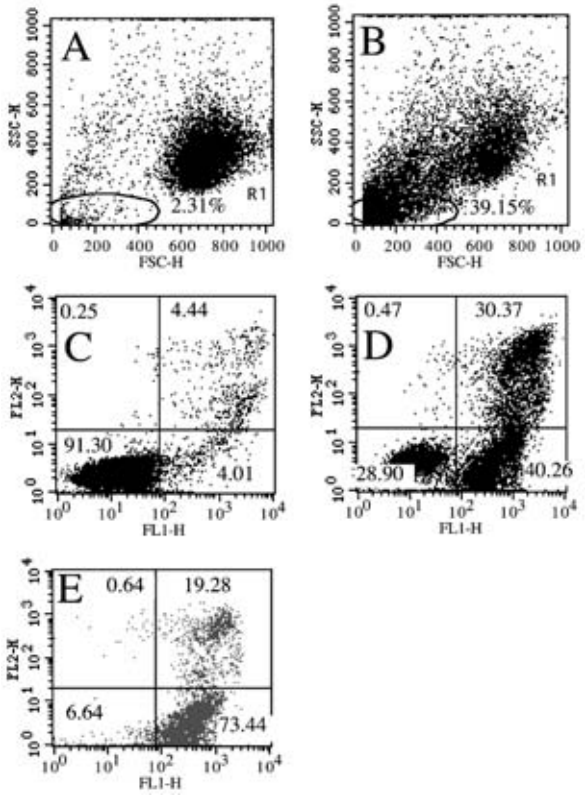


Fig. 9 Chlorpyrifos-induced reduction in the cell size of U937. **A**: dot plot of forward scatter (FSC) vs side scatter (SSC) in control U937 cells. **B**: dot plot of FSC vs SSC in chlorpyrifos-treated U937 cells: the horizontal axis (FSC) shows the size of U937 cell, while the vertical axis (SSC) shows the complexity. **C**: dot plot of FITC-Annexin V/PI in control U937 cells. **D**: dot plot of FITC-Annexin V/PI in chlorpyrifos-treated U937 cells. **E**: dot plot of FITC-Annexin V/PI in gate R1 in **Figure 9B** in chlorpyrifos-treated U937, the horizontal axis shows the intensity of fluorescence of FITC-Annexin V, whereas the vertical axis shows the intensity of fluorescence of PI. U937 cells were treated with chlorpyrifos at 0 or 142 μM for 4 hours, and then apoptosis was determined with FITC-Annexin V/PI staining detected with flow cytometry. Cited from Nakadai A et al., Toxicology, 2006; 224: 202–209[76].

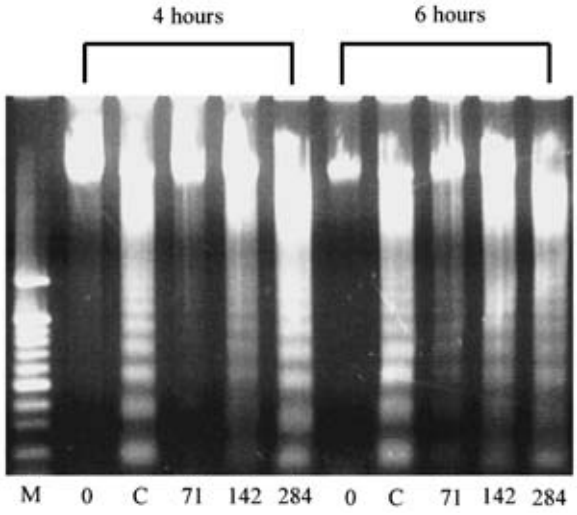


Fig. 10 Chlorpyrifos-induced DNA fragmentation in U937 cells determined with agarose gel electrophoresis. M: marker of the DNA ladder, C: a positive control, camptotecin at 6 μM. The concentrations of chlorpyrifos were 0, 71, 142, and 284 μM. Data shown are representative of three similar experiments. Cited from Nakadai A et al., Toxicology, 2006; 224: 202–209[76].

lymphocytic leukemia cell line through activation of caspase-3. In this study, pretreatment of EL4 cells with the caspase-9-specific inhibitor zLEHD-fmk attenuated paraoxon-induced apoptosis in a dose-dependent manner, whereas the caspase-8 inhibitor zIETD-fmk had no effect. Furthermore, activation of caspase-9, -8, and -3 in response to paraoxon treatment was completely inhibited in the presence of zLEHD-fmk, implicating the involvement of caspase 9-dependent mitochondrial pathways in paraoxon-stimulated apoptosis. Indeed, under both in vitro and in vivo conditions, paraoxon triggered a dose- and time-dependent translocation of cytochrome c from mitochondria into the cytosol. Investigation of the mechanism of cytochrome c release revealed that paraoxon disrupted the mitochondrial transmembrane potential. Neither this effect nor cytchrome c release was dependent on caspase activation, since zVAD-fmk, the general inhibitor of the caspase family, did not influence either process. Finally, paraoxon treatment also resulted in a time-dependent up-regulation and translocation of the proapoptotic molecule Bax to mitochondria. Inhibition of this event by zVAD-fmk suggests that the activation and translocation of Bax to mitochondria follows activation of the caspase cascades. The results indicate that paraoxon induces apoptosis in EL4 cells through a direct effect on mitochondria by disrupting the transmembrane potential, causing the release of cytochrome c into the cytosol and subsequent activation of caspase-9[75].

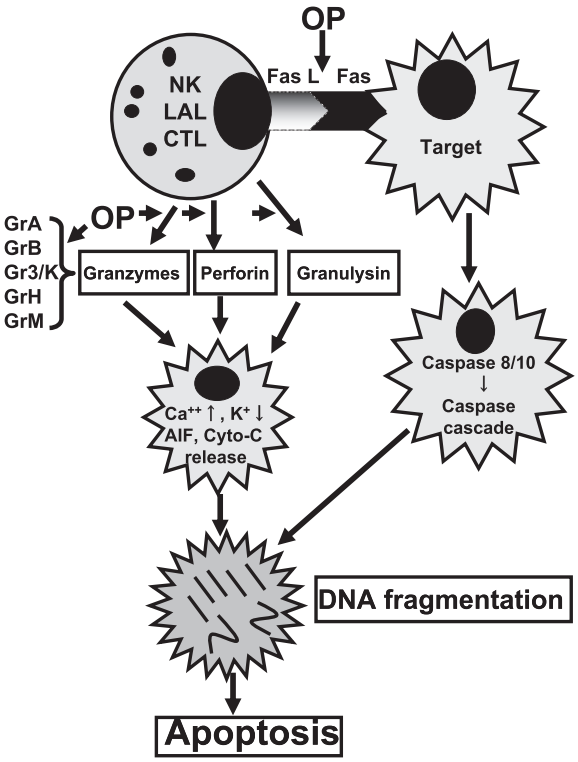


Fig. 11 OPs impair the granule exocytosis pathway (perforin/granzyme/granulysin pathway) and the FasL/Fas pathway of NK cells, LAK cells and CTLs. OP: organophosphorus pesticides. →: inhibition. Cited from Li Q and Kawada T, Cellular and Molecular Immunology 2006; 3: 171–178[56].

To explore the mechanism of OP-induced immunotoxicity, we also investigated whether OPs induced apoptosis in human immune cells and examined the underlying mechanism[76]. We treated human immune cells, a human monocyte like cell line (U937), with the OP chlorpyrifos and found that chlorpyrifos induced the cell death of U937 in a dose- and time-dependent manner, as shown by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasolium bromide (MTT) and lactate dehydrogenase (LDH) assays and propidium iodide (PI) uptake. We then investigated whether chlorpyrifos-induced cell death consisted of apoptosis, as determined by a DNA fragmentation analysis and by analysis of Annexin-V staining and the intracellular level of active caspase-3 by flow cytometry. We found that chlorpyrifos induced apoptosis in U937 in a time- and dose-dependent manner, as shown by Annexin-V staining **(Fig. 9)**. DNA fragmentation was detected when cells were treated with chlorpyrifos **(Fig. 10)**. Chlorpyrifos also induced an increase in intracellular active caspase-3 in U937 cells in a dose-dependent manner, and a caspase-3 inhibitor, Z-DEVD-FMK, significantly inhibited the chlorpyrifos-induced apoptosis. These findings indicate that chlorpyrifos induced apoptosis in U937 cells[76]. Das et al[77] have also reported that OPs, such as monocrotophos, profenofos, chlorpyrifos, and acephate, significantly induced apoptosis and necrosis in cultured human peripheral blood lymphocytes in vitro using DNA diffusion assay. Future studies should investigate whether OPs also induce apoptosis in NK cells, CTLs, and LAK cells and the relationship between OP-induced apoptosis and OP-induced inhibition of NK cell, CTL, or LAK cell activity.

In conclusion, the above findings indicate that OPs inhibit NK cell, LAK cell, and CTL activities mediated by at least the following three mechanisms[56]:

1. OPs impair the granule exocytosis pathway of NK cells, LAK cells, and CTLs **(Fig. 11)**[48–50];
2. OPs impair the FasL/Fas pathway of NK cells, LAK cells, and CTLs **(Fig. 11)**[51];
3. OPs induce apoptosis of immune cells[74–77].

Acknowledgements: This study was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 09877077, No. 10770178, No. 12770206 and No. 15590523). I am grateful to all staff at the Department of Hygiene and Public Health, Nippon Medical School for their assistances and advice.

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(Received, November 7, 2006)
(Accepted, February 1, 2007)