Contribution of the nuclear receptors PXR and CAR to gene expression modulations induced by fipronil in rodent liver

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Introduction: Fipronil, an insecticide widely used, is a thyroid disruptor in rat inducing a marked increase in thyroxine (T_4) clearance. Fipronil-induced thyroid disruption seems to require a bioactivation of fipronil via its biotransformation into fipronil sulfone by cytochromes P450 (CYP) and an induction of hepatic enzymes involved in thyroid hormone catabolism such as Udp-glucuronosyltransferases (UGT) and sulfotransferases (SULT). The nuclear receptors (NR) Pregnane X Receptor (PXR) and Constitutive Androstane Receptor (CAR) are transcription factors activated by many xenobiotics such as pesticides. Their target genes are involved in the metabolism of xenobiotics and endogenous hormones, including thyroid hormones, and, interestingly, fipronil is a ligand of the human PXR *in vitro*. We thus suspected that CAR and PXR are involved in the regulation of fipronil-induced thyroid disruption in rats. To test this hypothesis, we 1) obtained microarray gene expression profiles in fipronil-treated rats, 2) checked by RT-qPCR that the fipronil-induced mRNA expression modulations were similar in rats and mice in order to 3) investigate in wild-type, CAR and PXR-deficient mice the effects of fipronil on both T_4 clearance and hepatic expression of selected genes.
Materials and Methods: Fipronil were administered daily through feeding needles for 14 days. In experiment 1, rats were treated with vehicle (n=14) or fipronil 3 mg/kg/day (n=10). In experiment 2, mice were treated with vehicle or fipronil 5 mg/kg/day (n=8 each). In experiment 3, mice were treated with vehicle or fipronil 3 mg/kg/day (n=50 wild-type, 50 CAR-deficient and 50 PXR-deficient mice each). In experiment 4, mice were treated with vehicle or fipronil 10 mg/kg/day (n=8 mice/genotype each). Livers were collected for microarray gene expression profiling in rats (experiment 1), to compare the effects of fipronil on hepatic mRNA expression by RT-qPCR in rats and wild-type mice (experiments 1 and 2) and in wild-type and NR-deficient mice at different dosing regimen (experiments 3 and 4). For experiment 3, total blood was collected at 0.25, 2, 4, 8, 12 and 24h after an intraperitoneal bolus of $^{13}C_6$-LT_4 (n=5 to 10 mice/genotype/treatment/time) to determine the effect of fipronil on $^{13}C_6$-LT_4 clearance in wild-type and NR-deficient mice.
Results: In rats, fipronil increased the expression of known PXR and/or CAR target genes such as Cyp (Cyp3a1: 11-fold), Ugt and Sult (Sult1b1: 2.5-fold) and transporters involved in thyroid hormone cellular trafficking (Abcc2: 1.6-fold). T_4 clearance was higher in fipronil-treated than in vehicle-treated wild-type mice but not significantly. Furthermore, fipronil-induced mRNA expression modulations were lower in mice than in rats (6-fold, 1.5-fold and 1.2-fold increases for Cyp3a11, Sult1b1 and Abcc2, respectively). Although mice seemed to be less sensitive than rats to fipronil-induced thyroid disruption, fipronil-induced expression of PXR (Cyp3a, Abcb1a), CAR (Cyp2b, Ugt2b) or both (Ugt1a1, Abcc2) target genes were reduced in NR-deficient mice.
Conclusions: Thus, PXR and CAR contribute to modulate hepatic gene expression following fipronil treatment. Furthermore, our data combined with the known roles of PXR and/or CAR target genes suggest that these receptors could contribute to fipronil-induced thyroid disruption in rodents.

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ABSTRACTS