Toxicology in Vitro 26 (2012) 51–56

Contents lists available at SciVerse ScienceDirect

Toxicology in Vitro

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

Abamectin affects the bioenergetics of liver mitochondria: A potential mechanism of hepatotoxicity

Juliana C. Castanha Zanoli, Marcos A. Maioli, Hyllana C.D. Medeiros, Fábio E. Mingatto *

Laboratório de Bioquímica Metabólica e Toxicológica (LaBMeT), UNESP – Univ Estadual Paulista, Campus de Dracena, 17900-000 Dracena, SP, Brazil

ARTICLE INFO

Article history:
Received 28 August 2011
Accepted 8 October 2011
Available online 17 October 2011

Keywords:
Abamectin
Mitochondria
F_oF_1-ATPase
Oxidative phosphorylation
Adenine nucleotide translocator
ATP synthesis

ABSTRACT

Abamectin (ABA) is a macrocyclic lactone of the avermectin family used worldwide as an antiparasitic agent in farm animals and pets and as the active ingredient of insecticides and nematicides. In this study, the effects of abamectin on the bioenergetics of mitochondria isolated from rat liver were evaluated. Mitochondria are responsible for converting the energy released by electron transport and stored as the binding energy molecule ATP. Xenobiotics that interfere with its synthesis or utilization can be acutely or chronically toxic. Abamectin (5–25 μM) caused concentration-dependent inhibition of the respiratory chain without affecting the membrane potential or the activity of enzymes NADH dehydrogenase or succinate dehydrogenase. This behavior is similar to oligomycin and carboxyatractyloside and suggests direct action on F_oF_1-ATPase and/or the adenine nucleotide translocator (ANT). ABA more pronouncedly inhibited ATPase phosphohydrolase activity in intact, uncoupled mitochondria than in freeze–thawed disrupted mitochondria. ADP-stimulated depolarization of the mitochondrial membrane potential was also inhibited by ABA. Our results indicate that ABA interacts more specifically with the ANT, resulting in functional inhibition of the translocator with consequent impairment of mitochondrial bioenergetics. This effect could be involved in the ABA toxicity to hepatocytes.

1. Introduction

Abamectin (ABA) is obtained by natural fermentation of *Streptomyces avermitilis*, which provides a mixture of avermectins consisting of ⩾80% of avermectin B_{1a} and ⩽20% avermectin B_{1b} (Agarwal, 1998). B_{1a} and B_{1b} (Fig. 1) have similar biological and toxicological properties (Hayes and Laws, 1990). Abamectin is currently used in several countries as a pest control agent in livestock and as an active principle of nematicides and insecticides for agricultural use (Kolar et al., 2008). ABA is highly toxic to insects and may be highly toxic to mammals (Lankas and Gordon, 1989). Seixas et al. (2006) reported that ABA poisoning caused the death of 57 calves over 4 years. The authors noted that this number, caused by incorrect dosage to the animals, might be underestimated because signs of intoxication vary in intensity and many animals recover quickly. Despite its restricted use to animals and crops, several cases of accidental or intentional abamectin poisoning in human also have been described (Chung et al., 1999; Yang, 2008).

Due to its interposition between the digestive tract and the general circulation of the body, the liver has an important role in metabolism and biotransformation of exogenous substances. Therefore, it receives large amounts of nutrients and xenobiotics absorbed through the digestive tract and portal vein, becoming the target organ of several classes of toxicants and natural or synthetic toxins (Guillouzo, 1998). The most direct mechanism of liver toxicity, at the cellular and molecular level, is the specific interaction of the toxicant with a critical cellular component (mitochondria, for example) and subsequent modulation of its function (Meyer and Kulkarni, 2001).

ABA poisoning can impair the function of hepatocytes. Research conducted by Hsu et al. (2001) showed elevated levels of the enzyme aspartate aminotransferase (AST) in the blood serum of rats after exposure to ABA by gavage at doses between 1 and 20 mg/kg body weight. The maximum activity was obtained with a dose of 20 mg/kg of body weight 1 h after ingestion. Eissa and Zidan (2010), using a commercial product, also observed signs of abamectin liver toxicity, with increased activity of the enzyme AST in rats treated with doses equivalent to 1/10 or 1/100 of the LD_{50} (18 mg/kg) in the diet of animals over 30 consecutive days. In addition, El-Shenawy (2010) undertook a comparative study of the *in vitro* toxic action of some insecticides, including ABA at concentrations of 10 and 100 μM, on isolated rat hepatocytes. There was a significant increase in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity when hepatocytes were incubated for 30 min with either concentration of ABA. This activity persisted after 120 min, the longest time point for which data was collected.

* Corresponding author. Tel.: +55 18 3821 8158; fax: +55 18 3821 8208.
E-mail address: fmingatto@dracena.unesp.br (F.E. Mingatto).

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doi:10.1016/j.tiv.2011.10.007

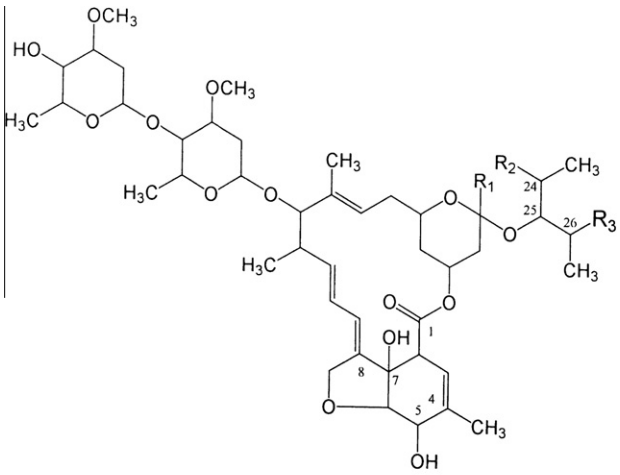

Fig. 1. Chemical structures of abamectin components avermectin B_{1a} and B_{1b}.

Mitochondria carry out a variety of biochemical processes, but their main function is to produce a majority (>90%) of cellular ATP. The proton motive force, whose major impetus is the membrane potential ($\Delta\psi$) generated by electron transport along the respiratory chain in the inner mitochondrial membrane, drives ATP synthesis via oxidative phosphorylation (Mitchell, 1961). Experimental evidence from our research group indicates that mitochondria represent a primary target critical for the action of drugs and toxins (Mingatto et al., 2000, 2007; Garcia et al., 2010). Here, we addressed the actions of ABA on mitochondrial bioenergetics by assessing its effect on respiration, membrane potential, ATP levels, activity of mitochondrial respiratory chain enzymes, ATPase and ANT in isolated rat liver mitochondria.

2. Materials and methods

2.1. Chemicals

Abamectin, containing 92% avermectin B_{1a} and 8% avermectin B_{1b}, was kindly supplied by the company Ourofino Agribusiness (Cravinhos, São Paulo, Brazil). All other reagents were of the highest commercially available grade. Dimethyl sulfoxide (DMSO) used to dissolve abamectin had no effect on the assays. The volume of DMSO added never exceeded 0.1% of the total volume of medium. All stock solutions were prepared using glass-distilled deionized water.

2.2. Animals

Male Wistar rats weighing approximately 200 g were used in this study. The animals, provenient from the Central Bioterium of the São Paulo State University, Botucatu, SP, Brazil, were maintained with a maximum of four rats per cage under standard laboratory conditions, while water and food were provided *ad libitum*. The experimental protocols were approved by the Ethical Committee for the Use of Laboratory Animals of the Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Dracena.

2.3. Isolation of intact and disrupted rat liver mitochondria

Mitochondria were isolated by standard differential centrifugation (Pedersen et al., 1978). Rats were sacrificed by decapitation, and the liver was immediately removed, sliced into 50 ml of medium containing 250 mM sucrose, 1 mM EGTA, and 10 mM HEPES-KOH, pH 7.2, and homogenized three times for 15 s at 1-min intervals with a Potter-Elvehjem homogenizer. Homogenate was centrifuged at 770*g* for 5 min, and the resulting supernatant further centrifuged at 9800*g* for 10 min. The pellet was suspended in 10 ml of medium containing 250 mM sucrose, 0.3 mM EGTA, and 10 mM HEPES-KOH, pH 7.2 and centrifuged at 4500*g* for 15 min. The final mitochondrial pellet was suspended in 1 ml of medium containing 250 mM sucrose and 10 mM HEPES-KOH, pH 7.2 and was used within 3 h. The mitochondrial protein concentration was determined by a biuret assay with BSA as the standard (Cain and Skilleter, 1987).

The disrupted mitochondria were obtained by heat shock treatment after three consecutive cycles of freezing in liquid nitrogen and thawing in a water bath heated to 37 °C. The membrane fragments were kept at 4 °C and were used in the assessment of mitochondrial enzymatic activity within 3 h.

2.4. Mitochondrial respiration assay

Mitochondrial respiration was monitored using a Clark-type oxygen electrode (Strathkelvin Instruments Limited, Glasgow, Scotland, UK), and respiratory parameters were determined according to Chance and Williams (1955). One milligram of mitochondrial protein was added to 1 ml of respiration buffer containing 125 mM sucrose, 65 mM KCl, and 10 mM HEPES-KOH, pH 7.4, plus 0.5 mM EGTA and 10 mM K_2HPO_4, at 30 °C. Oxygen consumption was measured using 5 mM glutamate + 5 mM malate, 5 mM succinate (+2.5 μM rotenone) or 200 μM N,N,N,N-tetramethyl-p-phenylene diamine (TMPD) + 3 mM ascorbate as respiratory substrates in the absence (state-4 respiration) or the presence of 400 nmol ADP (state-3 respiration).

2.5. Estimation of mitochondrial membrane potential ($\Delta\psi$)

The mitochondrial membrane potential ($\Delta\psi$) was estimated spectrofluorimetrically using model RF-5301 PC Shimadzu fluorescence spectrophotometer (Tokyo, Japan) at the 495/586 nm excitation/emission wavelength pair. Safranine O (10 μM) was used as a probe (Zanotti and Azzone, 1980). Mitochondria (2 mg protein) energized with 5 mM glutamate + 5 mM malate were incubated in a medium containing 125 mM sucrose, 65 mM KCl, 10 mM HEPES-KOH, pH 7.4, and 0.5 mM EGTA (2 ml final volume).

2.6. ATP quantification

ATP levels were determined using the firefly luciferin–luciferase assay system (Lemasters and Hackenbrock, 1976). After incubation in the presence of ABA, the mitochondrial suspension (1 mg protein/ml) was centrifuged at 9000*g* for 5 min at 4 °C, and the pellet was treated with 1 ml of ice-cold 1 M $HClO_4$. After centrifugation at 14000*g* for 5 min at 4 °C, 100 μl aliquots of the supernatants were neutralized with 5 M KOH, suspended in 100 mM TRIS–HCl, pH 7.8 (1 ml final volume), and centrifuged at 15000*g* for 15 min. The supernatant was worked up with a Sigma/Aldrich assay kit (Catalog Number FLAA) according to the manufacturer's instructions and measured using a SIRIUS Luminometer (Berthold, Pforzheim, Germany).

2.7. Mitochondrial ATPase activity

Mitochondrial ATPase activity was measured in intact-uncoupled and freeze–thawing-disrupted mitochondria according to the protocol of Bracht et al. (2003), with modifications. Intact mitochondria (1 mg protein/ml) were incubated in a medium contain-