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Revealing praziquantel molecular targets using mass spectrometry imaging: an expeditious approach applied to *Schistosoma mansoni*

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ABSTRACT

Finding specific molecular targets and the mechanism of action of praziquantel in the treatment of schistosomiasis remains a challenging task. Our efforts were focused on obtaining further information on worm composition before and after exposure to praziquantel in the treatment of schistosomiasis to elucidate the potential sites of action of this drug. Evidence indicates that the lipid bilayer is changed by treatment with praziquantel. Following this rationale, we employed a mass spectrometry imaging-based approach that helped to characterise lipids in specific locations, which are directly involved in the biochemical pathways of the BH strain of *Schistosoma mansoni*, as well as differentiating the molecular response that each worm sex presents in vivo. Our findings demonstrated significant differences between the chemical markers found in adult worms before and after praziquantel exposure, especially in phospholipids, which were predominantly identified as chemical markers in all samples. Results also indicate that distinct molecular pathways in both male and female worms could be differentially affected by praziquantel treatment. These data shine new light on the mechanism of action of praziquantel, taking a further step towards its full understanding.

1. Introduction

Praziquantel (PZQ) is the most commonly prescribed drug for all forms of schistosomiasis, since it is equally effective against *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma mekongi*, *Schistosoma intercalatum* and *Schistosoma haematobium* (Fenwick et al., 2003; Webbe and James, 1977). However, it does not show full efficacy against schistosomula and the juveniles stages of *S. mansoni,* resulting in lower cure rates in areas with high endemicity (Gönnert and Andrews, 1977; Pica-Mattoccia and Cioli, 2004; Sabah et al., 1986).

The PZQ mechanism of action is continually being studied (Andrews, 1985; Doenhoff et al., 2008; Hu et al., 2004; LoVerde et al., 2004); some physiological and morphological aspects have been understood for a relatively long time, such as rapid Ca^{2+} ion uptake (Pax et al., 1978) and vacuolation and blebbing near and on the surface of worms (Becker et al., 1980). In male worms, in addition to its effects on Ca^{2+} concentration, PZQ stimulates Na^{+} influx in a non-ionophore mechanism (Pax et al., 1978). Furthermore, PZQ induces modifications in membrane fluidity as well as in phospholipid (PL) composition, producing alterations in its permeability to ions or resulting in indirect effects on membrane receptors and channels (Harder et al., 1988; Lima et al., 1994). However, the full mechanism still remains unknown; for example, the pathway of Ca^{2+} homeostasis disruption by PZQ in adult schistosomes (Day et al., 1992; Redman et al., 1996) and the mechanism of PZQ binding to its molecular targets (Tallima and El Ridi, 2007; Troiani et al., 2007) are yet to be determined.

To better understand some of these mechanisms, modern analytical approaches, e.g. chromatographic techniques combined with MS, have been employed for chemical characterisation of adult schistosomes and PZQ metabolites in the host (Meier and Blaschke, 2000, 2001; van Balkom et al., 2005). More recently, MALDI-MS has been applied as the main analytical tool (Frank et al., 2012). Approaches using MS Imaging (MALDI-MSI) (Cornett et al., 2007) were developed to identify the spatial distribution of compounds in any physical sample such as tissue sections (Solon, 2007), single cell (Ferreira et al., 2014a), drug tablets (Rodrigues et al., 2014) and cosmetic products (de Oliveira et al., 2013).

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Moreover, MALDI-MSI was applied, for the first known time, in *S. mansoni* adult worms to demonstrate different chemical markers according to schistosome sex and strain (Ferreira et al., 2014b). In that study we localised each identified compound in the body of the worm. Since schistosomes show stage- and sex-dependent differences in susceptibility to PZQ (Pica-Mattoccia and Cioli, 2004), information on possible targets and pathways of the drug could be clarified by chemical marker localisation.

Based on this, the present work has employed the metabolomic platform to characterise both sexes of *S. mansoni* adult worms (BH strain) treated with PZQ. This report identifies the spatial distribution of chemical markers using MALDI-MSI technology, aiding the search for the understanding of possible targets and pathways of this anti-schistosomal drug.

2. Materials and methods

2.1. Mouse infection with S. mansoni

BALB/c albino female mice, 30 days-old, weighing 18–20 g, were individually infected with approximately 70 *S. mansoni* cercariae of the BH strain (from Belo Horizonte, MG, Brazil). At this stage, the utilised procedure was caudal immersion for 2 h, with light exposure and controlled temperature at 28 °C (Olivier and Stirewalt, 1952). After 45 days p.i., animals were divided into two groups ($n = 5$/group): (i) treated with PZQ (Merck, Darmstadt, Germany) and (ii) negative control. The first group received a single oral dose of 40 mg/kg of PZQ. The control group received 1% PBS solution. The oral dose of 40 mg/kg of PZQ used in the present study is considered curative in humans, as adopted by the schistosomiasis treatment and control programs in Brazil with a cure rate of 80–90%. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals. The protocol was approved by the International Ethics Commission for the Use of Animals (CEUA/ICCLAs, protocol n° 2170-1, University of Campinas, Brazil).

2.2. Recovery of S. mansoni worms

Two weeks after treatment, mice were subjected to cervical dislocation. *Schistosoma mansoni* adult worms ($n = 2$/mouse) were recovered by perfusion of the hepatic portal system and mesenteric veins (Pellegrino et al., 1962). All worms were recovered alive and washed carefully in a 0.9% saline solution at a temperature between 28 and 30 °C. Samples were sequentially transferred to an Eppendorf tube containing 1 mL of MilliQ H_2O at the same temperature. The maximum time of washing the worms was 30 min.

2.3. MALDI-MSI analysis

All adult *S. mansoni* worms were deposited on a TLC plate (Merck). Matrix coating was performed using a commercial airbrush, spraying α-cyano-4-hydroxycinnamic acid (Sigma–Aldrich, Pennsylvania, USA) (10 mg/mL in 1:1 Acetonitrile/Methanol solution). Images and MS were acquired in a MALDI-LTQ-XL instrument equipped with an imaging feature (Thermo Scientific, California, USA). The instrument uses a nitrogen laser as the ionisation source and a quadrupole-ion-trap analysing system. All data were acquired in the positive ion mode. For image acquisition, a 50 µm raster width was selected, with three shots taken per spectrum. Fragmentation data (MS/MS) were acquired by setting the collision-induced normalised energy to 40. Helium was used as the collision gas. Each ion was fragmented in triplicate. All imaging data were then processed using ImageQuest software v.1.0.1 (Thermo Scientific). Both spectral and imaging data were normalised according to a signal-to-noise ratio threshold of 3:1.

2.4. Statistical analysis and biomarker identification

Mass and intensity values for each spectrum were included in the Principal Component Analysis (PCA), which was performed using Unscrambler v.9.7 (CAMO Software, Trondheim, Norway). After discrimination by PCA, potential biomarkers were selected and, to identify the analytes, MS/MS reactions were performed to generate their fragmentation pattern. In addition to the spectrum, MALDI-MSI generated a chemical image allowing us to observe the spatial distribution of the analyte precursors, which were previously characterised as lipids. For lipid identification, MS/MS patterns and error values were considered, with the assistance of online databases such as Lipid MAPS (University of California, San Diego, CA, USA – www.lipidmaps.org) and METLIN (Scripps Center for Metabolomics, La Jolla, CA, USA), in order to guide the choice for potential lipid markers. Their structures were later proposed using Mass Frontier software v.6.0 (Thermo Scientific) (Urayama et al., 2010).

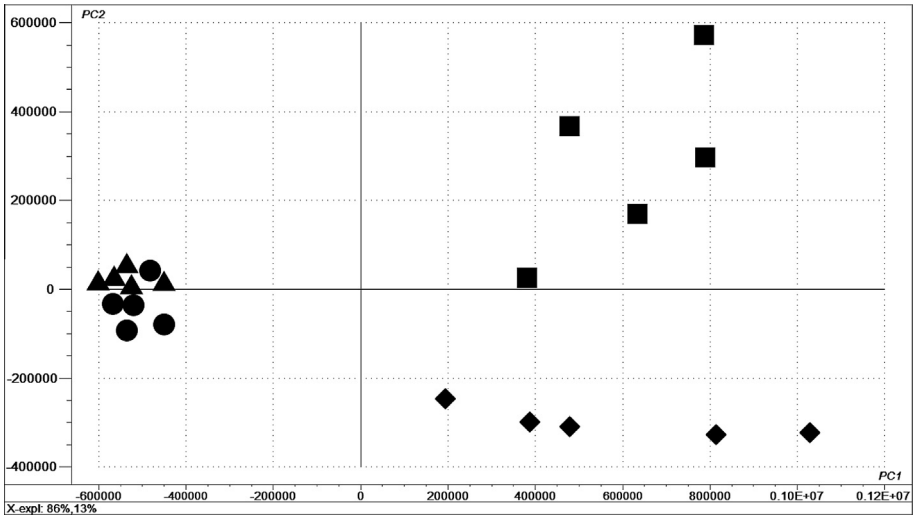

Fig. 1. Principal Component Analysis of all compounds of *Schistosoma mansoni* adult worms. Ion biomarkers for each group were separated by Principal Component Analysis ($n = 5$/group). The explained variances (X-expl) are shown below the figure. ▲, male negative control; ●, female negative control; ♦, male treated with praziquantel; ■, female treated with praziquantel.

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