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Original article

Synergistic effects of *in vitro* combinations of piplartine, epiisopiloturine and praziquantel against *Schistosoma mansoni*

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ABSTRACT

Schistosomiasis is a world health problem, and praziquantel is the only drug currently used for the treatment. There is some evidence that extensive monotherapy of praziquantel may be leading to drug resistance in the parasite. In order to find alternative treatments, the effects of the combination of epiisopiloturine (EPI), piplartine (PPT) and praziquantel (PZQ) were evaluated. Similarity analysis of these compounds was performed using optimized molecular structures to compare the shape and the charge modeling of combinations between PZQ and EPI or PPT. Supported by this data, *in vitro* association of PZQ-PPT, PZQ-EPI, and EPI-PPT was carried out, and the activity of these combinations against *Schistosoma mansoni* was assessed. The results showed synergistic activity with a combination index (CI) of 0.42 for the treatment with PZQ-PPT. Both PZQ-EPI and EPI-PPT combinations also showed synergistic effects, with CI values of 0.86 and 0.61, respectively. Surface alterations in the tegument of adult schistosomes after the treatments were observed using laser confocal microscopy and scanning electron microscopy. Additionally, the association of EPI-PPT decreased the cytotoxicity when compared with both isolated compounds in three different lines of mammalian cells. Thus, synergistic combinations of PZQ-PPT, PZQ-EPI, and EPI-PPT create the possibility of reduced doses to be used against *Schistosoma mansoni*.

1. Introduction

Schistosomiasis is a neglected disease that widely occurs in tropical and sub-tropical regions, and is caused by parasites from the *Schistosoma* genus, the specie *Schistosoma mansoni* being the main cause of this disease in humans [1]. The main symptoms of this disease are related to the production of eggs which can be found mainly in the liver and spleen. The inflammatory response mediated by the immune system against these eggs is the main

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factor which causes damage to the host, and it can lead to a serious health condition, and eventually to death [1]. This parasitosis is responsible for 280,000 to 500,000 annual deaths, ranking behind only malaria [2,3].

The only drug used in the treatment and control of schistosomiasis is praziquantel (PZQ). However, after decades of monotherapy, evidence of drug resistance in the parasite has been observed [4], it is an urgent necessity to discover new compounds or alternative therapies against schistosomiasis, such as drugs combinations [4,5].

Natural products have been considered as a source of alternative therapies against several diseases [6]. Compounds from plant extracts are promising candidates in the development of new drugs for treatment of microbial and parasitical diseases [7,8]. In this sense, the epiisopiloturine (EPI) (3-[hydroxy(phenyl)methyl]-4-[(3-methyl-1*H*-imidazol-5-yl)-methyl]-tetrahydrofuran-2-one), an alkaloidfound in the leaves from *Pilocarpus microphyllus* (Rutaceae) [9–11], promotes *in vitro* anti-helminthic activity *per se* or encapsulated in liposomes [12,13]. Likewise, piplartine (PPT) (5,6-dihydro-1[1-oxo-3-(3,4,5-trimethoxyphenyl)-*trans*-2-propenyl]-2(1*H*) pyridine), an amide found in several *Piper* species (Piperaceae) [14,15], induces *in vitro* activity against young and adult forms of *S. mansoni* [16,17].

Drug combinations as an alternative approach used in therapy of several diseases have demonstrated clinical importance due to increase in the efficacy of treatments, as well as decreases in eventual toxicity and drug resistance [18]. The interactions between drugs are commonly classified as synergistic, additive, or antagonist. Synergism can occur with drugs that have the same mechanism of action (an additive effect), which act by different modes (summation) or which act on different pharmacological receptors (potentiation). In order to define which type of these association occurs, interaction studies between drugs commonly has involved the generation of isobolograms, and determination of the combination index (CI) [6,19,20].

The aim of the present work is to assess the anthelmintic activity of pairwise combinations of PZQ, EPI and PPT against adult and young *Schistosoma mansoni* worms, based on the relationship between the drug lipophilicities, as well as to investigate morphological alterations in the parasite and cytotoxicity against host mammalian cells.

2. Material and methods

2.1. Active ligand-based similarity analysis

Similarity calculation is a computational method to identify compounds with similar properties to those of active compounds, based on a number of features including chemical fingerprints, physicochemical properties, and two and three dimensional structural features [21–23]. In order to identify similarity, MolShaCS [24], a C++ program developed to compare small molecules using shape and polarity attributes, was used. MolShaCS uses a Gaussian-based description of molecular shape and charge distribution to guide an overlay optimization and to compute similarity indexes, using the semi-empirical general AMBER force field (GAFF) [25]. Atomic charges were computed using the AM1 method, as implemented in the Antechamber [26] program, including desolvation. In order to evaluate the contact surface's similarity, calculations were performed by fixing the PZQ coordinates and optimizing the geometry of the other molecules in order to increase correlation between them. For this operation, the Van der Walls radii (VdW) radii, electrostatic potential (Q), and both simultaneously, were used as variables. The other structural parameters calculated were: van der Waals surface area; the molecular electrostatic potential maps (MEP), molecular lipophilicity potential (MLP) and the polar surface area (PSA), using the VEGA package [27,28].

2.2. Druglikeness evaluation

The *in silico* drug-like profiles of EPI and PPT were calculated using the program OSIRIS property explorer [29] to compare to the PZQ score. Data were generated on-line in the Osiris program, accessed by the link (http://www.organic-chemistry.org/prog/peo/accessed on October 1st 2014) and represented by druglikeness and drug-score. Druglikeness was calculated based on an equation summing up score values of the fragments present in the molecule under investigation. The fragments were identified from a list of 5300 distinct substructure fragments with associated druglikeness scores [30]. The results obtained at OSIRIS were compared with LogP data calculated by the ALOGPS [31] applet (VCCLAB, Virtual Computational Chemistry Laboratory, http://www.vcclab.org/accessed on October 01th 2014). Fig. 1 shows the molecular structures of EPI, PPT and racemate PZQ.

2.3. Drugs

The EPI was isolated from pilocarpine biomass production by jaborandi (*Pilocarpus microphyllus*) leaves, according to the previously described method [32]. The PPT was isolated from an extract of *Piper tuberculatum* (Piperaceae) roots, which was submitted to recrystallization with methanol in order to obtain the pure salt [33]. The PZQ was purchased from Merck (Rio de Janeiro, RJ, Brazil). A stock solution (2 mM) was prepared for all substances using dimethylsulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA). The plants were collected and transported for research purposes according to environmental license issued by IBAMA, number 5763956.

2.4. Animals and parasites

The BH strain of *Schistosoma mansoni* was maintained in *Biomphalaria glabrata* snails and *Mesocricetus auratus* hamsters at the Adolfo Lutz Institute (São Paulo, Brazil), according to standard procedures. Detailed methods for infection of mollusks and hamsters, as well as for the recovery of parasites, were previously described [34]. Briefly, the intermediate host snails were exposed

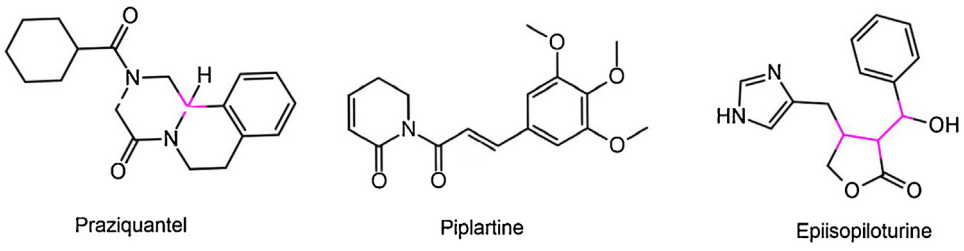

Fig. 1. Chemical structures of the compounds studied, with chiral centers in pink. A: Praziquantel, B: Piplartine and C: Epiisopiloturine.