Actinomycin Used as A Potent Inhibitor for Malaria Parasite Plasmodium Falciparum

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ABSTRACT

Malarial disease mainly caused by the Plasmodium falciparum is creating a worsened situation for mankind, and there were around 214 million new cases of malaria in 2015 (range 149-303 million) all over the world. Now, it has become the challenging task to sort out the problem because malarial parasites are becoming increasingly resistant to several antimalarial drugs. In vitro intercalated compounds Actinomycin, Cyclophosphamide, Camptothecin, and Genistein were evaluated on Plasmodium falciparum 3D7 strain using parasite culture assay and SYBER Green fluorescence assay. The 3D7 strain was used since genes from the parasite genome have been extensively used in vaccine development and other studies. Based on fluorescence assay, SYBR green I (SG) dye was used for screening the antimalarial compounds against malaria parasite 3D7. In the present study, we have studied the parasite growth inhibition by Actinomycin, Cyclophosphamide, Camptothecin, and Genistein to find out which intercalating agent is the most effective against Plasmodium falciparum 3D7 strain. We found in our observation that Actinomycin showed maximum inhibition of the parasite Plasmodium falciparum 3D7 strain growth. The analysis that was done for the effective and alternative antimalarial drugs with minimal side effects, had stimulated the identification of a novel targeted drug. We also analyzed and reported that the antiplasmodial activity of Actinomycin is novel as compared to the previously described Intercalating Compounds.

Key Words: Malaria, Actinomycin, Plasmodium falciparum, Anti-malarial drugs, Dactinomycin

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INTRODUCTION

Malaria is the major parasitic infection in many tropical and subtropical areas. It is a disastrous disease and a torture to mankind since the ancient times, which afflicts 300-500 million people worldwide and results in an estimated 880,000 deaths yearly [1,2]. Recently, malarial parasites have become growingly resistant to several antimalarial drugs, and this issue has worsened the situation in many ways [3]. So, finding alternative ways to suppress the Plasmodium spp which is widely known to infect humans, and Plasmodium falciparum which is the most virulent and causes the fatal form of malarial infection resulting in 95% of all malaria-related deaths, is an urgent neeed of the day. Hence, it is necessary to identify new targets for antimalarial drugs. Various efforts to identify a suitable drug that targets against malaria parasite infections are a global concern [4, 5] as many previous attempts have been unsuccessful to develop a new class of antimalarial drugs. Resistance to artemisinin has emerged in Southeast Asia [6, 7], and the resistance has been seen to the most artemisinin-based combination therapy partner drugs [8]. The development of new antimalarial agents with novel modes of action is urgently needed. Certain commonly used antibiotics have been reported to have antimalarial effects in vivo, either alone or in combination with other drugs, such as tetracycline [9], rifampin [10], clindamycin [7, 11], erythromycin [12, 13] and chloramphenicol [14].

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Based on these results, a range of selected antibiotics was tested for the antimalarial activity; the examined drugs included Actinomycin, Cyclophosphamide, Camptothecin, and Genistein.

Actinomycin D, also known as Dactinomycin, is an antibiotic of the Actinomycin group that exhibits high antibacterial and antitumor activity. Actinomycin D has been broadly used in clinical practice since 1954 as an anticancer drug for the treatment of many tumors, and it is also a useful tool in molecular biology and biochemistry. Various anticancer drugs in clinical practice interact with DNA through intercalation. When an intercalating molecule is transferred from an aqueous environment to the hydrophobic space between two adjacent DNA base pairs, the intercalation process starts. The hydrophobic property of this drug lets it diffuse across the lipid bilayer and concentrate in the parasite. It contains phenoxazine chromospheres attached to two cyclic depsipeptides which contain five amino acid residues. It can be considered as a hybrid compound that behaves both as a minor groove binding agent and a DNA intercalate.

This study reports the antimalarial profile and mechanism of action of drugs on the morphology of P. falciparum asexual blood stage parasites. Actinomycin D demonstrated a potent antimalarial activity [15], and biochemical studies identified its target as an inhibitor of the parasite. The present study aimed to analyze the in vitro activity of the compound's inhibition of Actinomycin, Cyclophosphamide, Camptothecin, and Genistein under SYBER Green dye fluorescence screening method to find out which intercalating agent is more effective on Plasmodium falciparum 3D7 strain.

MATERIALS AND METHODS

Parasite

The P. falciparum strain 3D7 was purchased commercially.

Parasite Culture

The compounds' effect was examined by using cultures with 1% infected red blood cells (RBCs) and 4% hematocrit. Fifty micromoles of the culture were centrifuged, and to this, various concentrations (1.0 to 50 µM) of compounds were added. In 96-well plates, parasite culture mixture was taken and incubated at 37 °C for different time periods. The smears were prepared with erythrocytic stages of the parasite growth at different time intervals. The slides were fixed in methanol for 20 s. It was then stained by dipping in diluted (1:20) Giemsa stain obtained from Sigma for 30 min. The slides were observed in a microscope under oil immersion after washing and drying for morphological study of the cultures. Images were captured in a bright field microscope. In addition to microscopic examination, SYBR green-based assay was also performed to examine the effect.

Screening of compounds on parasite culture

In this method, the relationship between parasite and SYBER Green dye fluorescence was examined. Using standard method [16], P. falciparum from parasite culture was extracted. The concentration of the parasite was serially diluted with DNase-free water for ranges of dilutions. Plasmodium falciparum 3D7 cultures were treated with various concentrations (1.0 to 50 µM) of compounds (Actinomycin, Cyclophosphamide, Camptothecin, and Genistein) and control containing DNase free water, followed by incubation with SG dye. Plates were incubated at room temperature in the dark for 1 h and intensity was measured. Then fluorescence units were plotted against the parasite concentration. The SYBR green-based assay reading was taken on ELISA reader, and the parasite inhibition assay was done in duplicates. The bar graph shows the inhibition growth of culture treated with compounds (Actinomycin, Cyclophosphamide, Camptothecin, and Genistein).

RESULTS

The effect of DNA interacting compounds on parasite culture P. falciparum

The effects of various compounds (1.0 to 50 µM) on parasite growth Plasmodium falciparum were analyzed. Results showed that only Actinomycin inhibits the parasite significantly by using at 50 µM (Fig. 2A, 1). The other compounds which inhibit the parasitic activity at a moderate level under in vitro conditions are Cyclophosphamide and Genestein (Fig. 2A, lanes 3 and 4, respectively). Among these three identified compounds, which inhibit the parasitic activity; Actinomycin inhibited the parasite at a low concentration compared with Cyclophosphamide, Camptothecin and Genestein. We further performed the assay in the presence of increasing concentration of Actinomycin to obtain the kinetics of inhibition of parasitic activity. The concentration of Actinomycin used in the parasite inhibition ranged from 1.0 to 50 µM (Fig.2B, lanes 1–6). The results showed that the inhibition is maximum at 50 µM (Fig.2B). Other inhibitors like Cyclophosphamide, Genestein and Camptothecin did not show to be significant while they inhibited >50% activity at 60-80 µM (Fig.2A, lanes 2, 3 and 4). The other compounds like Cyclophosphamide, Genestein and Camptothecin (Fig. 2A, lanes 2, 3, and 4, respectively) were not able to inhibit the parasite significantly at 50 µM concentrations under in vitro condition. Thus, in the present study, we have identified that the compound Actinomycin showed potential to inhibit P. falciparum. Among these four compounds, Actinomycin was demonstrated as a potent inhibitor.

The effect of DNA interacting compounds on the morphology of parasite growth

P. falciparum laboratory strains 3D7 were used in this study to investigate the effects of Actinomycin on parasites growth. Parasites (5% parasitemia, 2% hematocrit) maturation was monitored every hour for an entire Intra erythrocytic Intraerythrocytic Developmental Cycle (IDC) by Giemsa staining [17]. The fixed smears were studied for

comparison of parasite morphology at each time point. The stage-specific effects were monitored by incubating separately highly synchronized rings, trophozoite or schizont stages of P. falciparum 3D7 parasites with Actinomycin or without inhibitor (control) for 24 h duration which were sufficient to allow transition to the next stage [18]. After incubation, the treated as well as control culture was monitored at different time intervals at all the developmental stages using Giemsa staining. The control experiment (Figure 3) shows normal morphology of the parasite developmental stages. After treatment with Actinomycin, the parasite's morphology was less distorted during ring and trophozoite stage (Figure 3). Considerable inhibition was noticed during the late trophozoite to schizont stages of asexual development (Figure 3). The results showed that Actinomycin inhibits the parasitic growth especially during early schizont stage noticed from morphological changes in the growth of the parasite. The nucleus, cytoplasm, and other organelles within parasites were not detectable in stained parasites in trophozoite and schizont stages. In early trophozoite, the food vacuole's pigments were noticeable. They appeared as fine grains with a black or dark brown color spread throughout the digestive vacuole of the trophozoite. Parasites began to mature from the early trophozoite stage to mid-trophozoite stage, and later into the late trophozoite stage. The size of the pigment granules gradually increased in each stage. They became organized and appeared as clumps or rods [19]. In the early schizont stage, pigments were grouped similarly as in the late trophozoite stage. The clusters' numbers decreased, but they became larger in size with further maturation. The size seized when a considerable single mass with a golden color appeared in the late schizonts stage. The parasite growth inhibition was more prominent at second cycle as compared to the 45-46 hour of parasite development (Figure 3). Red blood cells' (RBC) rupture could not be detected, and schizonts expired within a few hours. These results demonstrated that Actinomycin is an extremely efficient inhibitor during the erythrocytic stages of the malaria parasite.

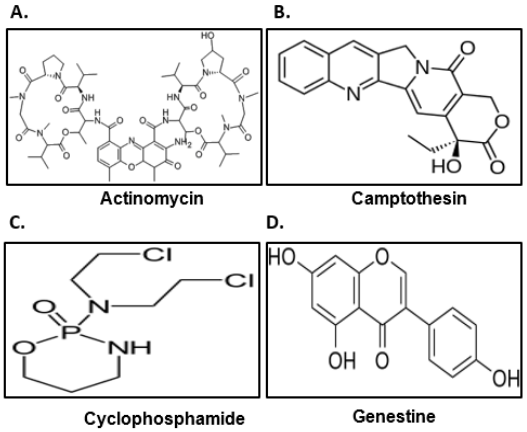

Fig.1. Chemical structures of DNA-intercalating compounds:
a) Camptothecin, b) Actinomycin, c)Cyclophosphamide, d) Genestein

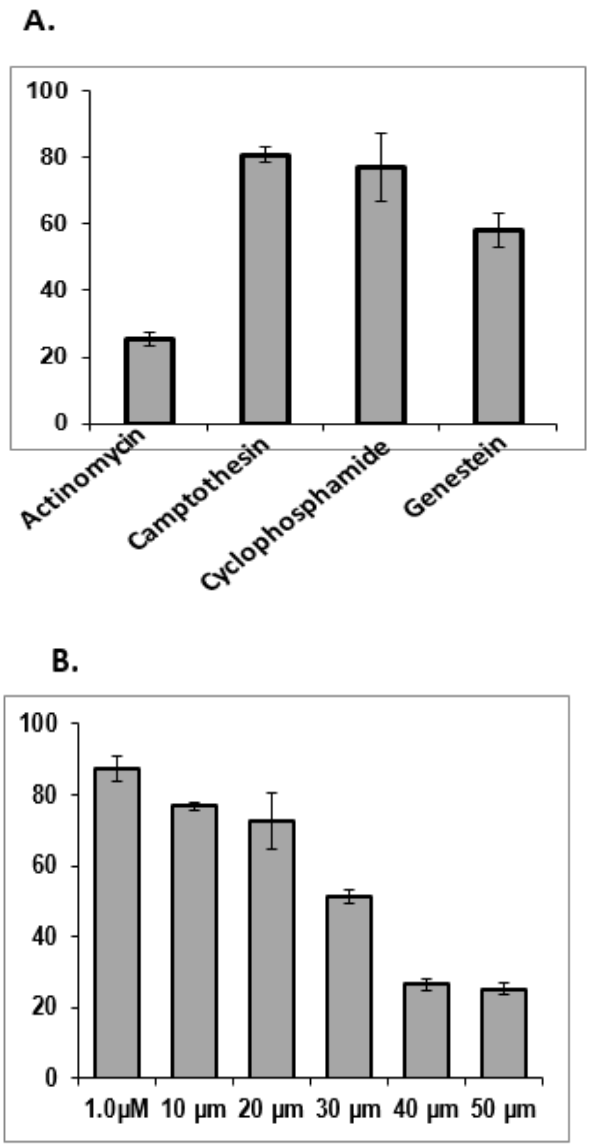

Fig.2.
A. Inhibition of P. falciparum growth by various drug inhibitors: Lane 1 actinomycin, Lane 2 camptothesin, Lane3 cyclophosphamide and Lane 4 genestein
B. Graph was plotted by using GraphPad Prism Software 5 (http://www.graphpad.com/scientific-software/prism/).
C. Increasing concentration of Actinomycin against parasite: Lanes 1–6 increasing conc. of compound actinomycin of 1, 10, 20, 30, 40, and 50uM
D. Graph was plotted by using GraphPad Prism Software 5 (http://www.graphpad.com/scientific-software/prism/)

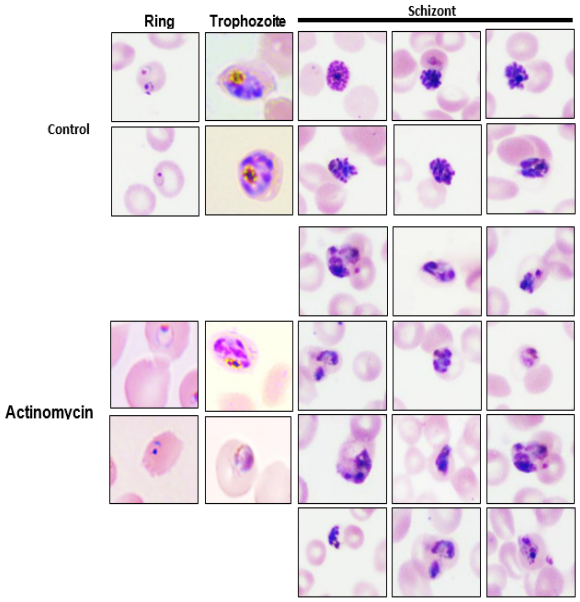

Figure-3
Giemsa-stained parasite infected RBC after Actinomycin treatment: Giemsa-stained parasite-infected RBC after compound treatment and Control lane culture without compound treatment.

Actinomycin treatment shows P. falciparum 3D7-infected RBCs at different developmental stages.

DISCUSSION

The four main drug classes presently used to treat malaria include, antimicrobials, antifolates artemisinin derivatives and quinoline-related compounds. However, no single drug that can eradicate all forms of the parasite's life cycle has been manufactured or discovered yet. Therefore, one or more classes of drugs often are given at the same time to combat malarial infection synergistically. Treatment regimens are dependent on the geographic location of the infection, the severity of disease presentation and Plasmodium species. It's a challenging task to identify and characterize a suitable chemotherapeutic target and develop of a newer class of antimalarial drug. Many drug targets that have been proposed in the past studies to screen the drug molecules are required to obtain an insight to design and develop novel inhibitor molecules with high specificity [20].

An effective drug treatment rapidly cleared asexual blood stage parasites. Different drugs differ markedly in their effects on gametocytes. Gametocytes become metabolically more inert as they develop, thus reducing the number of the drug targets available. Gametocytes mature over the course of approximately ten to fifteen days with each stage differing in the metabolic activity and drug susceptibility [21, 22]. In stages, I–IV, microvasculature, spleen or bone marrow are not present in the blood circulation. Stage V gametocytes have matured to the point of releasing from sequestered sites, becoming visible by blood smear. The parasite growth inhibition was more prominent at the second cycle as compared to the 45-46 hour of parasite development. Red blood cells' (RBC) rupture could not be detected, and schizonts expired within a few hours. Here, we simulated the effects of drugs against each developmental stage. In this study, we have assessed the antimalarial activity of different compounds, Actinomycin, Cyclophosphamide, Genistein and Camptothecin against the malaria parasite. Screening of Actinomycin against cultured P. falciparum identified as a potent antimalarial compound against multiple life-cycle stages of the parasite with excellent inhibition properties. It inhibited the parasitic activity during a screening at 1.0 μM concentration but did not appreciably inhibit at a lower concentration. It has potential to inhibit the growth of parasite with increasing concentration (1.0 to 40 μM) and maximum inhibition at 50 micromoles concentration. On the other hand, Cyclophosphamide, Genistein and Camptothecin inhibit the parasite with very less efficiency at the given concentrations. About 80% of the parasitic activity was inhibited with Actinomycin when assayed against parasitic growth, whereas 20-40% inhibition was seen with other compounds.

CONCLUSIONS

Actinomycin D is an antibiotic of the Actinomycin group that exhibits high antibacterial and antitumor activity and also known as Actinomycin. Actinomycin D has been broadly used in clinical practice since 1954 as an anticancer drug for the treatment of many tumors, and it is also a useful tool in molecular biology and biochemistry. This would reduce the initial level of infection, and with the prolonged activity eliminate the remaining parasites. Their activities on the sexual stages of the parasites show inhibition more prominently at the late trophozoite stage as compared to the initial hours of parasite development. Regarding treatment of the erythrocytic stage, Actinomycin fulfills the criteria as a long duration partner to complete the clearance of blood stage parasites.

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