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Fipronil induces CYP isoforms and cytotoxicity in human hepatocytes

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Abstract

Recent studies have demonstrated the potential of pesticides to either inhibit or induce xenobiotic metabolizing enzymes in humans. Exposure of human hepatocytes to doses of fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl) sulfinyl]-1H-pyrazole-3-carbonitrile) ranging from 0.1 to 25 μM resulted in a dose dependent increase in CYP1A1 mRNA expression (3.5 to ∼55-fold) as measured by the branched DNA assay. In a similar manner, CYP3A4 mRNA expression was also induced (10–30-fold), although at the higher doses induction returned to near control levels. CYP2B6 and 3A5 were also induced by fipronil, although at lower levels (2–3-fold). Confirmation of bDNA results were sought through western blotting and/or enzyme activity assays. Western blots using CYP3A4 antibody demonstrated a dose responsive increase from 0.5 to 1 μM followed by decreasing responses at higher concentrations. Similar increases and decreases were observed in CYP3A4-specific activity levels as measured using 6β-hydroxytestosterone formation following incubation with testosterone. Likewise, activity levels for a CYP1A1-specific substrate, luciferin CEE, demonstrated that CYP1A1 enzyme activities were maximally induced by 1 μM fipronil followed by dramatically declining activity measurements at 10 and 25 μM. Cytotoxic effects of fipronil and fipronil sulfone were examined using the adenylate kinase and the trypan blue exclusion assays in HepG2 cells and human hepatocytes. The results indicate both that HepG2 cells and primary human hepatocytes are sensitive to the cytotoxic effects of fipronil. The maximum induction of adenylate kinase was ca. 3-fold greater than the respective controls in HepG2 and 6–10-fold in the case of primary hepatocytes. A significant time- and dose-dependent induction of adenylate kinase activity in HepG2 cells was noted from 0.1 to 12.5 μM fipronil followed by decreasing activities at 25 and 50 μM. For fipronil sulfone, cytotoxic effects increased throughout the dose range. The trypan blue assay indicated that cytotoxic effects contributing to an increase of greater than 10% of control values was indicated at doses above 12.5 μM. However, fipronil sulfone induced cytotoxic effects at lower doses. The possibility that cytotoxic effects were due to apoptosis was indicated by significant time- and dose-dependent induction of caspase-3/7 activity in both HepG2 cells and human hepatocytes. Fipronil mediated activation of caspase-3/7 in concurrence with compromised ATP production and viability are attributed to apoptotic cell death.

Keywords: Fipronil; Human hepatic and HepG2 cells; CYP3A4; CYP2B6; CYP1A1; Cytotoxicity; Apoptosis

Abbreviations: AK, adenylate kinase; ATP, nucleotide adenosine triphosphate; Apaf-1, apoptotic protease activating factor-1; E_2, 17β-estradiol; Fas, death receptor; GABA, gamma-amino butyric acid; RXR, retinoid X receptor; XRE, xenobiotic responsive element

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1. Introduction

Fipronil (5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-[(trifluoromethyl) sulfinyl]-1H-pyrazole-3-carbonitrile) is a relatively new pesticide with widespread utility in control of many agricultural and domestic pests including many lepidopteran species as well as thrips, locusts, ants, cockroaches, fleas, and ticks [1]. Fipronil has also been designated as an alternative pesticide to organophosphates for termite and fire ant control by the U.S. Environmental Protection Agency [1,2]. Because fipronil is used both commercially and in home applications, recent concerns for potential adverse public health effects have been raised [1,3].

Fipronil toxicity is attributed to its ability to act at the GABA receptor as a noncompetitive blocker of the GABA-gated chloride channels of neurons in the central nervous system. The selective toxicity of fipronil to insects relative to vertebrates is primarily the result of differences at the target site [4,38–40]. Fipronil is moderately toxic to rats and mice, highly toxic to aquatic invertebrates, fish and upland game birds, but non-toxic to waterfowl and other bird species [5,6]. Rat organs affected by chronic fipronil exposure include the thyroid, liver and kidney [1]. Multi-generation rat studies showed reproductive toxicity and developmental delays at the higher doses tested, but no evidence of birth defects [6,7].

Relatively little is known of the ability of fipronil to be metabolized in vertebrates. In vivo mammalian studies indicate that the primary metabolic pathway for fipronil involves the oxidative formation of the sulfone metabolite [4]. Recent studies with human liver microsomes and recombinant CYP isoforms demonstrated that the formation of the sulfone is almost exclusively the result of CYP3A4 activity, although limited metabolism by CYP2C19 was also reported [8]. The sulfone metabolite, as well as a photodegradation product, fipronyl desulfinyl, have been reported to be more toxic to insects, mammals, fish and birds than the parent compound itself [5,9].

Fipronil is one of several pesticides known to induce thyroid cancer in rats, likely as a result of its ability to enhance hepatic thyroid hormone metabolism and excretion [10]. An analysis of several rodent cancer studies suggests that liver and thyroid tumors are often correlated, both within and between species [11]. It is possible that induction of microsomal enzymes and the accompanying symptoms associated with induction, including increased liver weights and heptocellular hypertrophy, may play a role in tumor development. The ability of chemicals to induce metabolic enzymes, including cytochrome P450 (CYP), has long been considered one of the most sensitive biochemical cellular responses to toxic insult [12,13], since it often occurs at much lower doses of the chemical than those known to cause lethal or overtly toxic effects.

Recent studies with human and rat hepatocytes have demonstrated that pesticides are capable of inducing many metabolic enzymes in these cells [14,15]. The present study was undertaken to examine the potential of fipronil to induce important xenobiotic metabolizing enzymes in human hepatocytes and to characterize the cytotoxic effects of fipronil that were discovered during the course of the study.

2. Materials and methods

2.1. Chemicals and reagents

Fipronil and fipronil sulfone were purchased from Chem Service (West Chester, PA) and AccuStandard, Inc (New Haven, CT), respectively. Williams E culture medium and medium supplements, dexamethasone and insulin, were obtained from Bio Whittaker (Walkersville, MD). Eagle's medium E without L-glutamine and phenol red, non-essential amino acid solution, L-glutamine solution, and other cell culture related products were purchased from Mediatech, Inc. (Herndon, VA). Certified fetal bovine serum, trypsin–EDTA solution, and HBSS buffers were obtained from GIBCO Invitrogen Corporation (Carlsbad, CA). Tissue culture flasks, 6-, 24- 48-, and 96-well culture plates along with other tissue culture related products, HPLC grade acetonitrile and water were purchased from Fisher Scientific (Pittsburgh, PA). Testosterone and 6β-hydroxytestosterone were purchased from Steraloids (Newport, RI). The ToxiLight™ assay kit was purchased from Cambrex Corporation (East Rutherford, New Jersey). Caspase-Glo™ 3/7 Assay kit was purchased from Promega Corporation (Madison, WI). Actinomycin D and Z-DEVD-FMK are products of Alexis Biochemicals supplied by AXXORA, LLC (San Diego, CA). Rifampicin, phenobarbital, and all other chemicals, unless specified otherwise, were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO). Polyclonal anti-rat CYP1A1 from goat, monoclonal anti-human CYP3A4 from mice were from BD Biosciences (Bedford, MA) while mouse anti-human Fas was from R&D Systems (Minneapolis, MN). All chemicals and reagents were used and disposed according to the NCSU safety protocols and guidelines.

2.2. Human hepatocyte primary culture

Primary cultures of mature human hepatocytes were obtained from Vesta Therapeutics (Research Triangle