Biochemical Pharmacology 86 (2013) 997–1039

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

The nuclear receptors pregnane X receptor and constitutive androstane receptor contribute to the impact of fipronil on hepatic gene expression linked to thyroid hormone metabolism

Béatrice B. Roques [a,b], Julien Leghait [a,b], Marlène Z. Lacroix [a,b], Frédéric Lasserre [a,c], Thierry Pineau [a,c], Catherine Viguié [a,b], Pascal G.P. Martin [a,c,*]

[a] INRA, UMR1331, Toxalim, Research Centre in Food Toxicology, F-31027 Toulouse, France
[b] Université de Toulouse, ENVT, INP, Toxalim, F-31076 Toulouse, France
[c] Université de Toulouse, INP, UMR1331, Toxalim, F-31000 Toulouse, France

ARTICLE INFO

Article history:
Received 24 June 2013
Accepted 8 August 2013
Available online 17 August 2013

Keywords:
Fipronil
Thyroid hormone metabolism
Pregnane X receptor
Constitutive androstane receptor
Hepatic metabolism
Rodents

Chemical compounds studied in this article:
Fipronil (PubChem CID: 3352)
Thyroxine (PubChem CID: 5819)
Triiodothyronine (PubChem CID: 5920)
$^{13}C_6$-levothyroxine (PubChem CID: 53442260)

ABSTRACT

Fipronil is described as a thyroid disruptor in rat. Based on the hypothesis that this results from a perturbation of hepatic thyroid hormone metabolism, our goal was to investigate the pathways involved in fipronil-induced liver gene expression regulations. First, we performed a microarray screening in the liver of rats treated with fipronil or vehicle. Fipronil treatment led to the upregulation of several genes involved in the metabolism of xenobiotics, including the cytochrome P450 Cyp2b1, Cyp2b2 and Cyp3a1, the carboxylesterases Ces2 and Ces6, the phase II enzymes Ugt1a1, Sult1b1 and Gsta2, and the membrane transporters Abcc2, Abcc3, Abcg5, Abcg8, Slco1a1 and Slco1a4. Based on a large overlap with the target genes of constitutive androstane receptor (CAR) and pregnane X receptor (PXR), we postulated that these two nuclear receptors are involved in mediating the effects of fipronil on liver gene expression in rodents. We controlled that liver gene expression changes induced by fipronil were generally reproduced in mice, and then studied the effects of fipronil in wild-type, CAR- and PXR-deficient mice. For most of the genes studied, the gene expression modulations were abolished in the liver of PXR-deficient mice and were reduced in the liver of CAR-deficient mice. However, CAR and PXR activation in mouse liver was not associated with a marked increase of thyroid hormone clearance, as observed in rat. Nevertheless, our data clearly indicate that PXR and CAR are key modulators of the hepatic gene expression profile following fipronil treatment which, in rats, may contribute to increase thyroid hormone clearance.

1. Introduction

The broad-spectrum phenylpyrazole insecticide fipronil (5-amino-1-(2,6-dichloro-4-(trifluoromethylphenyl)-4-(trifluoromethylsulfinyl)pyrazole-3-carbonitrile, CAS: 120068-37-3) is widely used in granular turf products, seed treatments, topical pet care products, gel baits and liquid termiticides in many countries leading to a high rate of potential contamination of the human domestic environment. It belongs to the second generation of insecticides acting at the γ-aminobutyric acid (GABA) receptor as

Abbreviations: Abc, ATP-binding cassette; CAR, constitutive androstane receptor; Ces, carboxylesterase; Cyp, cytochrome P450; ESI$^+$, electrospray ionization mode; FT3, free triiodothyronine; FT4, free thyroxine; GABA, γ-aminobutyric acid; GEO, gene expression omnibus; GO, gene ontology; Gst, glutathione *S*-transferase; LOQ, limit of quantification; MRM, multiple reaction monitoring; MRP, multidrug resistance-associated protein; OATP, organic anion-transporting polypeptide; PB, phenobarbital; PCN, pregnenolone-16alpha-carbonitrile; Polr2a, RNA polymerase II polypeptide A; PXR, pregnane X receptor; QC, quality control; RT-qPCR, real-time quantitative polymerase chain reaction; Slco, solute carrier organic anion transporter; Sult, sulfotransferase; T3, triiodothyronine; T4, thyroxine; Tbp, TATA-box binding protein; TSH, thyroid-stimulating hormone; TT3, total triiodothyronine; TT4, total thyroxine; Ugt, 5′-diphospho (UDP)-glucuronosyltransferase.

* Corresponding author at: INRA, UMR1331, Toxalim, 180 chemin de Tournefeuille, St-Martin-du-Touch, BP 93173, 31027 Toulouse Cedex 3, France. Tel.: +33 5 61 28 55 24; fax: +33 5 61 28 52 44.
E-mail address: Pascal.Martin@toulouse.inra.fr (Pascal G.P. Martin).

0006-2952
http://dx.doi.org/10.1016/j.bcp.2013.08.012

a noncompetitive blocker of the GABA-gated chloride channel [1]. It was developed as a substitute for the first generation of insecticidal chloride channel blockers such as the chlorinated cyclodienes and other polychlorocycloalkanes based on its selective toxicity for insects [2], thus lowering the risk for human health.

However, fipronil does not act only on the central nervous system (CNS). Regulatory toxicological evaluations and experimental investigations have shown that fipronil can act as a thyroid disruptor as it decreases plasma thyroid hormone concentrations in rats [3–5]. Because thyroid hormones regulate important biological processes including bone growth and development, cardiac function, CNS development, thermogenesis and hepatic metabolism [6], it is critical to understand the mechanisms underlying the thyroid disrupting effects of fipronil and their potential relevance to humans. From previous studies, we know that fipronil treatment of rats is associated with a marked increase in the clearance of free and total thyroxine (T_4) resulting, at least in part, from an increase in the activity and mRNA expression of two types of phase II hepatic enzymes potentially involved in T_4 elimination, the uridine 5′-diphospho (UDP)-glucuronosyltransferases (UGT) and the sulfotransferases (SULT) [4,5]. Moreover, the main fipronil metabolic pathway, leading to fipronil sulfone formation, is mediated by hepatic cytochrome P450 (CYP) enzymes [7] which are induced in the liver by fipronil treatment [5]. Upon exposure to xenobiotics, the increased expression and activity of hepatic enzymes involved in the metabolism of both xenobiotics and endogenous compounds often results from the activation of "xenosensors" such as the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR). These two nuclear receptors have been shown to regulate the expression of overlapping sets of genes including hepatic phase I enzymes such as CYP2B and CYP3A [8] as well as phase II enzymes [9,10] and membrane transporters [11,12] involved in hepatic xenobiotic and thyroid hormone metabolism. Additionally, fipronil has been shown to be a ligand of the human PXR *in vitro* [13].

Based on this information and on a screening of the impact of fipronil on the rat liver transcriptome presented here, we hypothesized that CAR and/or PXR could mediate the impact of fipronil on hepatic thyroid hormone metabolism in rodents. Based on this hypothesis, the goals of this study were: (1) to document the changes in liver gene expression profile occurring in rats treated with doses of fipronil which induce an increase of thyroid hormone clearance, (2) to identify a set of mouse hepatic transcripts regulated by fipronil in the same manner as in rat, (3) to evaluate in CAR-deficient and PXR-deficient mouse models the contributions of these receptors to the modulation of hepatic gene expression induced by fipronil and (4) to determine if the expression of these receptors was required for fipronil metabolism and fipronil-induced increase of thyroid hormone clearance.

2. Materials and methods

2.1. Animals and treatments

Female Wistar rats (Charles River, L'Abresle, France) and wild-type C57BL/6J male mice (Janvier, Le Genest St Isle, France) were acclimatized for at least 2 weeks to housing conditions (22 ± 2 °C, 12 h light/dark cycle, free access to water and food: A04 diet, Safe, Augy, France). Female rats were chosen for gene expression studies because the effect of fipronil on thyroid hormone clearance has been best described in this gender [4,5]. On the other hand, we used male mice for pharmacokinetic and gene expression studies because (i) most published studies on CAR/PXR activation by xenobiotics have been performed in male mice and (ii) we wanted to avoid the potentially confounding effect of the estrous cycle in the mouse pharmacokinetic study. This latter point is more critical for the mouse than for the rat pharmacokinetic study because only a single blood sample can be obtained from each mouse for the estimation of the labeled T_4 clearance. *In vivo* studies were conducted under E.U. guidelines for the use and care of laboratory animals. CAR−/− [14] and PXR−/− [11] male mice on a C57BL/6J genetic background are a generous gift from Pr David Moore (Baylor College of Medicine, Houston, TX, USA) and Pr Steven Kliewer (University of Texas Southwestern Medical School, Dallas, TX, USA) respectively. Colony founders were kindly provided by Pr Urs A Meyer (Biozentrum, University of Basel, Basel, Switzerland). Fipronil (95.6% purity, 3B Medical Systems, Libertyville, IL, USA) was administered orally as a suspension through feeding needles, daily for 14 days. Control animals received the vehicle alone (methylcellulose 0.5% (w/v) and Tween 80 0.01% (w/v) for rats and Arabic gum 3% (w/v) for mice). All administrations were performed under a volume of 2 mL/kg of body weight for rats and 2.5–3 mL/kg of body weight for mice. To evaluate T_4 clearance, adult male mice from the different genotypes were administered an intraperitoneal (ip) bolus (10.2 μg/kg) of levothyroxine labeled with a stable isotope ($^{13}C_6$-LT_4, Isosciences, King of Prussia, PA, USA) dissolved in 10 mM PBS pH = 7.4 containing 0.1% (w/v) bovine serum albumin. The following four different animal experiments were performed:

1. Two- to three-month-old female Wistar rats (202.4 ± 6.0 g body weight) treated with fipronil (3 mg/kg per day) or not were used for microarray (n = 7–8 per group) and qPCR liver gene expression studies (n = 10–14 per group including the animals used for the microarray study).
2. Nine-week-old male C57BL/6J mice (23.3 ± 0.9 g body weight) treated with fipronil (5 mg/kg per day) or not (n = 8 per group) were used for qPCR gene expression studies.
3. Fifteen to 22-week-old male C57BL/6J wild-type (30.7 ± 2.1 g body weight), CAR−/− (32.4 ± 4.2 g body weight) and PXR−/− mice (29.4 ± 3.4 g body weight) treated with fipronil (3 mg/kg per day) or not were used to estimate $^{13}C_6$-LT_4 clearance (n = 5 mice/genotype/treatment for 0.25 and 4 h and n = 10 mice/genotype/treatment for 2, 8, 12 and 24 h) and for qPCR gene expression studies (n = 5 mice/genotype/treatment).
4. Nine-week-old male C57BL/6J wild-type (25.7 ± 1.2 g body weight), CAR−/− (26.3 ± 2.1 g body weight) and PXR−/− mice (26.3 ± 2.1 g body weight) treated with fipronil (10 mg/kg per day) or not were used for qPCR gene expression studies.

Plasma parameters such as fipronil and fipronil sulfone, thyroid hormones or TSH were also obtained from these different studies as described throughout the manuscript.

2.2. Blood and tissue samples

Blood samples were collected in heparinized tubes by aortic puncture following euthanasia (rats) or intracardiac puncture under isoflurane anesthesia (mice). Plasma was prepared by centrifugation (4000 × *g*, 15 min, +4 °C) and stored at −20 °C until use. Following euthanasia, the liver was promptly removed, weighted, rinsed in ice-cold saline solution, dissected (∼100 mg fragments), snap-frozen in liquid nitrogen and kept at −80 °C until use.

2.3. Gene expression studies by RT-qPCR and microarrays

Total RNA was extracted using TRIzol reagent (Life Technologies, Saint Aubin, France). For real-time quantitative polymerase chain reaction (qPCR), 2 μg of total RNA were reverse transcribed with the High Capacity cDNA reverse transcription kit (Life Technologies, Saint Aubin, France). Amplifications with specific primers (Table 1) and SYBR Green fluorescence monitoring (Power SYBR Green PCR