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SUBCELLULAR LOCALIZATION OF THE AMIDE CLASS HERBICIDE 3,4-DICHLOROPROPIONANILIDE (DCPA) IN T CELLS AND HEPATOCYTES

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Abstract

3,4-Dichloropropionanilide (DCPA), or propanil, a post-emergent herbicide used on rice and wheat crops in the United States, is immunotoxic *in vivo* and *in vitro*. Although it has been documented that DCPA exerts differential effects on specific immune cell types and is toxic to the liver, the way in which DCPA modulates intracellular functions leading to these effects is less understood. In this study, Jurkat T cells and hepatocytes from C57Bl/6 mice were exposed to 100 μ M DCPA for 1.5 h. Following incubation, subcellular fractions of each cell type were isolated. DCPA, when present, was removed from each cell fraction by liquid–liquid extraction. The extraction product was then analyzed for the presence of DCPA using liquid chromatography–tandem mass spectrometry (LC-MS/MS). The cellular uptake of DCPA was monitored by detection of the molecular ion and product ion of DCPA. The analyses demonstrate that DCPA, a lipophilic compound, localizes primarily in the cytosol of T cells and hepatocytes. These results indicate that DCPA is able to cross the plasma membrane and is accessible to intracellular immunomodulatory effectors.

Propanil (3,4-dichloropropionanilide, DCPA) is an herbicide used extensively on rice and wheat crops in the United States. Individuals involved in agriculture, in particular, are at risk for high-level exposure to DCPA. DCPA can be absorbed through the respiratory tract, the gastrointestinal tract, and the intact skin (Richards et al., 2001). There are well-known immunotoxic effects on various compartments of the immune system following *in vivo* DCPA exposure (reviewed by Salazar et al., 2008). DCPA exposure decreases contact hypersensitivity responses, reduces proliferative responses to T- and B-cell mitogens, and impedes mixed lymphocyte reactions (Barnett & Gandy, 1989). In addition to immunotoxicity, reports have

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demonstrated that DCPA is hepatotoxic to humans (De Silva & Bodinayake, 1997) and rodents (Santillo et al., 1995).

Although it has been documented that DCPA exerts differential effects on specific immune cell types and is hepatotoxic, to date, the subcellular localization from which DCPA modulates immune cells and hepatocytes has not been examined. The high lipophilicity of DCPA has led to the suggestion of a higher affinity of DCPA for membranes (Corsini et al., 2007). The aim of this study is to determine the localization of DCPA in T cells and hepatocytes following *in vitro* exposure. Following DCPA treatment, subcellular fractions of each cell type were isolated. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) provides a definitive detection method for DCPA subcellular localization. Our results demonstrate that DCPA, a lipophilic compound (Finizio, 1997), localizes primarily in the cytosol of both T cells and hepatocytes. This study improves our understanding of the cellular location in which DCPA exerts its toxic effects.

MATERIALS AND METHODS

DCPA Treatment

Experiments were performed using the human T-cell leukemia cell line, Jurkat clone E6-1, obtained from the ATCC (American Tissue Culture Collection, Manassas, VA). Jurkat cells were maintained in complete RPMI media (Mediatech, Inc., Herndon, VA). The cultures were kept at 37°C in 5% CO₂. Cells in suspension were grown to obtain approximately 5×10^7 cells. Cells were stimulated with anti-CD3 (10 µg/ml) and anti-CD28 (2 µg/ml) (BD PharMingen, San Diego, CA) and at the same time exposed to 100 µM DCPA or ethanol (vehicle control) for 1.5 h at 37°C, 5% CO₂.

Female C57Bl/6 mice at 8–10 wk of age were obtained from Hilltop Lab Animals, Inc. (Scottsdale, PA). Normal mouse hepatocytes were isolated using the method described by Muller et al. (1972). Overnight cultures of isolated primary hepatocytes were incubated with 100 µM DCPA or ethanol for 1.5 h at 37°C, 5% CO₂.

Fractionation of T Cells and Hepatocytes

T cells were fractionated as described by Ramsby and Makowski (2005). Hepatocytes were fractionated as described by Graham (2000). The purity of each fraction was determined by Western blotting, in which cell-fraction-specific antibodies were used. Protein quantitation of each fraction was determined using the 2D Quant Kit (Amersham Biosciences).

Mass Spectrometric Analysis of Cell Fractions

Volumes of all fractions were adjusted using phosphate-buffered saline (PBS) to achieve a protein concentration of 0.5 µg/µl. DCPA was extracted from cell fractions using a liquid–liquid extraction procedure, with acetaminophen as an internal standard. A 5-point standard curve of a DCPA concentration range of 5–500 ng/ml was used to quantify DCPA in cell fractions. LC-MS/MS was used to determine DCPA localization in cells.

RESULTS AND DISCUSSION

Analysis of fractionated T cells following stimulation and treatment with 100 µM DCPA for 1.5 h demonstrated that DCPA localizes in the cytosol (Figure 1A and Table 1). DCPA was not detected in the membrane/organelle fraction (Figure 1B) or in the nuclear/cytoskeletal fraction (Figure 1C). Therefore, DCPA, a lipophilic molecule, passes through the plasma membrane and resides in the cytosol of T cells following 1.5 h of exposure. Analysis of fractionated hepatocytes following treatment with DCPA for 1.5 h demonstrated that the

molecule localizes mostly in the cytosol (Figure 1D) (Table 1). DCPA was also detected in the light mitochondrial fraction (Figure 1E), but the level was minimal relative to the level detected in the cytosol (Table 1). There was no DCPA detected in the peroxisomal fraction of hepatocytes (Figure 1F). Therefore, DCPA localizes predominantly in the cytosol of hepatocytes following 1.5 h of exposure.

The fivefold greater DCPA concentration observed in the hepatocyte cytosol compared to that in the T-cell cytosol is likely attributed to differences in cell size and cell composition (Table 1). Hepatocytes are among the largest of cell types, with an average diameter of 18–22 μm . In addition, they display an eosinophilic cytoplasm which contains numerous mitochondria. T cells are smaller than hepatocytes, with an average diameter of 7–12 μm . The nucleus is the major constituent of T cells; thus, there is little eosinophilic cytoplasm. The finding that DCPA is only detected in the cytosol of T cells, coupled with the size of this constituent, suggests that DCPA's mechanism of action has specificity.

We hypothesized that DCPA exerts its effects on immune cells and hepatocytes by interfering with cell activation and cell signaling processes at the cell membrane, due to DCPA being a highly hydrophobic, lipophilic compound. Our finding that DCPA is not in the membrane does not eliminate the possibility that DCPA is affecting membrane proteins or channels as it migrates into the cytosol. An earlier finding in our laboratory demonstrated that DCPA exposure does not disrupt the motional properties of lipid hydrocarbon chains in the bilayer, but does alter distribution of lipids in distinct motional environments in the membrane (Brundage et al., 2003). The present study provides an explanation for the limited effect observed in hydrocarbon chain mobility. The finding that DCPA is able to move through the membrane of T cells and hepatocytes and localize in the cytosol will aid in the understanding of DCPA's mechanism of toxicity to cellular functions.

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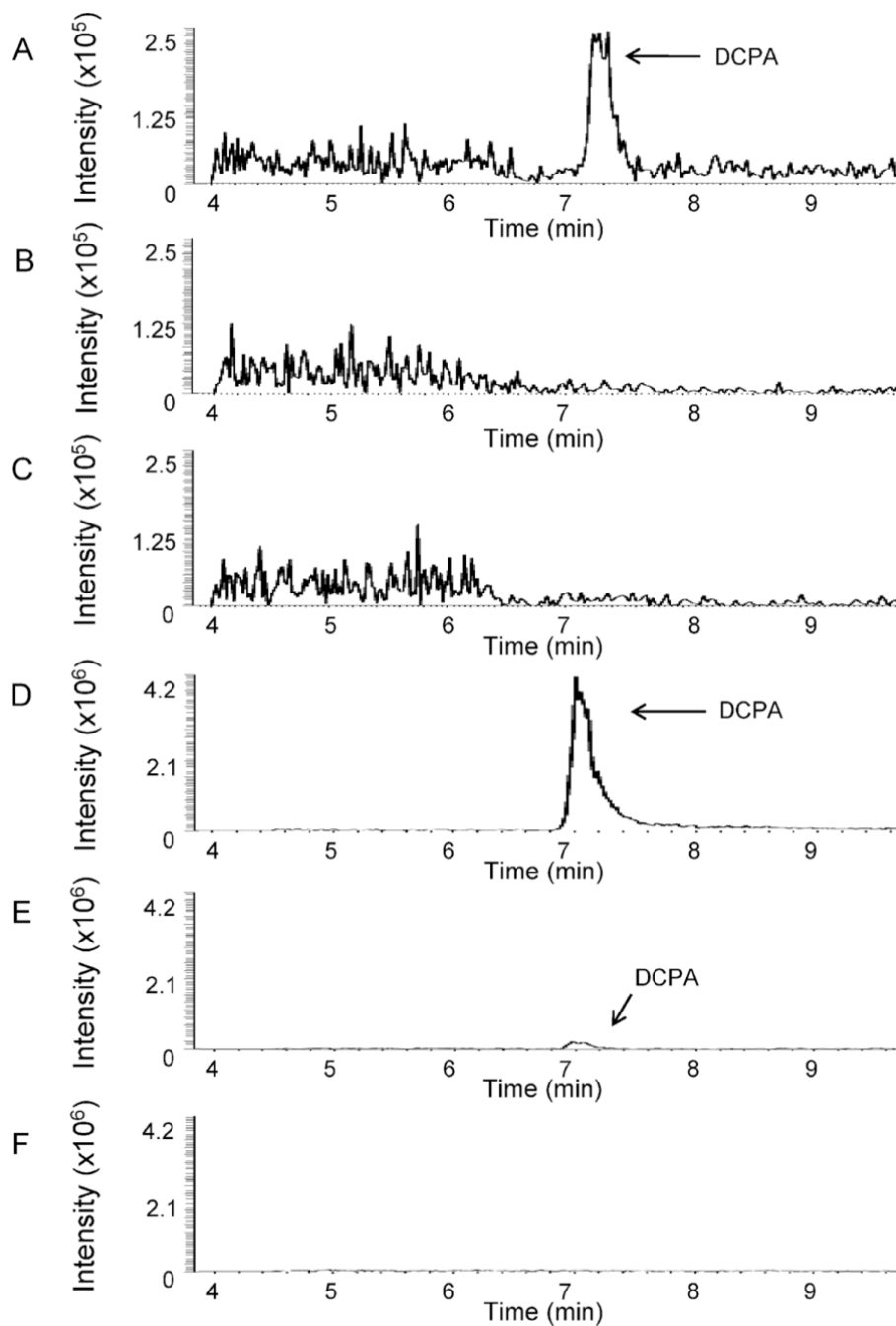


FIGURE 1.

Chromatograms of DCPA localization in T cells and hepatocytes. Jurkat T cells and mouse hepatocytes were exposed to 100 μ M DCPA for 1.5 h. Cells were fractionated using fractionation/homogenization and centrifugation. LC-MS/MS was used to determine DCPA location in cell fractions. Acetaminophen was used as an internal standard. Protein concentration of each fraction was 0.5 μ g/ μ l. (A) T-cell cytosol fraction, (B) T-cell membrane/organelle fraction, (C) T-cell nuclear/cytoskeletal fraction, (D) hepatocyte cytosol, (E) hepatocyte light mitochondrial fraction, and (F) hepatocyte peroxisome fraction. Purity of each fraction was verified through Western blot. All values are means of three separate experiments.

TABLE 1

DCPA Concentration in T-Cell and Hepatocyte Subcellular Fractions

T cell	DCPA concentration (ng/ml)^a	Hepatocyte	DCPA concentration (ng/ml)^a
Cytosol	26.3 ± 2.0	Cytosol	120 ± 5.8
Membrane/organelle	0 ^b	Light mitochondrial	25.3 ± 3.1
Nuclear/cytoskeletal	0	Peroxisomal	0

^aMean ± SEM, *n* = 3.^bbelow the level of detection.