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Trypanosoma rangeli: Effects of physalin B on the immune reactions of the infected larvae of *Rhodnius prolixus*

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Abstract

Physalins are seco-steroids obtained from plants of the family Solanaceae. Herein, we tested *Physalis angulata* L purified physalin B as an immunomodulatory compound in 5th-instar larvae of *Rhodnius prolixus*, which were systemically infected with the H14 *Trypanosoma rangeli* strain protozoan. In uninfected insects, the effective concentration of physalin B, which inhibited 50% of the blood ingested (ED_{50}) volume, was 15.2 ± 1.6 μg/ml of the meal. Ecdysis processes and mortality in uninfected larvae, treated orally with physalin B in concentrations ranging from 1 to 10 μg/ml, was similar to that observed in insects not treated with physalin B. However, *R. prolixus* larvae previously fed on blood containing 1.0, 0.1, and 0.01 μg of physalin B/ml exhibited mortality rates of 78.1, 54.3, and 12.7%, respectively, 6 days after inoculation of *T. rangeli* (1×10^3 parasites/insect), whereas only 7.2% mortality was observed in the control group, injected with sterile culture medium. The insects treated with physalin B (0.1 μg/ml) and inoculated with *T. rangeli* did not modify the phenoloxidase (PO) activity and total hemocyte count in the hemolymph. However, physalin B treatment caused a reduction in hemocyte microaggregation and nitric oxide production and enhanced the parasitemia in the hemolymph. These results demonstrate that physalin B from *P. angulata* is a potent immunomodulatory substance for the bloodsucking insect, *R. prolixus*.

Index Descriptors and Abbreviations: Seco-steroids; *Rhodnius prolixus*; *Physalis angulata* L; Physalin; *Trypanosoma rangeli*; Hemocytes; Microaggregation; Nitric Oxide; Prophenoloxidase

1. Introduction

Trypanosoma rangeli is a hemoflagellate protozoan parasite of triatomines and mammals. Unlike *Trypanosoma cruzi* which develops only in the gut of *Rhodnius prolixus*, *T. rangeli* also develops in the gut, but commonly invades the hemolymph. Development is completed in the salivary glands, in a key step prior to transmission by direct inoculation feeding on vertebrate host (Garcia et al., 1994; Hecker et al., 1990; Hoare, 1972; Takle, 1988; Tobie, 1970). Apparently, the parasite is harmless for humans and a variety of wild and domestic animals, however it can be pathogenic to the insect vector (Watkins, 1971).

Once in the hemocoel, *T. rangeli* must overcome its insect vector's defense system which depends on many aspects of the humoral and cellular responses, including lysozyme and trypanolytic activities (Mello et al., 1995), prophenoloxidase (proPO) activation (Gomes et al., 2003), phagocytosis and hemocyte microaggregate formation (Garcia et al., 2004; Mello et al., 1995; Takle, 1988), agglutination (Mello et al., 1995; Ratcliffe et al., 1996), and superoxide and nitric oxide production (Whitten et al., 2001).

Physalis angulata belongs to the Solanaceae family and is widely distributed throughout tropical and subtropical

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regions of the world. Extracts from this plant have been used in different countries in traditional medicines against a variety of diseases such as malaria, asthma, hepatitis, dermatitis, and rheumatism (Chiang et al., 1992; Lin et al., 1992). Several compounds from *P. angulata* have been isolated and chemically characterized. A group of seco-steroids, known as physalins, are found in *P. angulata* stems and leaves (Lin et al., 1992). Recently, Soares et al. (2003) reported that physalins inhibited macrophage activation and nitric oxide production as well as causing lipopolysaccharide-induced death in a murine model of endotoxic shock.

Since many aspects of physiological processes in mammals are mediated by physalins, we hypothesized that physalin B can alter the normal immunological responses of *R. prolixus* infected with *T. rangeli*. This is a suitable and interesting biological model for investigation since the hemocoelic infection by *T. rangeli* is similar to that occurring in nature. Herein, we provide evidence that insects previously fed on blood containing physalin B become more susceptible to systemic *T. rangeli* infection and that physalin B is a potential immunomodulatory compound for the triatomine *R. prolixus*.

2. Materials and methods

2.1. Insects

Fifth-instar *R. prolixus* larvae were used throughout these studies. After molting, insects starved for 15–20 days were randomly chosen. All insects were raised and maintained as previously described (Garcia et al., 1984) feeding on citrated rabbit blood through a membrane feeding apparatus (Azambuja and Garcia, 1997).

2.2. Parasites

Trypanosoma rangeli strain H14 (CT-IOC 038, supplied by Dr. M.A. Sousa, Fiocruz, Brazil) was grown in a Liver Infusion Tryptose (LIT, Difco) culture medium supplemented with 20% heat-inactivated fetal calf serum at 28 °C according to Garcia and Azambuja (1997). The short epimastigotes (99% purity) were obtained from the log-growth phase of the parasites (until day 7 of cultivation), washed and resuspended in sterile culture medium (Garcia et al., 2004).

2.3. Physalin purification

Physalins were purified as described by Soares et al. (2003). Basically, *P. angulata* L stem ethanolic extracts, obtained from dried plant material collected in Belém do Pará (Brazil), were dissolved in methanol and mixed with a lead acetate solution. Activated charcoal (Merck Darmstadt, Germany) was added to the mixture and stirred up. The solution was filtered, poured into a separatory funnel and then extracted with chloroform. The chloroform layer was evaporated under reduced pressure, producing a pool of physalins. This pool was chromatographed using a liquid medium pressure chromatographic column (MPLC). The elution was carried out with a gradient solvent system (hexane–ethyl acetate 7:3; through 100% ethyl acetate). The collected fractions, after evaporation, were assayed on thin layer chromatography (TLC) plates, against standard physalins. After recrystallization in ethyl acetate, the procedure yielded physalin B of 96% purity and other types of physalins. The chemical structure of physalin B is represented in Fig. 1.

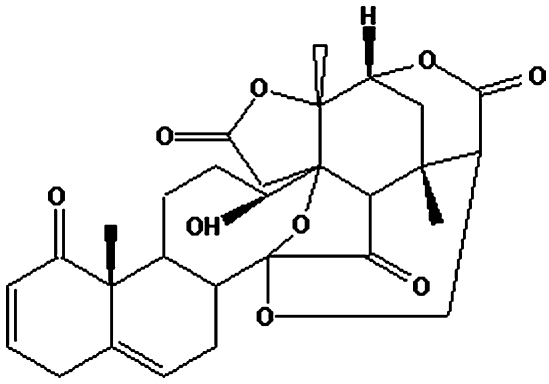

Fig. 1. Structure of the physalin B.

2.4. Effects of physalin B on blood ingestion rate and development

Before initiating the experiments of *T. rangeli* inoculation in *R. prolixus* (infected groups), we conducted a series of general control experiments and assessments to ensure that oral treatment with physalin B did not affect feeding, ecdysis processes, and death rate. First, to determine the antifeedant effect, physalin B was dissolved in ethanol–0.15 M NaCl (1:1) at a concentration of 4 mg/ml and added to the blood meal to achieve final concentrations varying from 1 to 30 μg/ml. Groups of 5th-instar larvae were allowed to feed through a membrane feeding apparatus (Azambuja and Garcia, 1997) for 30 min. Blood intake was determined by body weight difference just before (32.5 ± 3.4 mg) and after feeding. The effective antifeedant concentration (ED_{50}) was determined by using the linear regression, method of least squares (Snedecor, 1964), correlating the blood meal intake and the physalin B concentration. Secondly, to investigate possible physalin B toxicity and ecdysis affects we fed 5th-instar insect larvae on blood containing physalin B at concentrations varying from 1 to 10 μg/ml. A control group (not-treated with physalin) fed on blood with the solvent (ethanol/saline). Finally, we observed the mortality and molting during 30 days following feeding.

2.5. Insect infection with T. rangeli

Groups of 5th-instar *R. prolixus* larvae were allowed to feed for 30 min on a blood meal. For the treated groups the