Experimental Parasitology 176 (2017) 16–20

Contents lists available at ScienceDirect

Experimental Parasitology

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

Full length article

Activities of ectonucleotidases and adenosine deaminase in platelets of cattle experimentally infected by *Fasciola hepatica*

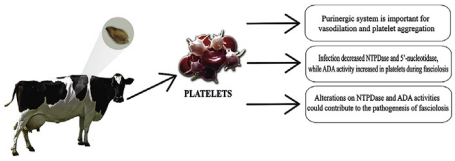
Mateus Fracasso [a, b], Aleksandro S. Da Silva [a, c, *], Matheus D. Baldissera [b], Nathieli B. Bottari [a], Mateus E. Gabriel [d], Manoela M. Piva [d], Fernanda A. Stedille [d], Ricardo Christ [d], Leandro A. Rhoden [d], Luan C. Henker [d], Vera M. Moresch [a], Maria Rosa C. Schetinger [a], Ricardo E. Mendes [*, d]

[a] Department of Biochemistry and Molecular Biology, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil
[b] Department of Microbiology and Parasitology, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil
[c] Department of Animal Science, Universidade do Estado de Santa Catarina, Chapecó, SC, Brazil
[d] Laboratory of Veterinary Pathology, Instituto Federal Catarinense (IFC), Concórdia, SC, Brazil

HIGHLIGHTS

- Fasciolosis is responsible for major economic losses in animal production, decreasing milk and meat production.
- *Fasciola hepatica* infection causes hepatic lesions.
- Liver plays a central role in the clotting process, since most coagulation factors are synthesized by liver cells.
- The study aimed to evaluated the activities of purinergic enzymes in platelets from cattle infected with *F. hepatica*.
- Altered NTPDase and ADA activities were found during *F. hepatica* infection in cattle.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 May 2016
Received in revised form
12 December 2016
Accepted 23 February 2017
Available online 24 February 2017

Keywords:
Fasciolosis
ATP
ADP
AMP

ABSTRACT

The enzymatic activities of NTPDase, 5′-nucleotidase and adenosine deaminase (ADA) are important in regulating the concentration of adenine nucleotides, molecules known to be involved on platelet aggregation. Fasciolosis causes coagulation disorders that have not been completely elucidated. Taking into consideration the association between the purinergic system and hemostasis, this study aimed to evaluate the enzymatic activities of NTPDase (hydrolyze ATP and ADP), 5′-nucleotidase (hydrolyze AMP) and ADA (deamination of adenosine) in platelets from cattle experimentally infected by *Fasciola hepatica* on days 20, 40, 60 and 80 post-infection (PI). For this study, 10 healthy Friesian steers were separated into two groups: the group A ($n = 5$) was used as uninfected control, and the group B was composed of steers experimentally infected by *F. hepatica* ($n = 5$). The number of platelets did not differ between groups in the periods evaluated. Reduction of NTPDase ($p < 0.05$) hydrolysing ATP (days 20, 40 and 60 PI), and ADP (days 40, 60 and 80 PI), and on 5′-nucleotidase hydrolyzing AMP (days 40 and 60 PI) was observed. A

* Corresponding authors. Department of Animal Science, Universidade do Estado de Santa Catarina, Chapecó, SC, Brazil; and Laboratory of Veterinary Pathology, Instituto Federal Catarinense (IFC), Concórdia, SC, Brazil.
E-mail addresses: aleksandro_ss@yahoo.com.br (A.S. Da Silva), ricardo.mendes@ifc-concordia.edu.br (R.E. Mendes).

http://dx.doi.org/10.1016/j.exppara.2017.02.014

Adenosine
Coagulation

reduction ($p < 0.05$) in ADA activity on day 20 PI, as well as an increase ($p < 0.05$) in ADA activity on days 40 and 60 PI was observed when compared to the control. Based on these results, we can conclude that ATP, ADP and AMP hydrolysis and adenosine deamination were altered in platelets of cattle infected by *F. hepatica*. Considering the importance of the purinergic system in hemostasis, it is believed that those changes may contribute to the coagulation impairment observed in acute fasciolosis described in the literature.

1. Introduction

Fasciolosis is a zoonotic disease caused by the trematode parasite known as *Fasciola hepatica,* an important parasitic disease affecting livestock and humans worldwide (Mas-Coma et al., 2005). *F. hepatica* is considered a cosmopolitan parasite, mainly found in Europe, the Americas, Australia and Africa (Mas-Coma et al., 2005) known as liver fluke. This disease is responsible for major economic losses in animal production, estimated at USD 2000–3000 billion per year due to decreased milk and meat production, and high costs to control parasitism (Mahami-Oskouei et al., 2012). Moreover, this disease represents a major public health concern with an increasing number of human cases (McCanus and Dalton, 2006). During the course of infection, alterations on coagulation factors were reported by Joachim et al. (2003), and according to this author, *F. hepatica* may cause coagulation impairment, confirmed by prolonged prothrombin time (PT), thrombin time (TT) and activated partial thromboplastin time (APTT).

The purinergic system has many important functions in the organism, such as vasodilation and platelet aggregation (Burnstock and Verkhratsky, 2010). The nucleotide enzymatic regulation initiates by NTPDase action, hydrolysing adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and adenosine monophosphate (AMP). Degradation continues with 5′nucleotidase activity that hydrolyses AMP into adenosine (Ado). According to Zimmermann (2010), ectonucleotidases and purine molecules have an important role in numerous physiological functions and diseases. Recently, alterations on NTPDase and 5′nucleotidase activities in serum and liver were observed in rats experimentally infected by *F. hepatica* (Doleski et al., 2016), however effect on platelets of infected animals was not studied.

The involvement of the purinergic system in coagulopathies is well documented, since ATP, ADP, AMP and adenosine are reported to regulate and modulate platelet aggregation (Godecke, 2008), due to its role on maintaining proper vascular hemostasis and thrombogenesis (Atikinson et al., 2006). ADP is primarily responsible for promoting this aggregation (Woulfe et al., 2001), while adenosine is a potent inhibitor (Anfossi et al., 2002). The role of ATP in coagulation impairment is unclear, but it is known that hydrolysis of ATP and ADP to AMP and/or adenosine inhibits platelets aggregation, leading to increased extracellular adenosine concentration (Birk et al., 2002). Therefore, considering the importance of adenine nucleotides hydrolysis in the coagulopathies, and the role of purinergic system in the pathophysiology of diseases, the aim of this study was to evaluate the activity of ectonucleotidases and adenosine deaminase in platelets of cattle experimentally infected by *F. hepatica.*

2. Materials and methods

2.1. Animals and experimental design

Ten Frisium steers (25–30 months old) were divided into two groups: group A consisted of five healthy animals (uninfected) used as a control group; the group B consisted of five steers that were experimentally infected by *F. hepatica*. Animals from group B were orally inoculated with a total of 200 metacercariae (Weybridge strain). The animals were kept in paddocks free of *F. hepatica* and its intermediate host, separated by group, and with free access to water. Animal diet was composed of pasture and concentrate feed (12% protein). The procedure was approved by the Animal Welfare Committee of the Instituto Federal Catarinense (IFC), under protocol number 001/2014.

2.2. Sampling

Animals were restrained in a squeeze chute, and blood samples were collected by jugular venipuncture on days 20, 40, 60, and 80 post-infection (PI) in order to evaluate the enzymatic activity in platelets. Fecal samples were collected directly from the rectum of all animals at the same time points, and analyzed by the sedimentation technique (Monteiro, 2010). Total blood (4 mL) was allocated in tubes containing citrate for platelets separation. On day 100 PI, animals were slaughtered in a commercial abattoir under state inspection, and liver fragments were collected for histopathology and to confirm fasciolosis.

2.3. Platelet count and preparation

Blood smears stained by the Romanowsky technique were used for platelet counts according to the methodology described by Stockham and Scott (2008). Platelets were prepared as previously described by Pilla et al. (1996) and modified by Lunkes et al. (2003). Briefly, blood was collected into 0.129 M citrate and centrifuged at 160×g for 15 min. The platelet-rich plasma was centrifuged at 1400×g for 30 min and washed twice with 3.5 mM HEPES buffer containing 142 mM NaCl, 2.5 mM KCl and 5.5 mM glucose. The washed platelets were resuspended in HEPES to determine enzymatic activities. Cell viability was estimated by lactate dehydrogenase activity in intact and disrupted platelets. Sample values were compared to the enzymatic activity obtained from cells lysed with 0.1% Triton X-100, as described by Bergmeyer (1983).

2.4. NTPDase and 5′-nucleotidase activities

The NTPDase enzymatic assay was carried out in a reaction medium containing 5 mM $CaCl_2$, 100 mM NaCl, 4 mM KCl, 50 mM glucose and 50 mM Tris-HCl buffer, pH 7.4, at a final volume of 200 μL as described by Lunkes et al. (2004). The protein content of platelets suspension was adjusted to 0.4–0.6 mg/mL (enzyme preparation). Twenty microliters of the enzyme preparation were added to the reaction mixture and pre-incubated for 10 min at 37 °C. The reaction was initiated by the addition of ATP or ADP at a final concentration of 1.0 mM, and incubated for 60 min.

Enzymatic (5′-Nucleotidase) activity was determined as described by Heymann et al. (1984) in a reaction medium