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Organochlorine pesticide acetofenate and its hydrolytic metabolite in rabbits: Enantioselective metabolism and cytotoxicity

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ABSTRACT

Acetofenate (AF) is a chiral organochlorine pesticide used for controlling hygiene pests. In this study, the metabolism of AF in rabbits in vivo and in vitro was investigated and the primary chiral metabolite acetofenate-alcohol (AF-A) was analyzed. The cytotoxicity of AF and AF-A was also determined. AF in rabbits in vivo was eliminated so rapidly that AF could not be detected within 10 min after intravenous administration at 20 mg/kg (body weight), and AF-A was quickly formed. In vitro metabolism assay, using plasma and liver microsomes, showed that AF was also quickly metabolized to AF-A and the metabolic process was significantly enantioselective with preferential degradation of (-)-AF and formation of (-)-AF-A. The cytotoxicity of AF and AF-A were investigated by assessing cell proliferation, apoptosis and generation of reactive oxygen species. The results showed that AF and AF-A induce enantioselective cytotoxicity. This study will be helpful for improving knowledge about the metabolism and toxicity of AF on an enantiomeric level and providing evidence to understand the potential environmental risk.

1. Introduction

Organochlorine pesticides (OCPs) have been widely used throughout the world and play a rather important role in controlling pests and vector borne diseases in agriculture and public health [1]. However, as a result of the negative impact on the environment and human health, most OCPs, such as DDTs, HCHs and chlordane, have been banned or severely restricted since the 1980s [2]. Acetofenate (AF, shown in Fig. 1), also known as plifenate or 7504, is a kind of OCP with outstanding effects against the pests having developed resistance to DDTs or HCHs when used alone or with pyrethroid insecticides [3]. Remarkably, AF is one of the few allowed OCPs and considered as a good alternative to those prohibited OCPs. It is currently used for controlling hygiene pests such as mosquitoes and flies both indoors and outdoors in China and most of southeastern Asia [3]. However, the understanding of AF is still limited.

AF is a chiral compound and consists of a pair of enantiomers. Enantioselectivity is a significant characteristic of chiral pesticides because enantiomers may behave differently in biological processes and the environment [4]. Many chiral pesticides displayed enantioselective toxicity [5]. For example, R-(−)-*o,p′*-DDT caused greater cell apoptosis in rat PC12 cells and had more potential endocrine disruption in human MCF-7 cells than its enantiomer S-(+)-o,p′-DDT [6,7]. R-(+)-*o,p′*-DDD also intensified apoptosis in rat PC12 cells compared to its enantiomer [8]. In addition, pesticide enantiomers may have different metabolic behaviors and accumulation in the biological system. For instance, α-HCH showed enantioselective degradation and accumulation in various tissues of mouse and quail, including blood, liver, brain, muscle spleen and kidney [9]. Similarly, (+)-α-HCH was preferentially metabolized in loaches, resulting in a gradual bioaccumulation of (−)-α-HCH [10]. Chlordane and heptachlor also have enantioselective metabolic behaviors in rat liver microsomes [11]. Therefore, enantiomeric differences are an essential consideration for the evaluation of chiral pesticides.

At present, most studies on AF have focused on its toxicity. The enantiomers of AF showed no difference in acute toxicity to zebrafish, but S-(+)-AF was more active than R-(-)-AF in developmental toxicity such as yolk bag edema and pericardial edema [12]. Besides, AF could induced obvious apoptosis in RAW 264.7 cells, and S-(+)-AF had stronger effects than R-(-)-AF on induction of intracellular reactive oxygen species and DNA damage [13,14]. Additionally, AF enantiomers could lead to enantioselective cytotoxicity in rat PC12 cells mediated by oxidative stress [15]. Moreover, S-(+)-AF had stronger potential effects on disrupting hormone secretion, maternal immune tolerance and steroidogenesis in the trophoblast compared to R-(-)-AF through a

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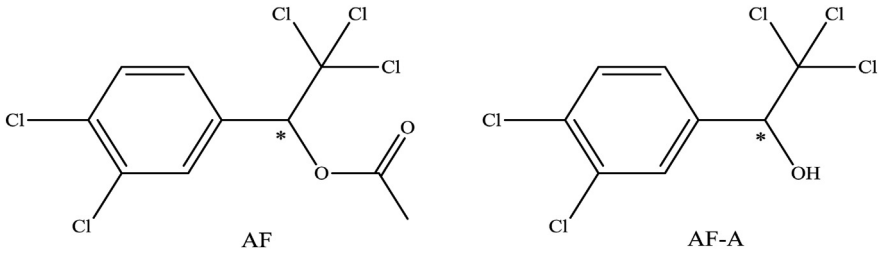

Fig. 1. Chemical structures of AF and its metabolite AF-A. Chiral center is denoted by an asterisk (*).

human MCF-7 and JEG-3 cell assay [16].

The metabolism of AF and related metabolites have not been reported in detail. Kang et al. has applied theoretical calculation to predict the degradation mechanism of AF in the atmosphere [17]. However, studies regarding the degradation of AF and the consequent metabolites in the organisms was rare. Methods to study pesticide metabolism in animals usually include in vivo and in vitro models. In vivo experiments are very effective in describing the relationship between time and concentrations of chemicals in blood (or plasma, serum), distribution and elimination in the body [18]. In vitro models are also very popular because of their easy control, low cost, great repeatability and flexibility [19]. Owing to the rich enzymes, plasma and liver microsomes are two common in vitro systems for investigating the mechanism of metabolism [20].

So far, the metabolic mechanism and enantioselective metabolism of AF in mammals are still not well known. In this work, the metabolism of AF in rabbits was studied in vivo, and the hydrolytic metabolite acetofenate-alcohol (AF-A, shown in Fig. 1) was monitored. To verify the in vivo results, AF metabolism in the in vitro assay including plasma and microsomes metabolism was investigated, and the enantioselectivity in metabolic processes was studied. For more comprehensive knowledge of AF and its metabolite AF-A, the enantioselective cytotoxicity of AF and AF-A was assessed by measuring the cell growth inhibition rate, cellular apoptosis and reactive oxygen species (ROS) generation.

2. Materials and methods

2.1. Chemicals and reagents

Rac-acetofenate (> 98%) was obtained from China Ministry of Agriculture's Institute for Control of Agrochemicals. The hydrolysis product of acetofenate, namely, acetofenate-alcohol (2,2,2-trichloro-1-(3,4-dichlorophenyl)ethanol, AF-A), was provided by the synthetic laboratory of China Agricultural University, and was over 99% purity. Dimethyl sulfoxide (DMSO) was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Tris, ethylene diamine tetra-acetic acid (EDTA), β-nicotinamide adenine dinucleotide phosphate (NADPH) and methyl thiazolyl tetrazolium (MTT) were purchased from Sigma-Aldrich (St Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from HyClone (Utah, USA). Heparinized tubes were purchased from Shandong Aosaite Medical Instrument Co. Ltd. (Shandong, China). Solvents including acetone, petroleum ether and ethyl alcohol (analytical-grade) were purchased from Beijing Chemical Reagents Company (Beijing, China). All the chromatographically pure mobile-phase reagents of were purchased from Fisher Chemicals (Fair Lawn, USA). Water was purified by a Milli-Q system. A cell culture incubator (Thermo Scientific 8000, USA), inverted microscope (Leica DMI3000B, Germany) and microplate reader (Thermo MK3, USA) were provided by Shanghai Rochen Biological Technology Co. Ltd. (Shanghai, China).

2.2. In vivo metabolism assay

2.2.1. In vivo metabolism procedure

Male adult Japanese white rabbits weighing 2–2.5 kg were purchased from Experimental Animal Research Institute of China Agriculture University (Beijing, China). They were given free access to commercial feed and water in a well-ventilated room with a cycle of 12/12 h light/dark. All animal experiments were carried out according to the standard ethical guidelines approved by the local ethics committee (Beijing Association for Laboratory Animal Science), ethical permit number 30749.

A certain amount of rac-AF dissolved in DMSO was added to alcohol (20% DMSO, v/v) and then administered to rabbits at a dose of 20 mg/kg body weight by intravenous injection in the ear vein. The injection volume for each rabbit was not > 0.2 mL. Blood samples were taken from the heart and collected in heparinized tubes at 10, 20 and 40 min and 1, 1.5, 2, 3, 5, 7, 12 and 24 h. Blank blood samples were collected from a rabbit never receiving drug administration. Samples were pretreated by centrifugation at 3300*g* for 10 min at 4 °C, and the supernatant plasma was separated and extracted immediately to avoid AF degradation.

An aliquot of 200 μL of plasma samples was transferred to a 15-mL polypropylene tube, and 4 mL of petroleum ether and acetone (9/1, v/v) was added. The polypropylene tube was vortexed for 3 min, and then the supernatant was transformed to a glass tube after centrifugation at 2500*g* for 5 min. The remaining plasma sample was re-extracted with another 4 mL of mixed solvent of petroleum ether and acetone (9/1, v/v) by vortex for 5 min, and the supernatant organic phase was combined. Subsequently, the supernatant organic phase was dried under a gentle nitrogen flow at 30 °C. Then, the residue was re-dissolved in 200 μL of isooctane, and finally filtered through a 0.22 μm syringe filter for GC-MS/MS analysis.

2.2.2. GC-MS/MS analysis

To ensure the accurate analysis for traces of targets in rabbit, gas chromatography-tandem mass spectrometry (GC-MS/MS, Thermo Fisher Scientific, Waltham, MA, USA) was applied for the quantification of AF and AF-A. Separation was performed using an HP-5 capillary column (30 m × 0.25 mm × 0.25 μm, Agilent). The GC injector was held at 270 °C in splitless mode, and the sample injection volume was 1 μL. The GC oven temperature program was set at 60 °C, increased to 160 °C at a rate of 10 °C/min and held for 1 min, then increased to 190 °C at a rate of 3 °C/min and held for 1 min, and finally increased to 270 °C at a rate of 20 °C/min and held for 1 min. The carrier gas was ultrapure helium at a constant flow rate of 1 mL/min. Electron impact ionization (EI^+) was achieved at 70 eV and, specific selective reaction monitoring (SRM) was chosen for analyzing the target compounds. The ion source and transfer line temperature was 250 °C. Other parameters were presented in Table S1.

2.3. In vitro metabolism assay

2.3.1. Sample preparations

The blood was taken from the heart of a drug-free rabbit and centrifuged at 3300*g* for 10 min at 4 °C to obtain plasma. The fresh plasma