Activation of PXR, CAR and PPARα by pyrethroid pesticides and the effect of metabolism by rat liver microsomes

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2014). The widespread use of pyrethroids has resulted in their frequent detection in humans, and their metabolites are utilized as biomarkers (Heudorf et al., 2004). In addition, several pyrethroids have shown estrogenic and anti-androgenic activities in in vitro studies (Andersen et al., 2002; Chen et al., 2002; Kojima et al., 2004; Du et al., 2010). Tange et al. (2014) reported that cis-/trans-permethrin exhibit estrogenic and anti-androgenic activities, which are modified by metabolism.

Pyrethroid pesticides are widely used throughout the world both in agriculture and in household applications (Casida et al., 1983). They act on axons in the peripheral and central nervous systems of insects, causing prolonged opening of sodium channels (Aldridge, 1990), but they have relatively low acute toxicity in mammals, and are not persistent in the environment (Soderlund et al., 2002). However, it has been reported that pyrethroids induce some adverse effects in mammals. In rodents, pyrethroids caused neurotoxicity upon acute as well as chronic exposure (Shafer et al., 2005). Deltamethrin is known to increase oxidative stress, resulting in wide toxic effects including hepatotoxicity and nephrotoxicity, and several protective substances against the toxicity have been found in rats (Abdel-Daim et al., 2013, 2014; Abdou and Abdel-Daim, 2014). The widespread use of pyrethroids has resulted in their frequent

1. Introduction

Pyrethroid pesticides are widely used throughout the world both in agriculture and in household applications (Casida et al., 1983). They act on axons in the peripheral and central nervous systems of insects, causing prolonged opening of sodium channels (Aldridge, 1990), but they have relatively low acute toxicity in mammals, and are not persistent in the environment (Soderlund et al., 2002). However, it has been reported that pyrethroids induce some adverse effects in mammals. In rodents, pyrethroids caused neurotoxicity upon acute as well as chronic exposure (Shafer et al., 2005). Deltamethrin is known to increase oxidative stress, resulting in wide toxic effects including hepatotoxicity and nephrotoxicity, and several protective substances against the toxicity have been found in rats (Abdel-Daim et al., 2013, 2014; Abdou and Abdel-Daim, 2014). The widespread use of pyrethroids has resulted in their frequent

In this study, we used reporter gene assays in COS-1 cells to examine the activation of rat pregnane X receptor (PXR), rat constitutive androstane receptor (CAR) and rat peroxisome-proliferator activated receptor (PPARα) by pyrethroid pesticides, and to understand the effects of metabolic modification on their activities. All eight pyrethroids tested in this study showed rat PXR agonistic activity; deltamethrin was the most potent, followed by cis-permethrin and cypermethrin. However, when the pyrethroids were incubated with rat liver microsomes, their rat PXR activities were decreased to various extents. Cis- and trans-permethrin showed weak rat CAR agonistic activity, while the other pyrethroids were inactive. However, fenvalerate showed dose-dependent inverse agonistic activity toward rat CAR, and this activity was reduced after metabolism. None of the pyrethroids showed rat PPARα agonistic activity, but a metabolite of cis-permethrin and phenothrin, 3-phenoxybenzoic acid, activated rat PPARα. Since PXR, CAR and PPARα regulate various xenobiotic/endobiotic-metabolizing enzymes, activation of these receptors by pyrethroids may result in endocrine disruption due to changes of hormone-metabolizing activities.
also hydrolyzed to PBAlc (Kaneko et al., 1984). Estrogenic activity of trans-permethrin has been reported, and the activity was maintained after metabolism, because the hydrolyzed metabolites have similar estrogenic activities to the parent compound. In contrast, the activity of cis-permethrin was markedly enhanced by metabolism, because cis-permethrin is mainly hydroxylated to 4'-OH cis-permethrin, which shows higher estrogenic activity than the parent compound (Tange et al., 2014). Thus, differences of metabolism between trans- and cis-permethrin could influence their endocrine-disrupting activities.

Pregnane X receptor (PXR), constitutive androstane receptor (CAR) and peroxisome proliferator-activated receptor α (PPARα) are members of the nuclear receptor superfamily. The induction of drug-metabolizing enzymes and transporters is predominantly regulated by these receptors (Omiecinski et al., 2011b). PXR and CAR have overlapping target CYP genes. Induction of CYP2B, CYP2C and CYP3A gene expression is mediated by both receptors (Maglich et al., 2002). In contrast, PPARα primarily induces the expression of CYP4A (Kroetz et al., 1998). These receptors regulate not only CYP but also phase II enzymes such as uridine 5'-diphospho-glucuronosyltransferases (UGTs), glutathione S-transferases and sulfotransferases (SULTs) (Kast et al., 2002; Kodama and Negishi, 2013; Bigo et al., 2013). In addition, PXR and CAR may contribute to the regulation of carboxylesterase (Staudinger et al., 2010). Because pyrethroids are metabolized by carboxylesterases and CYPs, the activation of PXR, CAR and PPARα could increase the extent of their metabolism.

Activation or inactivation of these receptors can also cause endocrine disruption indirectly, since endogenous hormones are metabolized by various enzymes, including CYPs, UGTs and SULTs. Thus, induction of these enzymes by PXR, CAR and PPARα promotes the inactivation of hormones. For example, CAR agonists reduce thyroid hormone levels by inducing UGTs (Schraplau et al., 2015). In addition, phthalates, which are PPARα agonists, reduce serum estradiol levels by inhibiting the gene expression of aromatase, which catalyzes the conversion of testosterone to estrogen (Lovekamp-Swan and Davis, 2003). Activation of mouse and human PXR by pyrethroids has been reported (Kojima et al., 2011; Abass et al., 2012), but the activation of CAR and PPARα by pesticides including pyrethroids has been little studied.

The effects of metabolic modification upon estrogen receptor and androgen receptor agonistic activities have been investigated. Indeed, various proestrogens, which are estrogenic only after metabolism by the microsomal CYP system, such as methoxychlor, trans-stilbene, diphenyl, 2,2-diphenylpropane, benzo[a]pyrene, 2-nitrofluorene and benzophenone-3, have been identified in in vitro estrogen screening tests (Kitamura et al., 2008; Watanabe et al., 2015). Thus, it is important to consider the effects of metabolic modification. However, the contributions of metabolic activation and inactivation to the PXR, CAR and PPARα agonistic activities of pyrethroids have not been investigated.

Pyrethroid pesticides can be mainly divided into two groups based on their chemical structures. Type I pyrethroids, such as cis-/trans-permethrin and allethrin, lack a cyano group, whereas type II pyrethroids, such as cypermethrin and deltamethrin, contain a cyano group. In the present study, we examined the ability of eight pyrethroids, i.e., cis-/trans-permethrin, allethrin, bioresmethrin and phenothrin (type I), and cypermethrin, deltamethrin and fenvalerate (type II) (Fig. 1), to activate rat PXR, CAR and PPARα. Furthermore, we examined the effects of metabolism by the rat liver microsomal system on their activities.

2. Materials and methods

2.1. Chemicals

The structure, source and purity of each of the eight pyrethroids and the authentic samples of their metabolites tested in the present study are shown in Fig. 1 and Table 1. 5-Pregnen-3β-ol-20-one-16α-carbonitrile (PCN; >97% pure) and artemisinin (>97% pure) were purchased from

![Fig. 1. Structures of pyrethroids used in this study. Type I: pyrethroids not containing a cyano group. Type II: pyrethroids containing a cyano group.](image-url)
activity was normalized to 2.3. Reporter gene assay (Fujino et al., 2016). Plasmids used in this study were as described previously. Cells were harvested with 25 μl of passive lysis buffer and 1% MEM NEAA (without FBS). After 24 h incubation with various concentrations of test compounds (trans-CAR), the cells were exposed to various concentrations of test compounds showing 20% of the agonistic activity of the positive control (1 μM PCN for rat PXR, 30 μM artemisinin for rat CAR and 30 μM BZF for rat PPARα), while REC20 means the concentration of test compound showing 80% of the activity of the vehicle control.

Table 1: Source and purity of pyrethroids and the metabolites used in this study.

| Compound               | Source            | Purity (%) |
|------------------------|-------------------|------------|
| allethrin              | Wako              | >98.0 (for Pesticide Residue Analysis) |
| biocresmethrin         | Wako              | 95.0 (for Pesticide Residue Analysis) |
| cypermethrin           | Wako              | >96.0 (for Pesticide Residue Analysis) |
| deltamethrin           | Wako              | 99.0 (for Pesticide Residue Analysis) |
| fenvalerate            | Wako              | 99.0 (for Pesticide Residue Analysis) |
| cis-permethrin         | Wako              | 98.0       |
| trans-permethrin       | Wako              | 98.0       |
| phenothrin             | Ehrenstorfer      | 97.5       |
| 3-phenoxybenzyl alcohol (PBAld) | Wako     | 98.0       |
| 3-phenoxybenzyl aldehyde (PBAlc) | Wako  | 97.0       |
| 3-phenoxybenzoic acid (PBAcid) | Wako     | 98.0       |

Sigma-Aldrich (St. Louis, MO, USA), and Tokyo Chemical Industry Co. Ltd. (TCl; Tokyo, Japan), respectively. Bezafibrate (BZF; >99.3% pure) and dimethyl sulfoxide (DMSO; >99.5% pure) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Cells and plasmids

COS-1 cells derived from African green monkey kidney were obtained from RIKEN BioResource Center (Ibaraki, Japan). Dulbecco’s modified Eagle’s medium (high glucose) with 1-glutamine, phenol red and sodium pyruvate (D-MEM) was purchased from Wako Pure Chemical Industries, Ltd. Fetal bovine serum (FBS) was purchased from Sigma-Aldrich. Anti-biotic-Antimycotic (Anti-Anti), MEM non-essential amino acids (MEM NEAA) and 0.5% trypsin-ethylenediaminetetraacetic acid (EDTA) disodium salt solution were obtained from Life Technologies, Inc. (Carlsbad, CA, USA).

Cells were routinely cultured in D-MEM supplemented with 10% FBS, 1% Anti-Anti and 1% MEM NEAA at 37 °C, in an atmosphere of 5% CO2/95% air under saturating humidity, as described in our previous report (Fujino et al., 2016). Plasmids used in this study were as described previously (Fujino et al., 2016).

2.3. Reporter gene assay

COS-1 cells were plated in 96-well plates (Thermo Fisher Scientific, Waltham, MA, USA) at 1×10^4 cells/well in D-MEM supplemented with 10% FBS, 1% Anti-Anti and 1% MEM NEAA. The cells were transfected with 6.25 ng/well of the expression plasmid, 100 ng/well of the reporter plasmid and 10 ng/well of the internal control plasmid using the jetPEI transfection reagent (PolyPlus Transfection, Illkirch, France) at the same time. After 24 h (assay for rat PXR and rat PPARα) or 12 h (assay for rat CAR), the cells were exposed to various concentrations of test compounds or 0.1% DMSO (vehicle control) in D-MEM supplemented with 1% Anti-Anti and 1% MEM NEAA (without FBS). After 24 h incubation with chemicals, cells were harvested with 25 μl of passive lysis buffer (Promega). Luciferase assays were performed using a Dual Luciferase Assay Kit (Promega), and luminescence was measured with the luminometer Luminoskan Ascent (Thermo Fisher Scientific). Firefly luciferase activity was normalized to Renilla luciferase activity of pHRL-4k. Results are expressed as means ± standard deviation (SD) from at least three independent experiments performed in duplicate. Details were described in our previous report (Fujino et al., 2016).

DMSO was used as a vehicle, and all test compounds used were dissolved in DMSO at a concentration of 30 mM. The final DMSO concentration in the culture medium did not exceed 0.1%, and this concentration did not affect cell yields. All compounds were diluted to the predetermined concentrations in appropriate medium immediately before use.

Relative activities toward rat PXR, CAR and PPARα are presented in terms of 20% relative effective concentration (REC20) for agonistic activities and 20% relative inverse agonistic active concentration (RIC20) for inverse agonistic activity. REC20 means the concentration of test compounds showing 20% of the agonistic activity of the positive control (1 μM PCN for rat PXR, 30 μM artemisinin for rat CAR and 30 μM BZF for rat PPARα), while RIC20 means the concentration of test compound showing 80% of the activity of the vehicle control.

2.4. Animals for the preparation of liver microsomes

Male Sprague Dawley rats (Slc:SD, 210–230 g, Japan SLC, Shizuoka, Japan), which are commonly used in studies on drug-metabolism and chemical toxicity, were used to prepare inducer-treated liver microsomes. The animals were housed at 22 °C and a relative humidity of 55% with a 12-h light/dark cycle, with free access to tap water and standard pellet diet MM-3 (Funabashi Farm, Funabashi, Japan). In this experiment, to prepare CYP-enriched liver microsomes, which efficiently metabolize chemical, as reported previously (Watanabe et al., 2015; Fujino et al., 2016), rats were co-treated with typical CYP inducers, phenobarbital (PB, which induces mainly CYP2B and CYP3A) and 3-methylcholanthrene (MC, which induces CYP1A1) once per day for 3 consecutive days at 80 mg/kg (i.p.) and 25 mg/kg (p.o.), respectively. These experiments were approved by “Animal Experiment Ethics Committee” of Niho Pharmaceutical University, and were conducted in accordance with the “Guide for the Care and Use of Laboratory Animals” of Niho Pharmaceutical University.

2.5. Preparation of liver microsomes

Rat liver was homogenized with four volumes of 1.15% potassium chloride, and the homogenate was centrifuged at 9,000 x g for 20 min. The supernatant was further centrifuged at 105,000 x g for 60 min to obtain cytosol and microsomes. These microsomes were washed by resuspension in two volumes of 1.15% potassium chloride solution at 105,000 x g for 60 min. The microsomal pellets were resuspended in the solution to make 1 ml equivalent to 1 g of liver.

2.6. Metabolism by rat liver microsomes

The incubation mixture consisted of 1 μmol of pyrethroid, an NADPH-generating system (1 μmol of NADPH, 5 μmol of d-glucose-6-phosphate disodium salt and 1 unit of glucose-6-phosphate dehydrogenase), which can sustainably regenerate NADPH, and rat liver microsomes of PB- and MC-treated rats (60 mg protein) in a final volume of 20 ml of 0.1 M K, Na-phosphate buffer (pH 7.4). Rat microsomes boiled for 10 min at 90 °C were used as the control. The incubation was continued for 30 min at 37 °C. After incubation, the mixture was extracted with 25 ml of ethyl acetate. This metabolism condition was reported previously (Tange et al., 2014). The extract was evaporated to dryness and the residue, which contains both unchanged pyrethroid and formed metabolites, was dissolved in DMSO at a concentration of 30 mM, determined as the initial concentration of unchanged form. This solution was diluted to predetermined concentrations as required. Aliquots were used to examine the effect of metabolism on the receptor agonistic activities.

2.7. Statistical analysis

Data analysis was conducted using Mini StatMate software (ATMS, Tokyo, Japan). Dunnett’s method was used to evaluate the significance of
Fig. 2. Agonistic activities of pyrethroids toward PXR (A), and the effects of metabolism (B). (A); PXR activation of pyrethroids was expressed as n-fold induction versus the vehicle control. Each value represents the mean ± SD of 3 individual experiments. *p < 0.05 indicates a significant difference from the vehicle control (Dunnett’s test). (B); Pyrethroids were incubated with native or boiled liver microsomes in the presence of NADPH, and extracts of the incubation mixtures were assayed. Each value represents the mean ± SD of 3 individual experiments. *p < 0.05 indicates a significant difference from the control experiment using boiled microsomes (Student’s t-test). Other details are described in Materials and Methods.

PCN: 5-pregnen-3β-ol-20-one-16α-carbonitrile.
differences in transcriptional levels between the experiment groups and the control group (0.1% DMSO alone). Student's t-test was used to evaluate the significance of differences in transcriptional levels between a test group (extract of incubation mixture with native microsomes) and the control group (extract of incubation mixture with boiled microsomes). Data are presented as means ± SD.

3. Results

3.1. Agonistic activities of pyrethroid pesticides toward PXR, and the effects of metabolism

The ability of the eight pyrethroids to activate rat nuclear receptor

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

**Fig. 3.** The activities of pyrethroids toward CAR (A), the activity of fenvalerate in the presence of artemisinin (10 μM) (B), and the effect of metabolism (C). (A); CAR activation of pyrethroids was expressed as n-fold induction versus the vehicle control. Each value represents the mean ± SD of 3 individual experiments. *p < 0.05 indicates significant differences from the vehicle control (Dunnett's test). (B); the activity was expressed as n-fold induction versus the activity of artemisinin (10 μM). *p < 0.05 indicates a significant difference from the control experiment in the presence of artemisinin (10 μM) (Student's t-test). (C); the activity was expressed as n-fold induction versus the vehicle control using COS-1 cells. Each bar represents the mean ± SD of 3 individual experiments. There is no significant difference between the boiled microsome group and the experimental group (Student's t-test).
PXr by reporter gene assay was examined. PCN, a positive control for rat PXr, enhanced luciferase activity 3.2-fold compared to DMSO-treated cells (control) at the concentration of 1 μM (Fig. 2A). All the pyrethroids used in this study showed PXr agonistic activity. The activities of cis-/trans-permethrin, allethrin, cypermethrin, deltamethrin and fenvalerate were enhanced about 2-fold at the concentration of 1 μM or lower, compared with the control and further increased to about 3-fold in the concentration range of 1–30 μM. However, bioresmethrin and phenothrin showed relatively weak activities. PBAlc, the hydrolysis product of cis-/trans-permethrin and phenothrin, showed weak PXr agonistic activity at the concentration of 30 μM, whereas the oxidized products, PBAld and PBAcid, were inactive (Fig. 2A). The effect of metabolism on the PXr activities of pyrethroids was examined using the mixed function oxidase system of microsomes from liver of PB- and MC-co-treated rats in the presence of NADPH. The PXr activities of all eight pyrethroids were decreased upon incubation with the microsomes (Fig. 2B). Among these pyrethroids, PXr activation by fenvalerate was markedly decreased at 3–30 μM. The activities of cis-/trans-permethrin, phenothrin and deltamethrin at high concentrations were also decreased, e.g. to the control level in the case of phenothrin (Fig. 2B).

3.2. Activities of pyrethroid pesticides toward CAR, and the effects of metabolism

In rat CAR activation assay, artemisinin, a positive control for rat CAR, enhanced luciferase activity 1.9-fold at the concentration of 30 μM (Fig. 3A). Among the pyrethroids tested, cis-permethrin and trans-permethrin showed CAR agonistic activity at higher concentrations. In contrast, bioresmethrin, phenothrin, deltamethrin and fenvalerate showed CAR inverse agonistic activity, i.e., they suppressed the constitutive activity of the control. In particular, fenvalerate decreased the activity in a dose-dependent manner in the concentration range of 1–30 μM. On the other hand, PBAlc, PBAld and PBAcid did not show CAR agonistic or inverse agonistic activity (Fig. 3A). Next, we examined the effect of fenvalerate on CAR activation induced by artemisinin (10 μM). As shown in Fig. 3B, fenvalerate significantly inhibited CAR activation by artemisinin in the range of 0.1–30 μM. However, after incubation of fenvalerate with liver microsomes in the range of 3–10 μM, its CAR inverse agonistic activity tended to decrease (Fig. 3C). Based on the Renilla luciferase activity, a cytotoxic effect was found at the concentration of 30 μM after metabolism.

3.3. Agonistic activities of cis-/trans-permethrin toward PP Ars, and the effects of metabolism

The activities of pyrethroids, as well as the hydrolysis product PBAlc and the oxidized products PBAld and PBAcid of cis-/trans-permethrin, toward rat PP Ars were examined. BZF, a positive control, enhanced luciferase activity 2.4-fold at the concentration of 30 μM (Fig. 4). None of the eight pyrethroids exhibited PP Ars agonistic activity (data not shown). However, PBAlc showed dose-dependent agonistic activity. PBAlc also showed weak activity at 30 μM (Fig. 4). We could not determine the effects of the metabolism, because the extract obtained after incubation without pyrethroids exhibited PP Ars agonistic activity (data not shown).

3.4. Relative activities of pyrethroids toward PXr, CAR and PP Ars

As the indicator of the relative activities of pyrethroids toward PXr, CAR and PP Ars, we used REC20 for agonistic activities and RIC20 for inverse agonistic activity in this study. The values of REC20 for the PXr, CAR and PP Ars agonistic activities of pyrethroids and their metabolites and RIC20 values for inverse agonistic activity for CAR are summarized in Table 2. Deltamethrin showed the lowest REC20 value for PXr agonistic activity (0.156 μM), followed by cis-permethrin (0.329 μM). The values toward pyrethroids examined in this study are within the range of 0.156–3.89 μM. The order of PXr activation in terms of REC20 was deltamethrin > cis-permethrin > cypermethrin > fenvalerate > allethrin > trans-permethrin > bioresmethrin > phenothrin. REC20 for CAR agonistic activity was evaluated only for trans-permethrin (4.19 μM). PBAlc showed a REC20 value of 26.8 μM for PP Ars agonistic activity, while fenvalerate showed a RIC20 value of 0.846 μM for CAR inverse agonistic activity.

4. Discussion

There are species differences in the ligand-dependent activation of nuclear receptors, such as PXr, CAR, and PP Ars. For example, rifampicin activates human PXr, but not mouse and rat PXr, and PCN is an agonist of mouse and rat PXr, but not human PXr. Furthermore, 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime (CITCO) and 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) act as agonists of human and mouse CAR, respectively. It has been reported that some pyrethroids possess agonistic activities toward human and/or mouse PXr, CAR and PP Ars. For example, Koijima et al. (2011) observed similar agonistic activities of deltamethrin, fenvalerate, cypermethrin and permethrin (a mixture of cis-permethrin and trans-permethrin) in human and mouse PXR-mediated transactivation assays using COS-7 cells. In addition, Baldwin and Roling (2009) reported that cypermethrin, deltamethrin and fenvalerate were agonists of both human and mouse CAR, although Abass et al. (2012) showed that fenvalerate possessed agonistic activity toward human but not mouse CAR. As for PP Ars activation, only pyrethrin, among 12 pyrethroid pesticides, activated mouse PP Ars (Takeuchi et al., 2006). However, there has been no comprehensive study on the in vitro activation of rat PXr, CAR and PP Ars by pyrethroids. In this study, we did not detect rat CAR activation by these pesticides, although cis-/trans-permethrin showed weak CAR agonistic activity (Fig. 3A). Furthermore, fenvalerate showed dose-dependent inverse agonist activity toward rat CAR in this study (Fig. 3B). On the other hand, Abass et al. (2012) reported that fenvalerate showed agonistic activity toward human but not mouse CAR. CITCO and TCPOBOP were reported to be ligands for human and mouse CAR,
respectively, but were inactive toward rat CAR (Omiecinski et al., 2011a). Thus, there appear to be marked species differences in the response to pyrethroids among human, mouse and rat CAR. These results are in line with those for rat PPARα activation (data not shown).

In this study, the PXR agonistic activity varied markedly among the pyrethroids tested (Fig. 2A), and some pyrethroids showed CAR activity (Fig. 2B), while most did not show PPARα activity. However, we could not identify structural requirements for PXR, CAR and PPARα activity. It is considered that PXR shows lower specificity than CAR, because the ligand-binding region in PXR is larger and more flexible than that of CAR (Timsit and Negishi, 2007). Actually, the number of chemicals known to activate PXR is much greater than that known to activate CAR (Kretschmer and Baldwin, 2005). Also, CAR activity is observed in the absence of ligand activation, as demonstrated in case of phenobarbital (Rencurel et al., 2005; Mutoh et al., 2013). Further study of ligand-independent activation of CAR by pyrethroids might be needed.

Metabolic modification of endocrine-disrupting agents can affect their toxicity. Further, hydroxyl metabolic metabolites of pyrethroids show greater estrogenic and anti-androgenic activity than the parent compounds (Tyler et al., 2000; McCarthy et al., 2006; Sun et al., 2007, 2014; Tange et al., 2014). Carboxylesterase is known to hydrolyze pyrethroids (Ross et al., 2006; Crow et al., 2007). Cis/trans-permethrin is hydrolyzed to PBAlc, and further oxidized to PBAlc and PBAcid by CYP and alcohol and aldehyde dehydrogenases (Choi et al., 2002; Nakamura et al., 2007). In this study, all the pyrethroid pesticides showed agonistic activity against PXR (Fig. 2A), but the activity was decreased after incubation with rat liver microsomes (Fig. 2B). It is reported that type I pyrethroid is more easily hydrolyzed than type II by carboxylesterase (Crow et al., 2007), but we observed no systematic difference in the metabolic modification of PXR activity between type I and type II pyrethroids (Fig. 2B). The PXR activity of phenoxyrin (type I pyrethroid) was significantly decreased, whereas those of allethrin and bioremethrin (type I pyrethroid) were slightly decreased by metabolism. Furthermore, the activity of fenvalerate (type II pyrethroid) was significantly decreased by metabolism. The results suggest that other metabolic reactions may contribute to the decrease of PXR activity. Exceptionally, the metabolites of cis/trans-permethrin and phenothrin, PBAlc and PBAcid, were more active PPARα agonists than the parent compounds (Fig. 4). We were unable to investigate the effect of the microsomal metabolism on PPARα activity, because the extract obtained after incubation without pyrethroids exhibited PPARα agonistic activity (data not shown). The enhanced activity may be due to the lipid derived from the microsomes. The metabolic pathways and the effects of cis/trans-permethrin and their metabolites on PXR, CAR and PPARα are summarized in Fig. 5.

Exposure of humans to pyrethroids has been reported. The urinary concentration of PBAcid is employed as an indicator of permethrin exposure, and PBAcid has been detected in urine of the general population (Leng et al., 1997; Barr et al., 2010; Le Grand et al., 2012; Wielogomas, 2013). The concentrations of pyrethroids and metabolites used in the present study are higher than the concentrations reported in vivo, but humans are exposed to pyrethroid pesticides on a daily basis, so it is important to consider the effect of chronic toxicity.

In conclusion, we found that all the pyrethroids examined showed PXR agonistic activity but were converted to inactive or less active metabolites upon incubation with rat liver microsomes. Fenvalerate showed inverse agonistic activity toward CAR, and this activity decreased after metabolism. PBAcid, a metabolite of cis/trans-

![Fig. 5. Metabolic pathways of cis/trans-permethrin, and summary of the effects of cis/trans-permethrin and their metabolites on PXR, CAR and PPARα. The metabolic pathways are taken from the reports of Choi et al. (2002), Hodgson (2003), Nakamura et al. (2007) and Tange et al. (2014). PBAlc: 3-phenoxybenzyl alcohol, PBAld: 3-phenoxybenzaldehyde, PBAcid: 3-phenoxybenzoic acid, ADH: alcohol dehydrogenase, ALDH: aldehyde dehydrogenase.](attachment:image.png)
permethrin, showed PPARα agonist activity, although pyrethroids themselves were inactive.

Declarations

Author contribution statement

Chieri Fujino: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yoko Watanabe: Analyzed and interpreted the data. Seigo Sanoh: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hiroyuki Nakajima, Naoto Uramaru, Hiroyuki Kojima, Shigeru Ohta: Contributed reagents, materials, analysis tools or data. Kouichi Yoshihara: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data. Shigeyuki Kitamura: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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