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Effects of avermectin on microsomal cytochrome P450 enzymes in the liver and kidneys of pigeons

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ARTICLE INFO

Article history:
Received 7 February 2014
Received in revised form
10 August 2014
Accepted 14 August 2014
Available online 23 August 2014

Keywords:
Avermectin
Cytochrome P450 enzyme
Pigeon

ABSTRACT

Residues of avermectin (AVM) drugs have toxic effects on non-target organisms. Analyses of cytochrome P450 enzymes are among the most frequently employed indicators in pharmacology and toxicology studies. In this study, the responses of cytochrome P450 enzymes and pathological changes in the liver and kidney tissues of King pigeons (*Columba livia*) following subchronic exposure to avermectin for 30, 60 and 90 d were investigated. Dose- and time-dependent decreases in the activities of P450 enzymes (i.e., aminopyrine-N-demethylase, erythromycin N-demethylase, aniline 4-hydroxylase and NADPH-cytochrome C reductase) and down-regulation of the P450 and b5 contents were observed. The microscopic structures were clearly altered, the severity of these alterations increased with the concentration of AVM and the exposure time. These results imply that AVM can inhibit the P450 enzyme systems in the liver and kidney tissues of pigeons. This research provides insight into the safe use of AVM and a comprehensive evaluation of the toxicological effects of AVM in birds.

1. Introduction

Avermectins (AVMs) are commonly used in veterinary medicine as anthelmintics against internal and external parasites of livestock, including nematodes, arachnids and insects (Huang et al., 2011; Molinari et al., 2009). As pesticides, AVMs are typically used in agriculture against mites, true bugs and other common pests (Novelli et al., 2012). Excessive application of AVMs and their poorly degradable nature have led to large amounts of avermectin drug residues and metabolites in livestock feces in the environment (Halley et al., 1989). A large number of studies have shown that AVMs have adverse effects on non-target organisms, including invertebrates that live in the soil, some water organisms and mammals (El-Shenawy, 2010; Na et al., 2009). To the best of our knowledge, few studies on the toxic effects of AVMs in birds have been reported (Halley et al., 1993). However, due to their strong sensitivities to environmental pollutants including pesticides, birds are one of the most important ecological pollution indicators (Rattner, 2009).

Abbreviations: AVM, avermectin; IVM, ivermectin; ABM, abamectin; CYPs, cytochrome P450 enzyme systems; AND, aminopyrine N-demethylase; ERND, erythromycin N-demethylase; PCBs, polychlorinated biphenyls; GABA, gamma-aminobutyric acid; AH, aniline 4-hydroxylase; CR, NADPH-cytochrome C reducatase; H&E, hematoxylin–eosin.

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http://dx.doi.org/10.1016/j.etap.2014.08.011
1382-6689/

Thus, research of the effects of pesticides in birds might be of particular significance to evaluations of the adverse effects of pesticides in the environment.

The microsomal cytochrome P450 enzyme system (CYPs) plays a primary role in the biotransformation of most exogenous compounds and is responsible for the primary modifications of xenobiotic compounds (Tompkins and Wallace, 2007). Ninety percent of exogenous poisons are chiefly metabolized and detoxified via the CYP enzymes (Bertz and Granneman, 1997). The CYPs is localized in the membrane of the endoplasmic reticulum (Neve and Ingelman-Sundberg, 2010) and includes CYP 450, b_5, CR, NADPH cytochrome b_5 reductase and phospholipids. The main CYPs include CYP3A4, which accounts for nearly 30% of the total CYP, CYP2C9 (~20%), and CYP1A2 (~15%) in mammals (Guengerich, 2003). The chicken CYP3A37 gene sequence is 60% homologous with the human CYP3A4, and the steroid hydroxylase profile of CYP3A37 exhibits a high degree of similarity to the mammalian 3A enzyme (Ourlin et al., 2000). Currently, the induction or inhibition of xenobiotic metabolism via CYP manipulations is well-established as a useful tool for ecotoxicology assays and the biomonitoring of environmental contamination (Dong et al., 2009).

Pigeons are one of the species that are recommended in the OECD Guidelines for the Testing of Chemicals. The early acute toxicity results of preliminary experiments completed in our laboratory revealed that pigeons are strongly sensitive to AVM; therefore, we chose pigeons for use as an experimental animal for the monitoring of environmental AVM pollution. Currently, only limited data are available that describes the effects of AVM exposure on the activities of cytochrome P450 enzymes and tissue damage in birds. Therefore, based on an established subchronic AVM poisoning model in pigeons, this experiment was designed to detect the CYP450 and b_5 contents, the aminopyrine-N-demethylase (AND), erythromycin N-demethylase (ERND), aniline 4-hydroxylase (AH) and NADPH-cytochrome C reducatase (CR) activities and the histopathological changes in the liver and kidneys.

2. Materials and methods

2.1. Preparation of animals

Eighty 60-day-old American King pigeons (*Columba livia*) were supplied by Harbin Zoo, Harbin, China. The animals were housed in the animal facility for 7 days prior to each experiment and fed a standard pigeon diet. The animal room was maintained at 24 ± 2 °C with 50% humidity and time-controlled lighting (12 h of light per day).

2.2. Chemicals

AVM (98.0% pure, containing 92% AVM_{1a}) was acquired from the New Technology Development Company (China Agricultural University, Beijing, China). Stock solutions of AVM were prepared in analytical grade acetone (purity 99%). The stock solutions were sprayed onto the standard pigeon diet at a proportion of 60 mg AVM:1 kg diet, and the pesticide diet was allowed to dry for approximately 12 h at room temperature in the dark before use. Cytochrome C-NADPH was obtained from Sigma–Aldrich Chemical Company (Saint Louis, USA); erythromycin was obtained from HuaShun Biological Engineering Company (Shanghai, China); CO gas was obtained from the Dawn gas company (Haerbin, China); coomassie brilliant blue kits were obtained from Nanjing Jiancheng Biotechnology Co., Ltd. (Nanjing, China). All other reagents were of analytically pure grade and were purchased from local reagent companies.

Table 1 – The data of the dietary LC_{50} for standard measure of the subacute toxicity test.

Group	Concentration of AVM (mg/kg diet)	Death rate (%)
Control	0	0
I	97.20	0
II	160.00	10
III	270.00	20
IV	450.00	30
V	750.00	60
VI	1250.00	90

2.3. Animal treatments

The animals were randomly divided into the following four groups (twenty pigeons/group): a control group, a low-dose group, a middle-dose group, and a high-dose group. These groups were fed diets spiked with 0, 20, 40 or 60 mg AVM/kg diet, respectively. Because this study focused on the toxicological effects of AVM rather than those of residual of AVM in the environment, the concentrations of AVM used in this study were determined according to the method of Helen and Ernest (2004) and not based on the actual residual concentrations in the environment, after a subacute toxicity test was performed to calculate the dietary LC_{50}. The dietary LC50 data from this standard subacute toxicity test are shown in Table 1. The pigeons were allowed free access to standard chow and water, and the actual ingested doses are provided in Table 2. The birds (six/group) were fasted for 12 h and then euthanized by cervical dislocation after they were exposed to AVM for 30, 60 or 90 days. The remaining two pigeons in each group were retains as standbys for any unexpected condition.

The livers and kidneys of the pigeons were promptly removed under ice-cold conditions and then thoroughly rinsing with an ice-cold physiological saline solution to wash off the blood stains. Next, the surface water was drained with filter paper, and the tissues were divided into two portions. One portion was weighed and then immediately frozen in liquid nitrogen and preserved at −80 °C for further microsomal CYP enzyme analyses, and the other portion was fixed in Bouin's solution for histological examination.

All experiments were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University under the approved protocol number SRM-06.

2.4. Preparations of liver and kidney microsomes

Liver and kidney microsomes were collected according to the method of Vrolijk (Vrolijk et al., 1994). The experimental operations were performed at 4 °C to prevent enzyme degradation. The livers and kidneys were homogenized in an