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Estudo da via de heme farnesilado e dos
inibidores desta via em estágios
intraeritrocitários de *P. falciparum*.

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Resumo

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Atualmente, linhagens *Plasmodium falciparum* tem apresentado resistência contra todos os antimaláricos em uso, o que leva a necessidade do desenvolvimento de novas drogas, para isso o nosso grupo tem trabalhado com a caracterização de produtos da biossíntese de isoprenoides em *P. falciparum*, uma via diferente da encontrada no hospedeiro vertebrado. O farnesil-PP (FPP), um intermediário da via de isoprenoides, pode ser substrato para a síntese de diversos isoprenoides, ou pode se ligar a um grupo heme. A investigação da via de biossíntese de heme farnesilado em *P. falciparum* é interessante, já que, os principais antimaláricos têm seu mecanismo de ação relacionado ao heme. Outro importante alvo derivado da via de isoprenoides, é a via de biossíntese de giberilina porque: é estudada em plantas; e já são conhecidos inibidores dessa via que não prejudiciais ao homem. Inabenfide (INA) e uniconazol-P (UNP) inibem a biossíntese de giberilina em plantas, e o crescimento de *P. falciparum*, por isso decidimos estudar um possível alvo dessas drogas no parasita. Inicialmente, identificamos no genoma do *P. falciparum* genes homólogos, *cox10* e *15*, que codificam as enzimas (HOS e HAS) necessárias para a síntese de heme O e heme A. O alinhamento das sequências de *P. falciparum* com a de outras espécies bem como as árvores filogenéticas, permitiram predizer que as candidatas, *cox10* e *15*, codificam HOS e HAS. Além disso, parasitas transgênicos expressando versões fusionadas a GFP permitiram identificar como possível localização de *cox10* o núcleo e de *cox15* a mitocôndria. Para caracterização do heme O, realizamos: marcações metabólicas com [1-³H]FPP ou com [U-¹⁴C]glicina, extração com acetona ácida de heme B e heme O; separação por coluna de afinidade; e um estudo com diferentes técnicas de espectrometria de massas. Os resultados obtidos nos permitiram identificar uma via de biossíntese ativa para heme B e O. Apesar dos esforços não identificamos uma via para biossíntese de heme A, o que é compreensivo já que alguns organismos só biossintetizam heme O. Além disso, como não observamos uma possível via ativa para produção de giberilina, resolvemos verificar se INA e UNP (assim como outros antifúngicos) atuam na via de biossíntese de heme e seus derivados. Resultados obtidos por estudo de oxidação e redução indicaram que estas drogas interferem na via de biossíntese do heme O; e experimentos com marcador de potencial de mitocôndria indicam que na presença da droga diminuiu a capacidade funcional desta organela no parasita. Os resultados obtidos nos levaram a fazer um estudo de marcação metabólica, com precursores radioativos da formação de heme B e O em parasitas tratados com INA e UNP, o qual nos permitiu concluir que na presença das drogas há uma diminuição da produção de heme O. Sendo assim, podemos dizer que a via de biossíntese de heme O é ativa em *P. falciparum* e que inibidores dessa via podem estar relacionados com a diminuição da parasitemia. A via de biossíntese de heme farnesilado é um importante alvo para drogas antimaláricas.

Palavras-chaves : heme O. Farnesilação. *Plasmodium falciparum*. Isoprenoides.

Abstract

Simão-Gurge RM. Study of the farnesylated heme synthesis pathway in intra-erythrocyte stages of *P. falciparum* and inhibitors of this pathway. [Ph. D. thesis (Parasitology)]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo, 2017.

The development of antimalarials is necessary because there are *Plasmodium* strains resistant to the drugs in use and an important target is the isoprenoid pathway. Targets derived from the isoprenoid pathway are: heme O as there are antimalarial drugs related to heme; And gibberillin, because there are inhibitors which are not harmful to man. Inabenfide (INA) and uniconazole-P (UNP) inhibit biosynthesis of gibberillin in plants and of growth the *P. falciparum*. Initially, we identified in *P. falciparum* genes homologous to *cox10* and *cox15* encoding the enzymes (HOS and HAS) for synthesis of heme O and A. We created transgenic parasite lines which had HOS and HAS tagged to GFP. These revealed that the subcellular location of *cox10* is in the nucleus and of *cox15* in mitochondria. We identified heme O by metabolic labeling and mass spectrometry. However, no heme A or gibberellin was detected. INA and UNP decreased heme O biosynthesis and parasitemia as observed by oxido-reduction and metabolic labeling. Our data point to heme O as an important target for antimalarials.

Keywords: heme O. Farnesylation. *Plasmodium falciparum*. Isoprenoids.

Introdução

A malária, em termos de morbidade e mortalidade em seres humanos é um importante problema de saúde pública. Principalmente nas regiões tropicais da África subsaariana, Ásia, Oceania e América (1). A estimativa da Organização Mundial da Saúde (OMS) é de 219 milhões de novos casos e 660.000 mortes por ano, a maioria crianças menores de 5 anos e mulheres grávidas (2).

A malária está presente em 97 países, e 3,3 bilhões de pessoas estão em área de risco, das quais 1,2 bilhões estão em risco elevado de contraírem a doença (3). As comunidades mais pobres e marginalizadas estão sujeitas ao maior risco de contrair malária, devido à falta de serviços eficazes de prevenção, diagnóstico e tratamento (4).

No Brasil a maioria dos casos de malária se concentra na região Amazônica (Acre, Amapá, Amazonas, Maranhão, Mato Grosso, Pará, Rondônia, Roraima e Tocantins), área endêmica para a doença. Entretanto, nas demais regiões se observa uma maior letalidade quando comparada a região Amazônica, motivo pelo qual a doença não pode ser negligenciada mesmo nas áreas não endêmicas (5). Uma redução gradual do número total de casos tem sido observada nos últimos cinco anos, porém em 2012 houve um aumento significativo no número de casos na Amazônia brasileira. Em 2013, segundo dados da OMS, foram registrados 308 mil casos de malária no Brasil (99% destes na região Norte) com 79 mortes (6).

A malária é causada pelo protozoário do gênero *Plasmodium*, a transmissão natural se dá pela picada das fêmeas dos mosquitos do gênero *Anopheles*. Os mosquitos transmissores da malária pertencem à: ordem Díptera; infra ordem Culicomorpha; família Culicidae; gênero *Anopheles*.

Segundo o Portal da Saúde – Ministério da Saúde, 2014 (7), o gênero *Anopheles* compreende cerca de 400 espécies, mas apenas cerca de 60 são capazes de transmitir a doença em condições naturais. No Brasil 11 das espécies têm importância epidemiológica na transmissão da doença: *Anopheles (Nyssorhynchus) darlingi*; *Anopheles (Nys.) aquasalis*; espécies do complexo *Anopheles (Nys.) albitarsis s. l.*; *Anopheles (Nys.) marajoara*; *Anopheles (Nys.) janconnae*; *Anopheles (Nys.) albitarsis s. s.*; *Anopheles (Nys.) deaneorum*; espécies do complexo *Anopheles (Nys.) oswaldoi*; *Anopheles (Kerteszia) cruzii*; *Anopheles (K.) bellator* e *Anopheles (K.) homunculus*. Os vetores da malária são

popularmente conhecidos por “carapanã”, “muriçoca”, “sovela”, “mosquito-prego” e “bicuda” (7).

Os anofelinos se criam em águas de remansos de rios e córregos, lagoas, represas, açudes, valas e valetas de irrigações, alagados, pântanos e em águas coletadas em plantas bromeliáceas.

Com relação ao parasita, este é transmitido ao hospedeiro vertebrado durante a picada de uma fêmea do mosquito infectada. Atualmente são conhecidas aproximadamente 100 espécies que pertencem ao gênero *Plasmodium*, estes infectam vários vertebrados, como aves, répteis, peixes, e primatas superiores. Apenas cinco espécies podem infectar o homem e essa alta especificidade entre hospedeiro e parasito indica uma longa associação entre esses organismos e uma possível evolução conjunta. Nas infecções por *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* e *Plasmodium knowlesi*, predomina a forma da doença caracterizada, classicamente, por: febre intermitente (paroxismo febril), sudorese, mal-estar, vômitos e intensa debilidade física. Já a maioria dos casos de malária grave e de óbitos é causada pelo *P. falciparum*, espécie mais virulenta, entretanto, existem relatos cada vez mais frequentes de infecções graves pelo *Plasmodium vivax* (8).

O ciclo completo de desenvolvimento do parasita da malária no homem e na fêmea do mosquito *Anopheles* foi descrito entre 1898 e 1899 pelos pesquisadores Ronald Ross, Amico Bignani, Giuseppe Bastianelli e Batista Grassi (9). Vários experimentos foram realizados nos anos seguintes para confirmar a transmissão da malária pelo mosquito, e todas as formas de desenvolvimento do parasito no inseto e no homem foram identificadas. Em 1976, William Trager conseguiu cultivar *P. falciparum in vitro* (9).

Conclusão

Podemos concluir que o parasita biossintetiza heme O e que a inibição da formação de heme farnesilado, por INA e UNP, leva a uma diminuição da parasitemia. INA e UNP parecem afetar o potencial de mitocôndria, o que seria fácil de compreender já que, inibem a formação de um componente da cadeia mitocondrial. Conseguimos por estudo *in silico* verificar que *cox10* e *cox15* de *P. falciparum* tem função HOS e HAS e identificamos a localização das enzimas, HOS e HAS na mitocôndria. Assim, este caminho pode se tornar um importante alvo para novos medicamentos e deve ser investigado mais a fundo. Não identificamos uma via de biossíntese de G4 e G3 por *Plasmodium*.

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