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Anti-plasmodial Activity of a Non-protein Amino Acid Taurine

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Abstract: Human malaria is caused by a few selected species of the genus Plasmodium. Among these, *Plasmodium falciparum* causes almost 90% of malaria-related mortality. Novel anti-malarial compounds are hence required to fight the anti-malarial drug-resistant *P. falciparum* parasites. The objective of this study is to analyze the effectiveness of Taurine (2-aminoethane sulfonic acid), a non-protein amino acid, in preventing the growth and development of both asexual and sexual stages of *in vitro* cultured *P. falciparum* parasites. We found that 200 mM concentration of Taurine almost completely (>80%) inhibited the propagation of asexual stages of *P. falciparum*. In contrast, it did not have any inhibitory activity against the maturation of sexual or gametocyte stages. However, the gametocytogenesis or the conversion of asexual to stage I gametocyte was blocked partially by this compound. The results suggest that derivatives of Taurine /2-aminoethane sulfonic acid could be considered to further improve the effectiveness of Taurine as an antimalarial compound against both the asexual and early sexual stages of *P. falciparum*.

Keywords: Malaria, *Plasmodium falciparum*, 2-amino Ethane Sulfonic Acid (Taurine), Drug Resistance and Gametocytes

1. Introduction

Protozoan parasites cause several pathological manifestations in humans, malaria being one of the most prevalent. Among the five species of Plasmodium parasites that cause malaria in humans, *Plasmodium falciparum* is the deadliest while P. *vivax* is the most widespread [1]. Numerous strategies have been developed and applied to control the spread of malaria parasites. Approaches such as spraying of insecticides both indoors and outdoors, use of insecticide treated bed nets (ITNs), and antimalarial drugs for prevention as well as treatment have long been used to control the spread of both Plasmodium parasites and the mosquito vectors which transmit malaria among humans. A greater success has been achieved by these approaches to control malaria but none of these approaches have effectively blocked the transmission of malaria parasites and prevented the spread of malaria. Also, *P. falciparum* parasites have acquired resistance to almost all anti-malarial drugs used so far [1-6]. Currently, the first line of treatment for most of the *P. falciparum* malaria is artemisinin-based combination therapy (ACT). But very recently, *P. falciparum* acquired resistance to artemisinin [1, 3, 7-10], and still the combination therapy is the only viable option available to treat malaria [1]. Development of anti-malarial vaccine against various targets and attenuated whole parasites is underway in several laboratories, but the preliminary clinical efficacy data are not very promising for an effective and complete control and elimination of malaria. Therefore, additional approaches and novel anti-malarial drugs are warranted to combat this deadly disease.

In this study, we analyzed whether certain nutraceuticals can be used to control the spread of *P. falciparum* malaria. For several decades, studies have been done on nutrition and nutritional supplements towards the control of several pathogens which inflict illness in human populations [11]. Several supplements have been found to effectively kill protozoan parasites which cause various diseases in humans, such as trypanosomiasis, which is caused by, *Trypanosoma brucei* Interestingly, Taurine, 2-amino ethane sulfonic acid, was tested against rodent malaria parasite *P. chabaudi.* It was found that high concentration of Taurine eliminates *P.*

chabaudi parasites in rodents [12]. Delic *et al.* [12] also found that mice defective in taurine transport (*taut* $^{-/-}$) have lost their ability to fight against blood-stage infections with *Plasmodium chabaudi* malaria, and 90% of the control *taut* $^{+/+}$ mice survive [12]. But the effect of taurine against *P. falciparum* growth was never tested previously. In this study, we tested the effectiveness of Taurine, a nutraceutical, against the asexual and sexual stages of growth and development of *P. falciparum* parasites. We found that Taurine blocked asexual growth and development of *P. falciparum* almost completely but did not affect sexual stages.

2. Materials and Methods

2.1. Materials

Taurine (Cat # T8691) was purchased from Sigma Aldrich (Thermo Fisher), RPMI medium supplemented with Hypoxanthine and glucose was gifted by K. D. Medicals (KD MEDICALS, Columbia MD, USA (Cat # CUS-0645)), Type A Human serum and type O^+ erythrocytes were obtained from Interstate Blood Bank (Interstate Blood Bank, Inc. Memphis, TN Center). Mixed gas containing 3% O_2, 5% CO_2 and 92% Nitrogen was purchased from Roberts Oxygen (Roberts Oxygen, Baltimore MD), Giemsa stain (cat # GS1L) was purchased from Sigma Aldrich. The concentrated Giemsa stain was diluted to 5% in H_2O at the time of use.

2.2. Methods

2.2.1. Preparation of Complete Culture Medium

Four hundred and fifty ml RPMI medium was mixed with 45 ml of serum, 16 ml of 7.5% sodium bicarbonate and 0.01% Gentamicin. The culture medium was filtered through 0.22 µM filter unit (Thermo Scientific, Cat # 5660020) and stored at 4°C for 2-3 weeks. Culture medium containing various concentrations of Taurine was prepared separately, by dissolving the required amount of Taurine in complete culture medium, filtered through 0.22 µM filter unit, when needed.

2.2.2. In Vitro Culture of P. falciparum

P. falciparum NF54 isolate was obtained from Johns Hopkins Malaria Research Institute. *P. falciparum* NF54 was cultured in RPMI medium supplemented with 8% O^+ human serum (heat inactivated) and Sodium bicarbonate. *P. falciparum* NF54 culture was maintained in RPMI by daily media change. The growth of NF54 was monitored by daily blood smearing, Giemsa staining of methanol fixed slides, and microscopy.

2.2.3. In Vitro Culture of P. falciparum Gametocytes

P. falciparum NF54 gametocytes were cultured as described previously [13]. The development and maturation of various stages of gametocytes were monitored by daily blood smearing, Giemsa staining of methanol fixed smears, and microscopy.

2.2.4. Analysis of the Maturation of Gametocytes

P. falciparum NF54 gametocyte culture was initiated in 6% hematocrit (HCT) and 0.2% of a sorbitol synchronized asexual ring stage parasite in complete culture medium. After 24 hours, the culture was diluted to 3% HCT and aliquoted into 6 flasks containing 20 ml of complete CM. Taurine treatment of these cultures were followed from Day 2 to 16 as described in Table 1.

3. Results

3.1. Effect of Taurine on P. falciparum Development

The growth of *P. falciparum* NF54 parasite was initiated from an established culture and tested the effect of Taurine on the growth and development of *P. falciparum* NF54. Actively growing *P. falciparum* NF54 culture was diluted to 0.2% parasitemia with 4% HCT in six 25 mm culture flasks containing 20 ml culture medium. Various concentrations, 1, 2.5, 5.0, 10 and 20 mM of Taurine (diluted from a stock of 500 mM Taurine in CM) were added and exchanged with 3% O_2 containing mixed gas. One flask containing 20 ml of CM with the same parasite and HCT without any added Taurine was set up as positive control. CM containing various concentrations of Taurine was exchanged every 24 hours for 4 days. The growth was monitored everyday by Giemsa-stained thin blood smears. Our results indicate that even with 20 mM concentrations of Taurine, there was no significant inhibition or acceleration of the growth of NF54 and the parasitemia was almost similar in all cultures (data not shown).

Since the first set of experiments, up to 20 mM concentration of Taurine, did not show any significant effect on the asexual growth of *P. falciparum*, we tested the effect of higher concentrations of Taurine on the growth and development of asexual stages. For this experiment, 25, 50, 100, 200- and 500-mM concentrations of Taurine were prepared in CM (Taurine was dissolved directly in culture medium, filtered through 0.22 µM filtration unit), and the cultures were initiated in 20 ml with 0.2% *P. falciparum* NF54. These cultures were maintained for 96 hours and the parasitemia was calculated from Giemsa-stained thin blood smears. Blood smears were blinded and the parasitemia was calculated by two independent investigators. As shown in figure 1, higher concentrations such as 100- and 200-mM taurine blocked more than 80% of the parasite growth in 96 hours. At the concentrations of 500-mM Taurine, the growth of NF54 was completely blocked. Additional experiments by using a few *P. falciparum* lines will be required to determine the IC50 of Taurine against the asexual growth and development of *P. falciparum*.

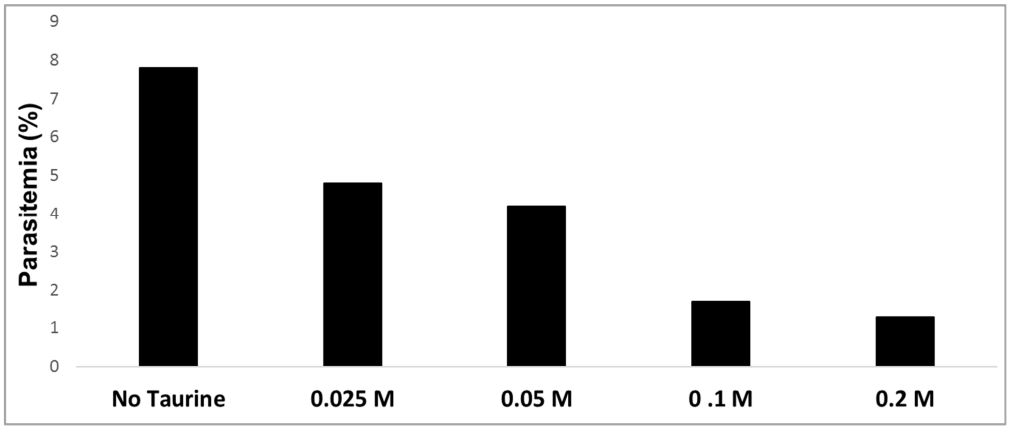

Figure 1. *Effect of Taurine on asexual stages of P. falciparum growth.*

P. falciparum parasite isolate NF54 was grown in complete culture medium containing 0.025, 0.05, 0.1 and 0.2 M concentrations of Taurine for 96 hours. Thin blood smears were prepared, fixed in methanol, stained with Giemsa and the parasitemia was calculated by counting *P. falciparum* infected red blood cells in 1000 red blood cells per experimental slide to determine the Parasitemia (number of parasite infected RBC in 100 RBCs.

3.2. Effect of Taurine on P. falciparum Sexual Stages

Gametocytes are the transmissible forms of malaria parasites to mosquito vectors from vertebrate hosts and are developed in red blood cells [14, 15]. All the available anti-malarial drugs, except primaquine, do not have an effect on gametocytes. To test the gametocidal activity of Taurine, gametocyte cultures were initiated as described previously in 6% HCT (stress induction by high HCT). After 24 hours, the gametocyte culture was diluted to 2.5% HCT and divided into 6 portions. We tested 200 mM concentrations of Taurine on various developmental stages of gametocyte as detailed in Table 1. The culture medium containing Taurine was exchanged every 24 hours for 15 days. The gametocyte development was monitored by blood smearing and microscopy. As shown in Table 1, 200 mM concentrations of taurine did not block the maturation of most stages of gametocytes and all Taurine treated cultures showed similar number of gametocytes except the one culture where asexual stages are treated with 200 mM concentrations of Taurine.

Table 1. *Effect of Taurine on the maturation of sexual stages of P. falciparum.*

Day-0	Day-2	Days-3&4	Days-5&6	Days-7&8	Days-9&10	Days-11-15	Gametocytemia (%) on Day 16
Fl-1	CM	CM	CM	CM	CM	CM	2
FL-2	CM	CM	CM	CM	CM-T	CM-T	2.1
FL-3	CM	CM	CM	CM-T	CM-T	CM-T	1.9
FL-4	CM	CM	CM-T	CM-T	CM-T	CM-T	2
FL-5	CM	CM-T	CM-T	CM-T	CM-T	CM-T	1.8
FL-6	CM-T	CM-T	CM-T	CM-T	CM-T	CM-T	1.0

P. falciparum NF54 was cultured to 6% of asexual ring stage and synchronized with D-sorbitol to lyse asexual tophozoites and schizonts. The synchronized ring-stage parasite culture was diluted to 3% and aliquoted into 6 flasks (FL) containing 20 ml of culture medium (CM), and marked as FL1, FL2, FL3, FL4, FL5 and FL6. Gametocyte maturation in these cultures were tested against 0.2 M Taurine as follows. FL-6 was set up to monitor the inhibitory effect on Stage-I, FL-5 was set up to monitor the inhibitory effect on Stage- I to II, FL-4 was set up to monitor the inhibitory effect on Stage –II to III, FL- 3 was set up to monitor the inhibitory effect on Stage III to IV, FL-2 was set up to monitor the inhibitory effect on Stage IV to V, FL-1 was set up to monitor the normal growth and development of PfNF54 without any added Taurine.

4. Discussion

P. falciparum developmental stage-specific growth inhibition by Taurine suggests Taurine interaction may be target specific on the asexual stage parasites. Importantly, Lutgen P [11] finds that, in mice, when the circulating Taurine concentration falls below the physiological level, Plasmodium chabaudi infected mice develop a 60% higher parasitemia [11]. Also, Taurine accumulation increased in Plasmodium infected RBCs compared with uninfected RBCs [11, 16], and this result suggests that Taurine accumulation in human RBCs may use nutrient permeability pathways (NPP) and EXP1 [16-18] for the transport of Taurine and this transport system may not support Taurine uptake in gametocytes.

5. Conclusion

The present study results demonstrate that there is a positive correlation between Taurine concentration and its inhibitory effect on the growth and development of asexual stages of *P. falciparum* parasite. Interestingly, Taurine did not show inhibitory activity on the maturation of *P. falciparum* sexual forms or gametocytes.

6. Recommendations

The findings of this study suggest that *P. falciparum* growth inhibition analysis by other compounds like Taurine

or modified derivatives of Taurine may provide valuable information on the mechanism of growth inhibition of *P. falciparum* parasites by Taurine and its derivatives. The evaluation of various other food supplements which contain Taurine on the growth inhibition of Plasmodium parasites may also be informative to design and develop novel class of anti-malarial compounds.

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Authors Contribution

TR and KH conceived and devised the study, designed experiments. AK and TR performed experiments, collected data and analyzed results. TR and KH wrote the manuscript.

Ethical Approval

The research study was conducted as per the National Institutes of Health (NIH) guidelines on using blood and blood borne pathogens. This study was conducted in a Bio Safety Level (BSL2) facility approved by the institution. Animals were not used in this study. Human subjects were not involved in this study.

Conflict of Interest

The author reports no conflict of interest.

Consent for Participation

Not applicable. Human subjects did not participate in nor were involved in this study. The blood and serum samples used in this study were obtained from Interstate Blood Bank (Interstate Blood Bank, Inc. Memphis, TN Center).

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