Parasitology International 66 (2017) 584–587

Contents lists available at ScienceDirect

Parasitology International

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

Short Communication

Activity of carboxylesterases, glutathione-S-transferase and monooxygenase on *Rhipicephalus microplus* exposed to fluazuron

Fabrício Nascimento Gaudêncio[a,*], Guilherme Marcondes Klafke[b], Vinícius Menezes Tunholi-Alves[a], Thaís Paes Ferreira[c], Cristiane Nunes Coelho[c], Adivaldo Henrique da Fonseca[d], Isabele da Costa Angelo[e], Jairo Pinheiro[a]

[a] Laboratório de Biofísica, Departamento de Ciências Fisiológicas, Instituto de Ciências Biológicas e da Saúde, Curso de Pós-Graduação em Ciências Veterinárias, IV, UFRRJ, Seropédica, RJ, Brazil
[b] Laboratório de Parasitologia, Instituto de Pesquisas Veterinárias Desidério Finamor, Eldorado do Sul, RS, Brazil
[c] Laboratório de Quimioterapia Experimental em Parasitologia Veterinária, Departamento de Parasitologia Animal, Instituto de Veterinária, UFRRJ, Seropédica, RJ, Brazil
[d] Laboratório de Doenças Parasitárias, Departamento de Parasitologia Animal, Instituto de Veterinária, UFRRJ, Seropédica, RJ, Brazil
[e] Departamento de Epidemiologia e Saúde Pública, Instituto de Veterinária, UFRRJ, Seropédica, RJ, Brazil

ARTICLE INFO

Keywords:
Ticks
Biochemistry
Detoxification

ABSTRACT

The objective of this study was to assess the effect of the exposure to fluazuron on the activity of common pesticide detoxification enzyme groups in the cattle tick (*Rhipicephalus microplus*). Engorged females of a susceptible strain (POA) and a resistant strain (Jaguar) were exposed *in vitro* to fluazuron and their eggs and larvae were used to compare the activities of the general esterases, mixed-function oxidases (MFO) and glutathione-S-transferase (GST). The results showed significant elevation in MFO contents and esterases activity in the resistant strain when compared with the susceptible strain, in eggs and larvae respectively. In the POA strain, the MFO activity in eggs was down-regulated by fluazuron exposure. Based on these results, it can be concluded that different detoxification enzymes can act in distinct pathways depending on the tick's development stage, and may be related to fluazuron detoxification in resistant strains.

1. Introduction

The tick *Rhipicephalus microplus* is one of the parasites with the greatest economic impact on cattle breeding [1]. According to Grisi et al. [2], the losses caused by this tick amount to US$ 3.24 billions per year in Brazil.

The acaricides belonging to the growth regulator class do not necessarily kill the ticks directly. Instead, they interfere in the parasites' growth and development process, interfering in molting and reproduction [3]. The benzoylphenyl ureas group is composed of molecules that inhibit the synthesis of chitin in arthropods [4]. One of the molecules belonging to this group is fluazuron. It inhibits the growth and development of arthropods by blocking the formation and deposition of chitin [5].

Many researchers have demonstrated the efficacy of fluazuron on larvae and nymphs of ticks, such as *R. microplus* [6] and *R. sanguineus* [7], besides other ectoparasites, like the mite *Sarcoptes scabei* [8]. In *Rhipicephalus sanguineus* nymphs, it was demonstrated the effects of the arthropod growth regulator, fluazuron, in the formation of the integument and digestive processes [7]. However, the application of chemical products leads to the selection of resistance in these organisms, hampering their control. Resistance of cattle ticks to this substance was confirmed by Reck et al. [9] in southern Brazil, corroborating data presented in technical reports by the Food and Agriculture Organization [10], which indicate that drug resistance is the main obstacle to the efficient control of these arthropod populations.

In ticks, three enzyme systems are known that detoxify xenobiotic compounds: (i) esterases, (ii) mixed-function oxidases (MFO) and (iii) glutathione-S-transferase (GST) [11]. These enzyme complexes act making the drug more hydrophilic and thus less toxic to the organism. Biochemical assays with different populations of ticks and insects have demonstrated an increase in the activities of these detoxification enzymes in resistant strains in comparison with susceptible strains [12,13,14,15,16] within different active ingredients, but the exactly detoxification mechanisms of benzoylphenyureas is still unclear.

Recently, Gaudêncio et al. [17] documented a series of alterations in

* Corresponding author at: Curso de Pós-Graduação em Ciências Veterinárias, Departamento de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Km 7, BR 465, Antiga estrada Rio-São Paulo, 23890-000 Seropédica, RJ, Brazil.
E-mail address: fabriciogaudencio@ufrrj.br (F.N. Gaudêncio).

http://dx.doi.org/10.1016/j.parint.2017.04.006
Received 24 August 2016; Received in revised form 25 February 2017; Accepted 19 April 2017
Available online 20 April 2017
1383-5769/

the oxidative metabolism of *R. microplus* in response to exposure to fluazuron. The results indicate an inversion of the oxidative metabolisms in exposed ticks, characterized by an overlap of the enzyme related to fermentative pathways, besides variations of the hemolymph concentrations of some carboxylic acids, indicating that under these conditions, maintenance of the redox balance is carried out mainly by anaerobic pathways. Despite these results, information on detoxification enzymes in *R. microplus* after exposure to fluazuron is still scarce, limiting knowledge of the role of these enzymes in the detoxification step and their possible involvement in tick resistance. Therefore, to complement the data previously published, the aim of this study was to analyze the influence of exposure to fluazuron on the enzyme activity of complexes related to the detoxification of pesticides in *R. microplus* during its ontogenetic development (eggs and larvae), comparing the enzymes' activities in resistant and sensitive strains.

2. Materials and methods

2.1. Ethical aspects

The present study was approved by the Bioethics Committee for Animal Experimentation of Rio de Janeiro Federal Rural University (CEUA-UFRRJ), under protocol number 023/2015.

2.2. In vitro exposure

The Guidelines for Resistance Management and Integrated Parasite Control in Ruminants, published by the Food and Agriculture Organization [10], establish a method to test the resistance to acaricides, based on the female immersion test [9].

The choice of the resistant strain (Jaguar strain) was based on the results obtained by Reck et al. [9], in which they observed traits denoting resistance of the Jaguar strain.

The *in vitro* tests were conducted with engorged females from a susceptible strain to fluazuron (POA strain, from Porto Alegre, RS) and a resistant strain (Jaguar strain, from a colony maintained at Instituto de Pesquisas Veterinárias Desidério Finamor, located in Eldorado do Sul, RS, Brazil), to compare the activities of the detoxification enzymes in eggs and larvae after laying. All the engorged females collected weighed between 200 and 300 micrograms.

The engorged females were separated into different experimental groups (control group and exposed group for each strain), each containing 10 ticks. The entire experiment was run in triplicate.

For the immersion tests, the active principle fluazuron, in powder form (Sigma Chemical Co., St. Louis, MO, USA), was diluted in a solution containing Triton X-100 at 2% diluted in sterile Milli-Q water and acetone (Merck, Darmstadt, Germany) to produce a stock solution of fluazuron at 1000 ppm. From this stock solution, a new dilution was carried out with sterile Milli-Q water to obtain a final fluazuron concentration of 50 ppm (0.02% Triton X-100 and 1% acetone). This concentration was chosen based on the results of [9], in which the authors observed that at the concentration of 50 ppm it was possible to easily observe the influence of fluazuron on the biological parameters evaluated. The control group did not contain the active principle fluazuron in the solution for the immersion test.

After one minute of immersion, the ticks were dried and stuck in Petri dishes, ventral side up, with double-sided adhesive tape and incubated in a climate chamber at 27° ± 1 °C at 80% relative humidity to monitor egg laying.

Pools of eggs from each dish were collected on the fifth day of laying and frozen at − 80 °C for evaluation of enzyme activity. The egg masses from the first to the fourth day were separated in portions in small glass jars enclosed with cotton balls to monitor larval hatching. The larvae with age between 14 and 16 days were frozen at − 80 °C for subsequent biochemistry analyses.

2.3. Preparing homogenates of eggs and larvae

Homogenates containing 50 mg of eggs from the fifth laying day were prepared by maceration in a microtube containing 300 μL of distilled water. The homogenates of larvae containing 50 mg from the pools of larvae were prepared by the same way. Then, the aliquots were removed from each sample to quantify the activity of monooxygenase (MFO). Subsequently, the samples were centrifuged at 10,000*g* for 1 min to perform the other enzyme assessments.

2.4. Determination of enzyme activities

The protocol to determine the enzyme activities followed the technical document from the Brazilian Ministry of Health, formulated by Oswaldo Cruz Foundation (2006) [22], containing the Method for Quantifying Activity Related to Pesticide Resistance of *Aedes aegypti*. This protocol is based on the method described by [18], with modifications.

A Biochrom EZ Reader 4000 was used for readings in these tests. The entire experiment was run in triplicate.

2.5. Determination of the activity of MFO

For the enzyme assay with MFO, each sample, containing a mixture of 20 μL of homogenate (before centrifuging), 60 μL of potassium phosphate buffer (90 mM, pH 7.2), 200 μL of a solution of 3,3′,5,5′-tetramethylbenzidine (TMBZ) (0.01 g TMBZ dissolved in 5 mL methanol plus 15 mL of 0.25 M sodium acetate buffer pH 5.0) and 25 μL of 3% hydrogen peroxide was placed in a well of a microplate (96-wells microplate). Then, the microplate was incubated while protected from light at room temperature for 2 h and the absorbance was read at 450 nm. The values were compared against a standard curve of purified cytochrome C. The results were expressed in equivalent units of cytochrome C/mg of protein.

2.6. Determination of the activities of carboxylesterases

The enzyme activities of the esterases were determined by addition of the substrates α and β-naphthyl. A mixture of 20 μL of the homogenate supernatant (for both α- and β-esterase), in 250 μL of a solution of α/β-naphthyl acetate (120 μL of 30 mM α- or β-naphthyl acetate) dissolved in 24.75 mL of sodium phosphate buffer (20 mM, pH 7.2) was placed in each well of a 96-wells microplate. The reaction was incubated for 30 min at room temperature. Then 50 μL of a solution of Fast Blue B (0.045 g of Fast Blue B in 4.5 mL of distilled water added to a solution of 15 mL of 5% sodium dodecyl sulfate, SDS) was added. The reaction was incubated again at room temperature for 5 min, after which 200 μL of α/β-naphthyl acetate/sodium phosphate and 50 μL of Fast Blue were added. The absorbance readings were performed at 570 nm. The results were compared with a standard curve with known concentrations of α/β-naphthol and expressed in mmol of product formed/min/mg of protein.

2.7. Determination of the activity of glutathione-S-transferase

The enzyme activity of GST was measured using 10 μL of the homogenate supernatant added to 200 μL of glutathione and a solution of 1-chloro-2,4-dinitrobenzene (CDNB) (10 mM, pH 6.5, and 3 mM CDNB originally dissolved in methanol). The enzyme kinetics readings were performed at intervals of 5, 10, 15 and 20 min at 340 nm. The results were expressed in mM/min/mg of protein.

2.8. Protein assay

The protein contents of each well were measured by adding 300 μL of Bio-Rad reagent for protein quantification (1:4 diluted with dd H_2O