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Chlorpyrifos induces apoptosis in human monocyte cell line U937

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Abstract

In order to investigate chlorpyrifos-induced cell death and its underlying mechanism in human immune cells, a human monocyte like cell line (U937) was treated with chlorpyrifos at 4.45–570 μM for 0.5–24 h at 37 °C in a 5% CO_2 incubator. We first found that chlorpyrifos induced cell death of U937 in a dose- and time-dependent manner, as shown by LDH and MTT assays and PI uptake. Then, we investigated if chlorpyrifos-induced cell death consisted of apoptosis, as determined by analysis of Annexin-V staining and the intracellular level of active caspase-3 by flow cytometry, and DNA fragmentation analysis. We found that chlorpyrifos induced apoptosis in U937 in a time- and dose-dependent manner, as shown by Annexin-V staining. DNA fragmentation was detected when cells were treated with 71 to 284 μM chlorpyrifos for 4 or 6 h. Chlorpyrifos also induced an increase of 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 can induce apoptosis in U937 cells, and this effect is partially mediated by activation of intracellular caspase-3.

Keywords: Annexin-V; Apoptosis; Caspase-3; Chlorpyrifos; DNA fragmentation; U937

1. Introduction

Chlorpyrifos is an organophosphorus pesticide and that is widely used throughout the world as an insecticide in agriculture and an eradicating agent for termites around homes (Richardson, 1993; Caroline, 1994). The main toxicity of chlorpyrifos is neurotoxicity, which is caused by the inhibition of acetylcholinesterase (Whitney et al., 1995; Moser, 2000; Chanda and Pope, 1996; Chakraborti et al., 1993). Chlorpyrifos-oxon, a metabolite of chlorpyrifos, is approximately 3000 times as potent against the nervous system as chlorpyrifos itself (Caroline, 1994). On the other hand, it has been reported that chlorpyrifos caused immunologic abnormalities in humans (Thrasher et al., 1993, 2002) and animals (Blakley et al., 1999; Navarro et al., 2001). Higher than usual frequencies of allergies and sensitivities to antibiotics together with a decrease in CD5 cells and increases in CD26 cells and autoantibodies were found in patients following chlorpyrifos exposure (Thrasher et al., 1993, 2002). Increased numbers of CD26 cells and decreased numbers of CD5 cells are associated with autoimmunity, in which an individual's immune system acts against itself, rather than against infections (Lambeir et al., 2003; Youinou et al., 2005). It also has been reported that chlorpyrifos was associated with multiple chemical sensitivity (Berkson, 1994; Ziem and McTamney, 1997) and that immunologic abnormalities have long been advanced as a potential mechanism for multiple chemical sensitivity (Mitchell

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et al., 2000). To explore the mechanism of chlorpyrifos-induced immunotoxicity, we investigated whether chlorpyrifos induces apoptosis in human immune cells, and examined the underlying mechanism. Although it has been reported that chlorpyrifos induced apoptosis in rat primary cortical neurons *via* a mechanism that is regulated by the balance between p38 and extracellular signal-regulated protein kinase (ERK)/c-Jun NH_2-terminal protein kinase (JNK) MAP kinases (Caughlan et al., 2004) and in murine preimplantation embryos (Greenlee et al., 2004), there have been no reports on human immune cells.

2. Materials and methods

2.1. Reagents

RPMI 1640 medium was purchased from Nissui Pharmaceutical (Tokyo, Japan). RPMI 1640 medium without phenol red was purchased from GIBCO. Fetal bovine serum (FBS) was purchased from JRH Biosciences (Lenexa, KS), and heat-inactivated at 56 °C for 30 min prior to use. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrasolium bromide (MTT), glutamine, 2-mercaptoethanol (2-ME) and propidium iodide (PI) were obtained from Sigma (St. Louis, MO). Fluorescein isothiocynate (FITC)-anti human Annexin V and FITC-anti human active caspase-3, Z-DEVD-FMK (a caspase-3 inhibitor), Z-FA-FMK (a negative control for Z-DEVD-FMK) and Cytofix/cytoperm solution were purchased from BD Pharmingen (San Diego, CA). Chlorpyrifos and chlorpyrifos-oxon were purchased from Wako Pure Chemical Industries (Osaka, Japan). (*S*)-(+)-Camptotecin as a positive control of apoptotic inducer was purchased from Sigma (St. Louis, MO). Chlorpyrifos and camptotecin were prepared as stock solutions in DMSO at 57.05 and 11.48 mM, respectively.

2.2. Cells

The U937 cell line was obtained from American Type Culture Collection (ATCC) (Manassas, VA) and was maintained in RPMI 1640 medium containing 10% FBS.

2.3. Chlorpyrifos-induced cell death determined by PI uptake analyzed by flow cytometry

U937 cells at 5×10^5/ml were treated with chlorpyrifos or chlorpyrifos-oxon at 0 (1% DMSO as a control), 8.91, 17.83, 35.65, 71.31, 142.61, or 285.23 μM for 2, 4 or 6 h at 37 °C in 5% CO_2 incubator. Then, the treated cells were harvested and suspended in RPMI 1640 medium without phenol red containing 1% FBS. The dead cells were stained with PI and analyzed by flow cytometry (Li et al., 1999).

The reduction of cell size was observed by Forward Scatter (FSC) of flow cytometry.

2.4. Chlorpyrifos-induced cell death determined by MTT metabolism

The MTT assay relies primarily on mitochondrial metabolic capacity of viable cells and reflects the intracellular redox state. U937 cells at 2×10^5 cells/ml were treated with chlorpyrifos at 0 (1% DMSO), 8.91, 17.83, 35.65, 71.31, 142.61, 285.23, or 570.45 μM for 4 h at 37 °C in 5% CO_2. Then 10 μl of MTT dye was added, and the cells were incubated for another 4 h at 37 °C. Then 100 μl of acidic isopropanol (0.04N HCl in isopropanol) was added and mixed thoroughly with the cells. The absorbance was measured at 570/620 nm as described previously (Okada et al., 2003). Results were expressed as inhibitory rate of MTT reduction induced by chlorpyrifos, which represents cell death.

2.5. Chlorpyrifos-induced cell death determined by measurement of lactate dehydrogenase (LDH)

The LDH released in the culture medium (extracellular LDH) was used as an index of necrotic cell death (Kim et al., 1997; Zhang et al., 2004). U937 cells at 2×10^5/ml were treated with chlorpyrifos at 0 (1% DMSO), 8.91, 17.83, 35.65, 71.31, 142.61, 285.23, or 570.45 μM for 4 h at 37 °C in 5% CO_2. Then, LDH activity released from U937 cells into the culture medium, which represents cell death was determined with an LDH kit (Roche) as described previously (Li et al., 2005a).

2.6. Chlorpyrifos-induced apoptosis in U937 determined by FITC-Annexin V staining

It has been reported that Annexin-V staining is able to detect apoptosis in the early stage based on the alteration of the cell membrane (Dong et al., 2005). Therefore, we used Annexin-V staining assessed by flow cytometry to investigate chlorpyrifos-induced apoptosis.

The U937 cells at 5×10^5/ml were treated with chlorpyrifos at 0 (1% DMSO), 4.45, 8.91, 17.83, 35.65, or 71.31 μM for 1, 2, 4, 14 or 24 h at 37 °C in a 5% CO_2 incubator, and then harvested and washed twice with PBS. The treated cells were suspended in binding buffer at 3×10^5 cells/100 μl, and supplemented with 5 μl of FITC-Annexin-V and 5 μl of PI, and incubated for 15 min at room temperature in the dark. Flow cytometric analysis was performed with a FACScan flow cytometer (Becton Dickinson, San Jose, CA) as described previously (Dong et al., 2005).

2.7. DNA extraction and detection of DNA fragmentation

U937 cells at 5×10^5/ml were treated with chlorpyrifos at 0 (1% DMSO), 71, 142 or 284 μM or Camptotecin at 2 μM for 4 or 6 h. The treated cells were harvested and washed with PBS. DNA was extracted with a Wizard genomic DNA purification kit (Promega). DNA fragmentation was detected by electrophoresis through an agarose gel and the bands were stained with ethidium bromide for UV light visualization.