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*In vitro* cultivation of *Plasmodium falciparum*: Studies with modified media supplemented with Albumax and hypoxanthine.

Shubhra Singh, Pratibha Singh, S K Puri and Kumkum Srivastava

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*In vitro* Cultivation of *Plasmodium falciparum*: Protein profile of parasites grown in RPMI and RPNI (modified) media with human and animal sera.

In vitro cultivation of *Plasmodium falciparum*: Studies with modified medium supplemented with ALBUMAX II and various animal sera

Kum Kum Srivastava *, Shubhra Singh, Pratibha Singh, S.K. Puri

Department of Parasitology, Central Drug Research Institute, Lucknow, India

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Abstract

RPNI, a combination of three commercially available growth media (RPNI-1640, NCTC-135 and IMDM) has been found to support long term continuous cultivation of 3D7 strain of *Plasmodium falciparum* in the presence of 10% bovine calf serum. During the present study, the suitability of this medium was evaluated for the development of *P. falciparum* in the presence of horse, goat and rabbit sera as well as various concentrations of ALBUMAX II. RPNI medium supplemented with 10% bovine calf serum (RPNI-BCS) was used as control. The cultures were maintained in candle jar protocol and parasitaemia was monitored daily up to day 7. Horse, goat and rabbit sera all supported the development of *P. falciparum*. Horse serum gave best results in RPNI medium and supported continuous culture up to day 100. The parasitaemia in the presence of ALBUMAX was significantly higher in RPNI than in RPNI-1640. Addition of hypoxanthine in RPNI-1640 caused an increase in parasitaemia whereas no obvious advantage could be observed in RPNI. The findings exhibited that medium RPNI has an edge over conventional RPNI-1640 medium for in vitro cultivation of *P. falciparum*.

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Index Terms and Abbreviations: Malaria; Protozoa; Horse serum; Rabbit serum; Goat serum; RPNI; RPNI-1640; Hypoxanthine

1. Introduction

*In vitro* cultivation of malaria parasites (Trager and Jensen, 1976) has become an easy method to screen compounds against the target parasite. Recent findings have shown that RPNI, a combination of three commercially available growth media (RPNI-1640, NCTC-135 and IMDM) supplemented with 10% bovine calf serum (BCS) supports long term continuous cultivation of *Plasmodium falciparum* (Srivastava and Puri, 2004) and overcomes the risks associated with use of human sera. As RPNI is a new combination of media, no information is available about its utility when used with ALBUMAX or other serum supplements. We have therefore investigated the potential of this media for cultivation of *P. falciparum* after supplementation with ALBUMAX and different animal sera.

2. Materials and methods

Studies were carried out mainly using laboratory maintained CHQ sensitive 3D7 strain of *P. falciparum*; however, on a few occasions, different parasite lines (NF-54, JDP-8 and RKL-9) were also used. JDP-8 and RKL-9, the Indian field isolates (Okrey et al., 1999) were obtained from International Centre for Genetic Engineering and Biotechnology, New Delhi, India and maintained at C. D. R. I. Lucknow. RPNI medium was prepared by combining RPNI-1640, NCTC-135 and IMDM media (Sigma) as described by Srivastava and Puri (2004). These three media were prepared separately. RPNI-1640 powdered medium was supplemented with Hepes buffer (25 mM), 0.2% NaHCO3, gentamycin at 40 μg/ml, (Sigma) and Fungizone

*Corresponding author. Fax: +91 522 223405.
E-mail address: kumkum1105@yahoo.com (K. Srivastava).

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Synthesis of 2-[3-(7-Chloro-quinolin-4-ylamino)-alkyl]-1-(substituted phenyl)-2,3,4,9-tetrahydro-1H-β-carbolines as a new class of antimalarial agents

Leena Gupta,a Kumkum Srivastava,b Shubhra Singh,b S. K. Puri and Prem M. S. Chauhanab*

aDivision of Medicinal and Process Chemistry, Central Drug Research Institute, Lucknow 226001, India
bDivision of Parasitology, Central Drug Research Institute, Lucknow 226001, India

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Abstract—a series of hybrid molecules 2-[3-(7-Chloro-quinolin-4-ylamino)-alkyl]-1-(substituted phenyl)-2,3,4,9-tetrahydro-1H-β-carbolines have been synthesized and screened for their in vitro antimalarial activity against chloroquine-sensitive strains of Plasmodium falciparum. Compounds 26, 32, and 34 have shown MIC in the range of 0.05–0.11 μM and are in vitro several folds more active than chloroquine.

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Despite over 100 years of drug development efforts, malaria remains one of the most devastating infectious diseases in the world.1,2 Furthermore, the worldwide burden of malaria is increasing in part due to the spread of resistance to the available anti-malarials especially chloroquine (CQ) and related quinoline-based agents.3,4 CQ had been the prime therapy for nearly half a century. It was safe, effective, widely available, and remarkably inexpensive and could also be administered to pregnant women and infants, but P. falciparum, the cause of the most deadly variety of malaria, is now CQ-resistant (CQR) in all epidemic regions of malaria. This rapid spread of resistance has highlighted the need to identify alternative anti-malarials.

Recent approaches are aimed to increase the potency of quinoline-based anti-malarials against the resistant parasite, have included the design and synthesis of quinoline-containing dual inhibitors that would potentially inhibit haemoglobin formation and also another target within P. falciparum.5 The 7-chloroquinoline moiety binds to haematin in parasite’s acidic food vacuole, thus inhibiting haematin formation, and also increases accumulation of the drug due to the protonatable quinoline nitrogen.6

Moreover, a number of alkaloids containing a β-carboline moiety, derived from marine sponges, represent important lead structures for the development of anti-infectives.7,11 Manzamine alkaloids, the most unique group of β-carboline, first isolated in 1986, was found to exhibit significant anticancer activity against P-388 mouse leukemia.12 More recently, manzamine A has been found to have potent antimalarial activity.13–15 These β-carbolines interact with DNA through GC-selective intercalation,14 therefore compounds containing β-carboline moiety can bind to the plasmidial DNA and thereby the inhibition of DNA synthesis is the malarial parasites.

On the basis of these above observations, we have designed and synthesized a class of hybrid molecules containing 7-chloroquinoline (haematin inhibitor) as a base moiety and tetrahydro-β-carboline moiety (DNA intercalator)-linked diamino alkyl chain at 4-position of 7-chloroquinoline. This paper describes the synthesis and in vitro antimalarial activity of these hybrid molecules.

Keywords: Malaria; Plasmodium falciparum; Chloroquine; Tetrahydro-β-carboline.

*Corresponding author. Tel.: +91 522 2262411; fax: +91 522 2262405; e-mail addresses: prem_chauhan_2000@yahoo.com
premchauhan@hotmail.com

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Treatment and control of mycoplasma contamination in Plasmodium falciparum culture

Shubhra Singh · S. K. Puri · Kunikum Srivastava

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Abstract A comparative efficacy of four antibiotics, plasmocin (macrolid), Biomyc-1, -2, (tetracycline), and Biomyc-3, and Mycoplasma Removing Agent (quinolone derivatives) was determined for elimination of mycoplasma from Plasmodium falciparum culture. Presence of mycoplasma was detected using enzyme-PCR-based mycoplasma detection kit and survival of malaria parasite was determined in Giemsa’s stained smear made from treated and untreated cultures. It was observed that a combination of Biomyc-1 and -2 killed malaria parasites within 24 h, whereas plasmocin and Biomyc-3 caused slow death of malaria parasite stretched over a period of 6 days. The only compound which did not kill malaria parasite and eradicated mycoplasma from P. falciparum culture was observed to be MRA.

Introduction

Mycoplasma are common contaminants of cell cultures (Razin and Barile 1985). The first report of mycoplasma contamination of Plasmodium culture was published in 1997 by Turrini et al. who had found mycoplasma contamination in several strains of Plasmodium falciparum directly originating from the Malaria Strain Bank in Edinburgh, UK (Turrini et al. 1997). Mycoplasma does not grow in mammalian erythrocytes, but they attach to human erythrocytes (Loomes et al. 1984) by adhering to steric acid-containing proteins (Roberts et al. 1989) and glycolipids (Loomes et al. 1985). Mycoplasma are very small, can pass through 0.1 μm pore-size membrane filters, have no cell wall, and are invisible in smears of malaria cultures stained by Giemsa or Acidine orange. The low mycoplasma contamination had an inhibitory effect on parasite invasion or maturation and for this reason their frequent presence in parasite cultures may easily escape notice. But in the presence of heavy contamination of mycoplasma, within 2–4 weeks, malaria parasites do not survive. Thus, mycoplasma contamination is a major hindrance for long-term in vitro cultivation of P. falciparum and may be a source of artifactual results in several ways. Metabolic pathways, specific enzymes, or products of mycoplasma origin may be falsely attributed to P. falciparum and the AT richness (61–76%) of the mycoplasma genome is similar to that of P. falciparum (Bove 1993), thus making this a potential pitfall for any researcher working with mycoplasma-contaminated material.

Mycoplasma is typically resistant to antibiotics such as penicillin and streptomycin and antibiotics such as gentamicin, tylnoxin, lincomycin, and spectinomycin do not eliminate mycoplasma contamination (Visser et al. 1999). Three classes of antibiotics, i.e., tetracycline, macrolids, and quinolones, have been shown to be highly effective against mycoplasmas, both in human/veterinary medicine and in cell culture. So far, no efforts have been made to observe the usefulness of these antibiotic classes for elimination of mycoplasma from P. falciparum culture as most of the antibiotics process antimicrobial activity. The present study was planned to determine a comparative efficacy of plasmocin (macrolid), Biomyc-1, -2 (tetracycline), and -3, and Mycoplasma Removing Agent (quinolone derivatives) for elimination of mycoplasma from P. falciparum culture.