A Comparative in vitro Study of the Effect of Eosin B on Asexual Blood Stages and Gametocyte of Plasmodium falciparum

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ABSTRACT

Background and Aim: Malaria is one of the most life-threatening infectious diseases worldwide. Transmission of the parasite from human to vector mosquitoes is carried out by the gametocyte of the Plasmodium parasite, while these cells are not involved in the symptoms of the disease. The control of the human to mosquito transmission stage of the parasite life cycle by antigametocyte drugs is currently under increasing scrutiny in an effort to eradicate the disease globally. In this study the gametocytocidal activity of eosin B on asexual blood stages and gametocytes of Plasmodium falciparum has been probed.

Materials and Methods: The parasite Plasmodium falciparum 3D7 was first cultured and then gametocytogenesis was induced to obtain gametocyte cells. After microscopically studying the developmental stages of the parasite during the culture of the parasite, eosin B was tested on blood and sexual parasites and the viability was assessed by lactate dehydrogenase assay and the results were compared for the two groups.

Results: Mature gametocytes were produced during 12 days. The blood parasites in culture were increased until day 4 and then gradually diminished. The results of lactate dehydrogenase assay showed a stronger effect of eosin B on gametocytes (IC50 = 23 nM) than asexual blood stages (IC50 = 133 nM).

Conclusion: Due to the rarity of anti-gametocyte drugs and importance of these intervention on malaria elimination, eosin B appears to be a suitable candidate for combination therapy against Plasmodium falciparum gametocytes.

Keywords: Malaria, Plasmodium falciparum, Gametocyte, Eosin B, Lactate dehydrogenase

Introduction

Malaria is a preventable, treatable, and life-threatening disease caused by disease-related parasites, which are transmitted to humans by mosquito bites. According to the World Health Organization (WHO) annual report on malaria, in 2018 there were about 228 million cases of malaria and 405,000 deaths due to this disease worldwide (1). According to the official report of the WHO in 2017, 57 cases of native malaria were registered in Iran, which shows a significant decrease compared to 1800 cases...
in 2010 and 12000 cases in 2000. In addition, no deaths from malaria were reported in Iran in 2017 (2).

In the complex life cycle of *Plasmodium* parasites, asexual blood stage parasites are responsible for the development of clinical signs and symptoms of the disease, and therefore major antimalarial drugs target this part of the parasite’s life cycle and are used to improve the symptoms of the disease. Gametocytes, which are involved in the sexual part of the parasite’s life cycle, play no role in causing the symptoms of the disease, but they play a role in transmission of the parasite from the human host to the Anopheles mosquito vector (3).

Drugs that can reduce gametocytogenesis, or can kill gametocytes, called gametocytocides, are very effective in counteracting the spread of malaria but due to lack of proper quantitative high throughput screening assays are still being studied. These transmission-blocking antimalarial drugs can work by targeting the following: 1. Effective and complete killing of adult gametocytes when they form in a mosquito vector targeting the following: 1. Effective and complete killing of adult gametocytes when they form in a human host. 2. Inhibition of gametocyte growth to oocytes and eventually sporozoites in mosquitoes. This requires enough medicine to reach the midgut of mosquito from the blood sample (4).

Gametocyte production occurs through five stages of maturation (I to V), and stage V is the only form that can infect mosquitoes. For *P. falciparum*, these mature gametocytes appear 12 days after symptoms and circulate for an average of 2.5 to 6.5 days, lasting up to 22 days. Thus, circulating gametocytes can maintain the process of malaria transmission from host to vector after drug treatment, which eliminates the symptoms of the disease (5).

Most currently approved antimalarial drugs, including artemisinin (ART)-based combination therapies (ACT), are effective only against blood stages and early-stage gametocytes up to stage III and possibly stage IV gametocyte maturation. In addition, some drug treatments such as chloroquine (CQ) and sulfadoxine-pyrimethamine induce gametocytogenesis and therefore effectively increase the number of cases of disease transmission and the rate of new infections (6).

Currently, the only antimalarial drug that has effective gametocytocidal activity is primaquine, which acts against gametocytes of all *Plasmodium* spp. and is the WHO recommended option against *P. falciparum* gametocytes. Unfortunately, the possibility of using this drug is also limited - due to the possibility of acute hemolytic anemia associated with primaquine treatment, resulting in much less false-positive diagnosis due to the continued presence of a biomarker after removal of the infection (12).

To achieve malaria elimination, antimalarial drugs or combination therapies must not only eliminate the asexual stages of the parasite that are responsible for the clinical symptoms of disease, but must also be able to clear the sexual stages of the parasite that maintain the host-to-vector transmission stage. Therefore, the results of such studies can be a way to prepare novel transmission-blocking antimalarial drugs.

Eosin B (EO) is a laboratory dye that has previously been proposed for its anti-parasitic ability through molecular docking methods and its antimalarial effect has been studied in *in vitro* and rodent malaria models (13-16). In the present study, after culturing *P. falciparum* gametocytes and carefully examining the blood and sex stages of the parasite during the culture period, the EO compound was investigated as a new anti-gametocyte agent and the results of treatment of asexual and sexual stages of the parasite by eosin B using LDH test eosin B has been compared.

**Materials and Methods**

*In vitro Culture of P. falciparum Asexual Stage Parasites*

Culture of *P. falciparum* strain 3D7 was performed at 37°C in human type O+ RBCs at 5% haematocrit. Culture medium contained complete medium including RPMI 1640 medium (Sigma-Aldrich), 25 mM HEPES (Sigma-Aldrich), 0.2% D-glucose (Sigma-Aldrich), hypoxanthine 200 μM (Sigma-Aldrich), 0.2% Sodium hydrogen carbonate (Sigma-Aldrich), gentamicin (Invitrogen) 40 mg. L⁻¹, 0.5% albumex...
The culture medium was daily gassed and replaced with a new medium (heated to 37°C). Parasite proliferation on each day of the culture period was microscopically examined by taking Giemsa-stained thin blood smear. To synchronize asexual culture, 5% D-sorbitol was used to prepare the parasite in the early trophozoite stage (ring) (19).

**Induction of Gametocytogenesis and Gametocyte Culture**

Asexual parasites were cultured to increase the parasitemia to 6-10%. Then the parasitemia was descended to 0.5% (in 6% hematocrit). Cultures were maintained in an atmosphere containing 90% N₂, 5% O₂ and 5% CO₂ without shaking. The cultures were also maintained at 37°C during daily media change. After 72 hours, the hematocrit was reduced to 3% (day 0). Gametocytogenesis was subsequently monitored by daily microscopic examination of the culture medium. On days 6 to 9, asexual forms were removed by treatment with 100 µg. mL⁻¹ heparin (Sigma-Aldrich). Gametocytes were monitored daily by examining Giemsa-stained thin blood smear until they reached stage V and were prepared for further testing (20).

**Quality Control of Functionally Viable Mature Stage V Gametocyte Production and Male Gamete Exflagellation**

After production of stage V gametocytes, during daily change of culture medium, the precipitated blood cells were resuspended. 200 µL was taken from the culture medium and quickly transferred to a 1.5 ml tube prewarmed to 37°C. After treating the sample with 50 µM xanthurenic acid in an exflagellation buffer (RPMI 1640 with 25 mM HEPES, 0.2% sodium bicarbonate, pH 8), the culture medium was placed in a microcentrifuge at 2000 g for 30 s at room temperature. Then a thin blood smear was prepared from it and stained with Giemsa and then a light microscope was used to control the quality of exflagellation (21).

**Eosin B Test on the P. falciparum Ring Stage**

In a 96-well plate, 20 µL of culture containing added asexual parasites was added to all rows except one row belonging to the control group. All samples were repeated in triplicate and placed in a CO₂ incubator for 48 hours.

**Eosin B Test on Plasmodium Falciparum Gametocyte**

In a 96-well plate, 20 µL of gametocyte-containing culture was added to all rows except one row belonging to the control group. All samples were repeated triplicate and placed in a CO₂ incubator for 42 hours.

**Parasitemia and Gametocytemia Evaluation by P. falciparum Lactate Dehydrogenase (PfLDH) Assay**

Parasitemia evaluation by lactate dehydrogenase assay is a high-throughput screening assay for antimalarial agents. For this purpose, 100 µL of Malstat reagent (1.57 g Tris HCl, 2 g L-lactic acid, 200 µL Triton X-100 in 85 ml double-distilled water, 66 mg 3-acetylpyridine adenine dinucleotide (APAD), pH 9.1) was added to the 96 –well microplate. Then 20 microliters of infected or uninfected red blood cells were added. The plate was incubated at room temperature and gently shaken for a few minutes to dissolve the red blood cells. During incubation, equal volumes of Nitro Blue Tetrazolium (NBT) and Phenazine Ethosulphate (PES) were mixed away from light and 20 µL of the mixture was added to the wells. The plate was placed away from light. After 30 to 60 minutes, the color change was controlled so that the color tended to dark purple. The plate was read using a BioTek PowerWave XS Microplate Reader at 650 nm. Uninfected red blood cells were used as a reference. Viability percentage was calculated from the following formula (9, 22, 23)

\[
\text{Viability\%} = 100 \times \frac{\text{OD_{treated\ sample}} - \mu_{c-}}{\mu_{c+} - \mu_{c-}}
\]

\[
\mu_{c+} = \text{means (µ) of OD control gametocytes (c+)}
\]

\[
\mu_{c-} = \text{means (µ) of OD blank uninfected RBCs (c-)}
\]

**Statistical analysis:** In this study, the tests were repeated 3 times and the results were analyzed by one-way ANOVA at a significance level of p-value <0.05 using GraphPad Prism Version 7.05 software.

**Results**

**Culture of Asexual Parasites**

After adjusting the culture conditions and using the appropriate protocol, asexual parasites were cultured. Figure 1 shows the number of parasites during the culture period.
As can be seen in the diagram, the number of parasites gradually increases from the beginning of the culture period and reaches its maximum on day 4. Then their number gradually decreased and the parasitemia of asexual sex blood parasites was diminished.

**Gametocyte Culture**

*Figure 2* shows which stages of the parasite are found in the culture medium on different days of the culture period.

The diagram shows the asexual stages around the first to fifth days and the early (I, II and III) and late stage (IV and V) gametocytes were observed in the culture on the second to eighth and seventh to twelfth days, respectively.

**Changes in the Number of Gametocytes During the Culture Period**

As can be seen in *Figure 3*, the number of gametocytes gradually increases during the culture period and reaches its maximum on day 12 at the same time as the gametocyte matures (stage V). In this condition, the gametocytes are capable of infecting the vector and are ready to perform an anti-gametocyte reagent test.

**The Effect of Eosin B on Asexual Stages of *P. falciparum***

*Figure 4* shows the effect of EO on the asexual stages of *P. falciparum*. Compared to the control group, the viability decreased for each test group by increasing the concentration of EO. The IC50 of EO for the asexual stages of the parasite is 133 nM.

*Figure 5* compares the effects of EO and the control drugs CQ and ART on the parasite ring of *P. falciparum* 3D7. The IC50 level of EO is higher than standard control drugs and is 133 nM. IC50 values for CQ and ART were 6.8 and 7.6 nM, respectively.
The Effect of Eosin B on *P. falciparum* Gametocytes

Figure 6 shows the effect of EO on *P. falciparum* parasite gametocytes. Compared to the control group, the viability decreased for each test group by increasing the concentration of EO. The IC50 of EO for the parasite gametocyte is 23 nM.

Figure 7 compares the effects of EO and the control drugs CQ and ART on *P. falciparum* 3D7 gametocytes. The IC50 value of EO is lower than 23 nM compared to standard control drugs. IC50 values for CQ and ART were 41 and 85 nM, respectively. Contrary to the results of the EO test on the blood stages of the parasite, it was observed that EO inhibited the gametocyte growth at a lower dose than standard drugs, indicating a potent inhibitory effect of EO on the *P. falciparum* 3D7 gametocyte.

**Discussion**

Sustained control of malaria is achieved if, in addition to using therapeutic strategies that target the asexual forms that cause the symptoms of malaria, the sexual forms of the parasite, which are the same as gametocytes, are also targeted by drugs that control the parasite transmission stage (24). In the present study, by an improved protocol of using heparin, induction of gametocytogenesis and production of parasite sex cells was performed to test a new compound in the treatment of *P. falciparum* strain 3D7. It should also be noted that the control of cultured gametocytes should be done carefully so that at least 90% of gametocytes have reached full IV-V growth stage in order to test the drug composition (25). Also, direct comparison of data from studies of drug discovery against gametocytes is difficult due to several factors, including the following: 1- Parasite strain used; 2- Protocol used to induce gametocytogenesis; 3- Combination of culture medium Used; 4- Gametocyte isolation protocols; 5- Developmental stage of gametocytes in the study; 6- Principles of assays used in the experiment; 7- Presence or absence of erythrocytes; 8- Number of gametocytes in each test well; 10- Concentration of tested compounds; 11- Duration of drug administration; 12- How to express the obtained data such as inhibition percentage in a certain concentration or IC50 alone, etc. (14).

EO has shown a significant inhibitory effect on *Toxoplasma gondii* and the blood stage of *P. falciparum*. IC50 values for EO in *T. gondii* and *P. falciparum* asexual blood stages were 180 μM and 124 nM, respectively (13, 14). Since the IC50 of EO for *P. falciparum* gametocytes is 23 nM, EO can inhibit gametocytes more severely than asexual blood parasites. Due to the fact that many antimalarial drugs do not have much ability to eliminate the sexual stage of the parasite and even some (such as CQ) induce...
gametocyte production and increase the number of gametocytes [26], the significant effect of EO on gametocytes and its anti-gammocyte effect in vitro can be considered for the preparation of drugs that block human-to-mosquito transmission.

Methylene blue, which is a dye used in the laboratory, has an inhibitory effect against all stages of *P. falciparum* [4] and has been proposed as a strong inhibitor of the transition from host to vector [27]. Also, its significant effect on gametocytes of this parasite through morphological deformation of gametocytes has been recently reported [28]. The IC<sub>50</sub> value of methylene blue for *P. falciparum* gametocyte in vitro is 12.49 nM [24]. The IC<sub>50</sub> value for EO is close to this combination, indicating the high efficacy of EO against the sexual stage of the parasite.

Compared to the main antimalarial drugs, it can be mentioned that ART, which is an important antimalarial drug, especially in combination therapies, as well as cases of severe and drug-resistant malaria, cannot directly inhibit gametocytes in the patient’s body, but it reduces the gametocytemia indirectly by eliminating the blood stages of the parasite [6]. The effect of Artesunate, a derivative of ART, on *P. falciparum* gametocytes has been previously reported in vitro with an IC<sub>50</sub> of 102.3 nM [24]. Also, primaquine, which is the only drug used clinically to remove gametocytes, has a much higher IC<sub>50</sub> in vitro than EO (IC<sub>50</sub> 15 μM). Unfortunately, the use of this drug is also limited due to the possibility of hemolytic anemia in people with deficiency in glucose-6-phosphate dehydrogenase [7]. Other drugs used clinically against malaria blood stages include quinine and mefloquine, each of which has a higher IC<sub>50</sub> than eosiin with an IC<sub>50</sub> of 50 nM [29]. Hydroxychloroquine is another derivative of the important antimalarial drug (CQ) in vitro with an IC<sub>50</sub> of 22.78 nM against gametocytes has shown its inhibitory effect [24], which is very close to IC<sub>50</sub> EO. Therefore, the efficacy of EO against the sexual stage of *P. falciparum* is close to or higher than some of the important known antimalarial drugs. Therefore, due to the possibility of using EO as an oral drug and its strong anti-gametocytic effect compared to some conventional drugs, this combination can be considered as a suitable candidate for use as a drug that blocks the transmission from host to vector.

**Conclusion**

Given the significant effect of EO on the number of gametocytes and also the comparison of its effect on the blood stage of *P. falciparum* in vitro, this combination will probably be able to effectively control the transmission from human host to mosquito vector. This effect can be tested by infecting Anopheles mosquitoes, which can be considered in future research using the standard Membrane-Feeding Assay test, which is the gold standard test for blocking transmission to be examined [30]. Given the lack of known pharmacological agents that block human-to-vector transmission, identifying the EO compound as an antigamocyte agent could be important for future research.

**Acknowledgment**

None.

**Conflict of Interest**

The authors declared no conflict of interest.
یک مطالعه مقایسه‌ای در مورد تاثیر انواع B بر مراحل خونی غیرجنسي و کامتوستی انگل پلاسمودیوم فالسپاروم در شرایط برون‌تنی

ظریه صادقی، زهرا زمانی، مرجان صباغیان، محمدرضا نژاد، علیرضا شادی و محمد ارجمند

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مقدمه

مادی‌های ملایاری که بیماری قابل پیشگیری و قابل درمان و در عین حال تهدید کننده حیات انسان است که از طریق انگل‌های مربوط به این بیماری که توسط میش به انسان منتقل می‌گردد، ابتدا می‌شود. مطلوب گزارش سالانه سازمان بهداشت جهانی (WHO) در مورد ملایاری در سال ۲۰۱۸ حدود ۴۲۸ میلیون بیمارا و ۴۵ هزار مورد مرگ با دلیل این بیماری در سطح جهان رخ داده است (۱). طبق گزارش رسیم سازمان بهداشت جهانی در سال ۲۰۱۷ الی ۲۰۱۲ مورد بیماری ویریوسید به ایران بین دو سال ۲۰۱۱ و ۲۰۰۹ و ۲۰۰۹ مورد شده است که نسبت به ۱۷۰۰ مورد در سال ۲۰۱۳ و ۲۰۰۹ کاهش محسوسی را نشان می‌دهد. علی‌رغم این هیچ

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روش پژوهش
کشت بروینتی اینگل‌های مرحله غیرجنسی پلاسمودیوم فالسیپاروم
کشت پلاسمودیوم فالسیپاروم در محیط 3D7 در دمای 37 درجه سلسیوس با استفاده از نرم‌افزار میکروآت و کاتالیزور سونیک 5 با هم‌اکنونت‌گری

دوره‌هایی که معادل گونمودگی‌زونر را کاهش دهنده می‌باشند، آن‌ها باعث کشته شدن گونمودگی‌زونر و گونمودگی‌زونر نامی‌شده می‌شوند. از نظر زیستی، که گونمودگی‌زونر باعث افزایش سطوح تئزر، تئزر قشری درمان انسانی و انسانی انگل‌های فعال می‌شود. لکه‌های روده (LDH) از میانه‌های مرحله‌های اول و دوم می‌شود و میزان روده‌های اینگل انسانی را در آن‌گونه روده‌ها، با کاهش و گسترش بیماری، توده روده‌های اینگل در مرحله‌های اول و دوم روده‌های اینگل بازگردن، و در این‌جوار میزان روده‌های اینگل در مرحله‌های اول و دوم روده‌های اینگل بازگردند. این‌گونه روده‌ها در مرحله‌های اول و دوم روده‌های اینگل بازگردند.

تلودی گونمودگی‌زونر از طریق الت که در مرحله یک زمان کمتر از 15-20 دقیقه می‌باشد. این‌گونه روده‌ها در مرحله اول و دوم روده‌های اینگل بازگردند.

در حالت حاضر، نه تنها در اینگل‌های دارویی مد از مرحله‌های بالاتر، در بیماری‌های درون‌بدنی وجود دارد. در این‌جوار، میزان روده‌های اینگل در مرحله‌های اول و دوم روده‌ها بازگردند.

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در پیام 94004048 و با توجه به حالت و شرایط نمونه، تغییرات در بسط رنگ و ضریب جلیقه نخواهد بود.

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با استفاده از نرم افزار GraphPad Prism Version 7.05 آنالیز گردید.

یافته‌ها

کشت انگل غیر جنسی

بعد از تنظیم شرایط کشت و استفاده از پروتکل مناسب انگل‌های غیر جنسی کشت داده شدند. شکل 1 تعداد انگل‌ها را در دوره کشت نشان می‌دهد.

تجزیه و تحلیل آماری

در این مطالعه آزمون‌ها 3 بار تکرار شدند و نتایج توسط آزمون آنالیز واریانس یک طرفه (One-way ANOVA) در سطح معنی‌داری P<0.05 کرد.

آنالیز واریانس یک طرفه (One-way ANOVA)

در زمان تکرار آزمون

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هم‌فاز

روز

Sheek 1. درصد پارازیتم در طول کشت دوره‌ای گامتوسیت.

کشت گامتوسیت

شکل 2 نشان می‌دهد که در روز‌های مختلف دوره کشت کدام مراحل انگل در محیط کشت پاکت شوند.

دان که در شکل مشاهده می شود تعداد انگل‌ها از آغاز دوره کشت به تدریج افزایش یافته و در روز 4 به حداقل مقدار خود می‌رسد. سپس به تدریج تعداد آن‌ها رو به کاهش می‌گردد و درصد پارازیتم انگل‌های خونی غیر جنسی کاهش می‌یابد.

Sheek 2. مراحل مختلف انگل پلاسمودیوم فلاسپاروم در طول دوره کشت گامتوسیت.
تشکل ۳. تغییرات تعداد گامتوسیت‌ها در طول دوره کشت با بلع گامتوسیت (مرحله V) به حداکثر مقدار خود می‌رسد. در این وضعیت گامتوسیت‌ها توان آلوده کردن ناقل را دارند و آماده انجام تست ترکیب ضد گامتوسیت هستند.

تشکل ۴. تاثیر اونژین B بر رنگ اکل پلاسمودیوم فلسپیازوم. درصد زندگی باین در مقایسه با گروه کنترل برای هر گروه تست نشان داده شده است.

(Mean ± SEM (n=5 & P<0.05), One Way-ANOVA test with Graph Pad prism version 7.05)
تأثیر انژه B بر مراحل غیرجنسي ا نگل پلاسمودیوم فالسپاروم

در شکل ۵ ناتوان انژه B و داروهای کنترل کلروکین و آرتمیزین بای انگل پلاسمودیوم فالسپاروم ۳D7 مقایسه سه مقدار IC50 انژه B نسبت به داروهای کنترل استاندارد بالاتر و بالای IC50 برای ۱۳۳ nM است. در شرایط آزمایش مقادیر B کلروکین (CQ) و آرتمیزین (ART) به ترتیب برای ۶.۸ و ۷.۶ به دست آمد.

شکل ۴ تأثیر انژه B بر مراحل غیرجنسي ا نگل پلاسمودیوم فالسپاروم را نشان می‌دهد. درصد گردش زندگی در مقایسه با کنترل، برای هر انژه تست با افزایش غلظت انژه B کاهش می‌یابد. مقدار IC50 انژه B برای مراحل غیرجنسي ا نگل پلاسمودیوم فالسپاروم ۱۳۳ nM است.

شکل ۵ مقایسه ناتوان انژه B و داروهای کنترل کلروکین و آرتمیزین بای انگل پلاسمودیوم فالسپاروم ۳D7. مقادیر IC50 برای انژه B کلروکین (CQ) و آرتمیزین (ART) در زیر هر انژه نشان داده شده است.

(Means ± SEM (n=5 & P< 0.05), One Way-ANOVA test with Graph Pad prism version 7.05)
ائری نژاتون B بر گامتوستین اکل پلاسمودیوم

فالسپاروم

شکل 6- تاثیر انوژین B بر گامتوستین اکل پلاسمودیوم

فالسپاروم را نشان می‌دهد. در مقایسه با گروه کنترل، برای هر گروه تست با افزایش غلظت انوژین کاهش می‌یابد. مقدار IC50 B در مورد انوژین اکل 23 nM بود. است. (Means ± SEM (n=5 & P<0.05), One Way-ANOVA test with Graph Pad prism version 7.05)

تأثیر انوژین B بر گامتوستین اکل پلاسمودیوم

در مرحله IV-V مقدار مقایسه مثبت مصرف اکل پلاسمودیوم و انوژین B در مورد درمانی دایره‌ای اشاره دارد. در شرایط آزمایش عملی در فاصله 23 nM مقدار میزان درصد مراحل در دستگاه اکل متفاوت بود. در مقایسه با گروه کنترل، برای هر گروه تست با افزایش غلظت انوژین کاهش می‌یابد. مقدار IC50 B در مورد انوژین اکل 23 nM بود. است. (Means ± SEM (n=5 & P<0.05), One Way-ANOVA test with Graph Pad prism version 7.05)

بت‌حد

کنترل بیماری‌های مالاریا در صورت محیط می‌گردد که علاوهبر استفاده از استرائژالهای درمانی هدف گیرند اشکال غیرچینی مولد علت بیماری مالاریا، اشکال جنسی اکل که همان

تأثیر انوژین B بر گامتوستین اکل پلاسمودیوم

فالسپاروم

شکل 6- تاثیر انوژین B بر گامتوستین اکل پلاسمودیوم

فالسپاروم را نشان می‌دهد. در مقایسه با گروه

کنترل، برای هر گروه تست با افزایش غلظت انوژین کاهش می‌یابد. مقدار IC50 B در مورد انوژین اکل 23 nM بود. است. (Means ± SEM (n=5 & P<0.05), One Way-ANOVA test with Graph Pad prism version 7.05)

تأثیر انوژین B بر گامتوستین اکل پلاسمودیوم

فالسپاروم

شکل 6- تاثیر انوژین B بر گامتوستین اکل پلاسمودیوم

فالسپاروم را نشان می‌دهد. در مقایسه با گروه کنترل، برای هر گروه تست با افزایش غلظت انوژین کاهش می‌یابد. مقدار IC50 B در مورد انوژین اکل 23 nM بود. است. (Means ± SEM (n=5 & P<0.05), One Way-ANOVA test with Graph Pad prism version 7.05)

تأثیر انوژین B بر گامتوستین اکل پلاسمودیوم

فالسپاروم

شکل 6- تاثیر انوژین B بر گامتوستین اکل پلاسمودیوم

فالسپاروم را نشان می‌دهد. در مقایسه با گروه کنترل، برای هر گروه تست با افزایش غلظت انوژین کاهش می‌یابد. مقدار IC50 B در مورد انوژین اکل 23 nM بود. است. (Means ± SEM (n=5 & P<0.05), One Way-ANOVA test with Graph Pad prism version 7.05)
جنسي انجکتل پلاسمودیوم فالسیپاروم نزدیک و یا بالاتر از بعضی داروهای مه شناخته شده ضدمالاریا است. لذا ب توجه به امکان استفاده از ب‌بصورت دراوی خوراکی و اثر قوی ضدگامتوستیش‌ها در مقایسه با بعضی داروهای مرسوم، این ترکیب می‌تواند به عنوان کاندیدای مناسبی برای استفاده ب‌بصورت دراوی مسدود کننده انتقال از میزان به ناقل مورد توجه قرار گیرد.

نتایج گیری

با توجه به تاثیر معنی‌دار انجکتل B بر تعداد کامورستی‌ها و اهمیت مقایسه تاثیر آن بر مرحله خونی پلاسمودیوم فالسیپاروم در شرایطی این ترکیب احتمال‌دار به کنترل موتور انتقال می‌باشد (که خواهد بود که لازم است این امر از طریق آلوهه کرد و این نتایج مورد بررسی گردد) با این توجه که مانند نتایج بعدی مورد توجه در انتقال مورد رفت و با که Membrane-Feeding Assay استفاده از آزمون استاندارد آزمون استاندارد طالبی برای بررسی مسدود کردن انتقال است (200 مورد بررسی قاره گیرد. با توجه به کمبود عامل دارویی شناخته شده مسدودکننده انتقال از انسان به ناقل، شناسایی ترکیب انجکتل B به عنوان عامل اتی کامورستی می‌تواند برای تحقیقات آتی حائز اهمیت باشد.

سیاستگزاری

نادر.

تعارض در مناطق

نویسنده‌گان هیچگونه تعارضی در مناطق گزارش نکرده‌اند.

مراجع مالی

نادر.

Referance

1. World Health Organization. "World Malaria Report 2019. 2019." Reference Source. https://www.who.int/malaria/publications/world-malaria-report-2019/en/
2. World Health Organization. Update on the E-2020 Initiative of 21 Malaria-Eliminating Countries, Report and Country Briefs. 2018. Reference Source.
3. https://www.who.int/malaria/publications/atoz/e-2020-progress-report/en/
4. Gaur D, Chitnis CE, Chauhan VS, editors. Advances in malaria research. John Wiley & Sons, Incorporated; 2017. [DOI:10.1002/9781118493816]
5. Biamonte MA, Wanner J, Le Roch KG. Recent advances in malaria drug discovery. Bioorg Med Chem Lett. 2013 May 15;23(10):2829-43. [DOI:10.1016/j.bmcl.2013.03.067] [PMID] [PMCID]
6. Abdul-Ghani R, Basco LK, Beier JC, Mahdy MA. Inclusion of gametocyte parameters in anti-malarial drug efficacy studies: filling a neglected gap needed for malaria elimination. Malar J. 2015 Dec;14(1):1-41. [DOI:10.1186/s12936-015-0936-4] [PMID: [PMCID]]

7. Pukrittayakamee S, Chotivanich K, Chandra A, Clemens R, Looaeresuwan S, White NJ. Activities of artesunate and primaquine against asexual- and sexual-stage parasites in falciparum malaria. Antimicrob Agents Chemother. 2004 Apr 1;48(4):1329-34. [DOI:10.1128/AAC.48.4.1329-1334.2004] [PMID: [PMCID]]

8. Staines HM, Krishna S, editors. Treatment and prevention of malaria: antimalarial drug chemistry, action and use. Springer Science & Business Media; 2012 Jan 5. [DOI:10.1007/978-3-0346-0480-2]

9. World Health Organization (2012) Management of severe malaria: a practical handbook, 3rd edn. WHO, Geneva, http://www.who.int/iris/bitstream/10665/79317/1/9789241548526_eng.pdf?ua=1

10. D’Alessandro S, Silvestrini F, Dechering K, Corbett Y, Parapini S, Timmerman M, Galastri L, Basilico N, Sauerwein R, Alano P, Taramelli D. A Plasmodium falciparum screening assay for anti-gametocyte drugs based on parasite lactate dehydrogenase activity. J Antimicrob Chemother. 2013 Sep 1;68(9):2048-58. [DOI:10.1093/jac/dkt165] [PMID]

11. Markwalter CF, Davis KM, Wright DW. Immunomagnetic capture and colorimetric detection of malarial biomarker Plasmodium falciparum lactate dehydrogenase. Anal Biochem. 2016 Jan 15;493:30-4. [DOI:10.1016/j.ab.2015.10.003] [PMID]

12. Brown WM, Yowell CA, Hoard A, Vander Jagt TA, Hunsaker LA, Deck LM, Royer RE, Piper RC, Dame JB, Makler MT, Vander Jagt DL. Comparative structural analysis and kinetic properties of lactate dehydrogenases from the four species of human malarial parasites. Biochemistry. 2004 May 25;43(20):6219-29. [DOI:10.1021/bi049892w] [PMID]

13. Iqbal J, Siddique A, Jameel M, Hira PR. Persistent histidine-rich protein 2, parasite lactate dehydrogenase, and panmalarial antigen reactivity after clearance of Plasmodium falciparum monoinfection. J Clin Microbiol. 2004 Sep 1;42(9):4237-41. [DOI:10.1128/JCM.42.9.4237-4241.2004] [PMID: [PMCID]]

14. Atreya CE, Johnson EF, Irwin JJ, Dow A, Massimine KM, Coppers I, Stempliuk V, Beverley S, Joiner KA, Shoichet BK, Anderson KS. A molecular docking strategy identifies Eosin B as a non-active site inhibitor of protozoal bifunctional thymidylate synthase-dihydrofolate reductase. J Biol Chem. 2003 Apr 15;278(16):14092-100. [DOI:10.1074/jbc.M212690200] [PMID]

15. Massimine KM, McIntosh MT, Doan LT, Atreya CE, Gromer S, Sarwaraporn W, Elliott DA, Joiner KA, Schirmer RH, Anderson KS. Eosin B as a novel antimalarial agent for drug-resistant Plasmodium falciparum. Antimicrob Agents Chemother. 2006 Sep 1;50(9):3132-41. [DOI:10.1128/AAC.00621-06] [PMID: [PMCID]]

16. Zamani Z, Tafreshi AS, Nahrevanian H, Lame-Rad B, Pourfallah F, Eslamifar H, Sadeghi S, Vahabi F, Iravani A, Arjmand M. Efficacy of eosin B as a new antimalarial drug in a murine model. Malar Res Treat. 2012;2012. [DOI:10.1155/2012/381724] [PMID] [PMCID]

17. Federal Register, D&C Red no. 21 and D&C Red no. 22. Federal Register vol. 47, pp. 53843-53846, 1982.

18. Trager W, Jensen JB. Human malaria parasites in continuous culture. J Parasitol. 2005 Jun;91(3):484-6. [DOI:10.1645/0022-3395(2005)091[484:HMPICC]2.0.CO;2]

19. Allen RJ, Kirk K. Plasmodium falciparum culture: the benefits of shaking. Mol Biochem Parasit. 2010 Jan 1;169(1):63-5. [DOI:10.1016/j.molbiopara.2009.09.005] [PMID]

20. Fivelman QL, McRobert L, Sharp S, Taylor CJ, Saeed M, Swales CA, Sutherland CJ, Baker DA. Improved synchronous production of Plasmodium falciparum gametocytes in vitro. Mol Biochem Parasit. 2007 Jul 1;154(1):119-23. [DOI:10.1016/j.molbiopara.2007.04.008] [PMID]

21. Carter R, Ranford-Cartwright L, Alano P. The culture and preparation of gametocytes of Plasmodium falciparum for immunochemo, molecular, and mosquito infectivity studies. Protocols in Molecular Parasitology. 1993 (pp. 67-88). Humana Press. [DOI:10.1385/0-89603-239-6:67] [PMID]

22. Reader J, Botha M, Theron A, Lauterbach SB, Rossouw C, Engelbrecht D, Wepener M, Smit A, Leroy D, Mancama D, Coetzer TL. Nowhere to hide: interrogating different metabolic parameters of Plasmodium falciparum gametocytes in a transmission blocking drug discovery pipeline towards malaria elimination. Malar J. 2015 Dec 1;14(1):213. [DOI:10.1186/s12936-015-0718-z] [PMID] [PMCID]

23. Makler MT, Hinrichs DJ. Measurement of the lactate dehydrogenase activity of Plasmodium falciparum as an assessment of parasitemia. Am J Trop Med Hyg. 1993 Feb 1;48(2):205-10. [DOI:10.4269/ajtmh.1993.48.205] [PMID]
24. Makler MT, Ries JM, Williams JA, Bancroft JE, Piper RC, Gibbins BL, Hinrichs DJ. Parasite lactate dehydrogenase as an assay for Plasmodium falciparum drug sensitivity. Am J Trop Med Hyg. 1993 Jun 1;48(6):739-41. [DOI:10.4269/ajtmh.1993.48.739] [PMID]

25. Peatey CL, Spicer TP, Hodder PS, Trenholme KR, Gardiner DL. A high-throughput assay for the identification of drugs against late-stage Plasmodium falciparum gametocytes. Mol Biochem Parasit. 2011 Dec 1;180(2):127-31. [DOI:10.1016/j.molbiopara.2011.09.002] [PMID]

26. Ngwa C, Rosa TF, Pradel G. The biology of malaria gametocytes. Current Topics in Malaria. 2016 Nov 30:117-44. [DOI:10.5772/65464]

27. Dechy-Cabaret O, Benoit-Vical F. Effects of antimalarial molecules on the gametocyte stage of Plasmodium falciparum: the debate. J Med Chem. 2012 Dec 13;55(23):10328-44. [DOI:10.1021/jm3005898] [PMID]

28. Adjalley SH, Johnston GL, Li T, Eastman RT, Ekland EH, Eappen AG, Richman A, Sim BK, Lee MC, Hoffman SL, Fidock DA. Quantitative assessment of Plasmodium falciparum sexual development reveals potent transmission-blocking activity by methylene blue. P Natl A Sci. 2011 Nov 22;108(47):E1214-23. [DOI:10.1073/pnas.1112037108] [PMID] [PMCID]

29. Wadi I, Pillai CR, Anvikar AR, Sinha A, Nath M, Valecha N. Methylene blue induced morphological deformations in Plasmodium falciparum gametocytes: implications for transmission-blocking. Malar J. 2018 Dec 1;17(1):11. [DOI:10.1186/s12936-017-2153-9] [PMID] [PMCID]

30. Peatey CL, Skinner-Adams TS, Dixon MW, McCarthy JS, Gardiner DL, Trenholme KR. Effect of antimalarial drugs on Plasmodium falciparum gametocytes. J Infect Dis. 2009 Nov 15;200(10):1518-21. [DOI:10.1086/644645] [PMID]

31. Delves MJ, Straschil U, Ruecker A, Miguel-Blanco C, Marques S, Dufour AC, Baum J, Sinden RE. Routine in vitro culture of P. falciparum gametocytes to evaluate novel transmission-blocking interventions. Nat Protoc. 2016 Sep;11(9):1668-80. [DOI:10.1038/nprot.2016.096] [PMID]