REVIEW ON: INTRODUCTION TO NEW POTENTIAL ANTIMALARIAL DRUGS USED IN FALCIPARUM AND REVIEW OF VARIOUS ANALYTICAL METHODS FOR THEIR DETECTION FROM PHARMACEUTICAL FORMULATIONS

NEHAL SHAH¹, *MR. PINAK PATEL

¹Department of Pharmaceutical Chemistry, Indubhai Patel College of Pharmacy and Research Centre, Dharmaj, Gujarat-388430, Indubhai Patel College of Pharmacy and Research Centre, Dharmaj, Gujarat-388430. Email: pinakqa@gmail.com

Received: 30 May 2012, Revised and Accepted: 04 July 2012

ABSTRACT

Current paper describes potential therapies available to treat falciparum which is the most common and fatal disease of endemic regions along with review of various chromatographic methods available for their detection from various pharmaceutical formulations and from biological samples. It also suggest about the other alternate potential therapy to treat falciparum.

Keywords: Falciparum, Artemether, Lumefantrine, Amodiaquine, Piperaquine, Pyronaridine, RP-HPLC.

INTRODUCTION

Introduction to Malaria and malarial parasite

Malaria is caused by the protozoan parasite Plasmodium and transmitted by mosquitoes. At least five species of the parasite have been shown to infect humans: P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi. While they share a basic life-cycle, certain distinctive features relate to the virulence of each species. P. falciparum causes the most severe manifestations of malaria including coma, anaemia and multi organ failure. The severity of P. falciparum infection has been attributed to the relatively high parasitemias during infection and to the adherence of P. falciparum infected erythrocytes to the endothelium of capillaries and venules, a process known as sequestration.[1,2]

Background of falciparum

Half of the world’s population is at risk of contracting malaria. Out of the estimated 200-300 million episodes in 2006, the vast majority (86%) occurred in Africa. There were nearly a million malaria deaths the same year, of which 85% were children under the age of five.[3,4]

In the face of growing drug resistance, the WHO has issued recommendations strongly encouraging the use of combination therapies to combat uncomplicated malaria (WHO, WHO Guidelines for the treatment of malaria. 2006.). Amongst the most effective treatments are those which combine an artemisinin derivative with a longer acting component.[5,6]

Approaches to treat Falciparum

Artemisinin, a sesquiterpene lactone, was first isolated from the herb Artemisia annua L., Sweet Wormwood, in 1972. The artemisinins are also termed endoperoxides for the presumptive pharmacophore: an endoperoxide bridge. The endoperoxide moiety is believed to interact with intraparasiticheme to form reactive C-centered radicals that disrupt parasite proteins. A major target appears to be the parasite-encoded sarco-endoplasmic reticulum Ca²⁺-ATPase. Artesunate and artemether are semi synthetic derivatives of artemisinin. While artemenate is water soluble and suitable for all routes of administration (oral, rectal, intramuscular and intravenous) artemether is lipophilic and unsuitable for intravenous use. Both artemesine and artemether are readily absorbed from the gastrointestinal tract. The oral bioavailability of artesunate is low due to the rapid and extensive conversion to DHA.[10,11] DHA is further metabolized through glucuronidation.

Amodiaquine is a 4-aminoquinoline similar to chloroquine. The 4-aminoquinolines act by inhibiting the degradation of haemoglobin in the food vacuole of the parasite.[13] Amodiaquine is rapidly metabolized to the active metabolite N-desethylamodiaquine (N-DEAQ) through a reaction catalyzed by CYP2C8. Both amodiaquine and N-DEAQ possess antimalarial activity in vitro. Due to the rapid conversion of amodiaquine to N-DEAQ the metabolite is assumed to be responsible for the main clinical effect. In vitro studies, however, suggest a synergism between amodiaquine and N-DEAQ.[15,16,17]

Lumefantrine is an aryl-amino alcohol that prevents detoxification of haem, such that toxic haem and free radicals induce parasite death. Lumefantrine is a fluorene derivative discovered at the Academy of Military Medical Sciences in China. The mechanism of action of Lumefantrine is unclear. Lumefantrine is slowly absorbed with an estimated absorption half-life of 5 hours.[18]
Piperaquine was first synthesized at Rhone Poulenc, France, in the 1950s as compound 13228 RP, but was abandoned due to lack of commercial interest. It was later produced by the Shanghai Research Institute of Pharmaceutical Industry in 1966 under the name piperaquine. Piperaquine disposition is characterized by a multiphasic profile with an exceptionally long terminal half-life which may exceed one month in the adult.

**Fig. 4: Structure of Piperaquine**

Pyronaridine is a Mannich base anti-malarial with demonstrated efficacy against drug resistant Plasmodium falciparum, *P. vivax*, *P. ovale* and *P. malariae*. Recently its combination with artemisinin is approved by EMA. It showed consistently high levels of in vitro activity against a panel of six *P. falciparum* drug-sensitive and resistant strains. The data from various sources suggest that the combination of pyronaridine and artemisinin has completed development as a once daily, 3-day treatment for uncomplicated *P. falciparum* and blood stage *P. vivax* malaria in infants, children and adults.

**Fig. 5: Pyronaridine**

New Combination therapies available to treat Falciparum

Arterolane melate and Piperaquine

Literature survey revealed that the newer approaches to treat falciparum is the combination of Arterolane and Piperaquine. The study was done to assess the antimalarial efficacy and safety of a combination of 150 mg of arterolane maleate and 750 mg of piperaquine phosphate in comparison to Coartem (Artemether and Lumefantrine) in patients with acute uncomplicated *P. falciparum* malaria. Methods in (Methods) this open label, randomized, multicentric, parallel group clinical trial, 240 patients were randomized to receive arterolane maleate + Piperaquine phosphate (160 patients) or Coartem (80 patients). Patients with *P. falciparum* mono-infection and initial parasite densities ranging from 1000 to 100,000 asexual parasites/μL of blood were followed-up for 28 days. PCR-corrected ACPR (Adequate Clinical and Parasitologic Response) on day 28, parasite clearance time (PCT) and liver clearance time (FCT) were evaluated. Results total of 151 (94.4%) out of 160 patients completed the trial in arterolane maleate + piperaquine phosphate group while 77 (96.3%) out of 80 patients in Coartem group completed the trial.

No treatment failure was noted in arterolane maleate + Piperaquine phosphate group while one patient receiving Coartem was identified as treatment failure on Day 28. There was no difference in the median PCT (30 hours in both groups) and median FCT (24 hours in both groups) after administration of the two study treatments.

**Conclusion** The available data support the evaluation of drug combination in a larger population as fixed dose combination.

Tartemether and Lumefantrine

Artether-Lumefantrine combination therapy is now a day most widely preferred because of the resistance reasons for the available regimens. Now days this combination is considered as the line treatment for the treatment of plasmodium falciparum malaria. Artemisinin-based combination therapy (ACT) is the treatment of choice for uncomplicated falciparum malaria. Artemether-Lumefantrine (AL), a fixed dose co-formulation, has recently been approved for marketing in India, although it is not included in the National Drug Policy for treatment of malaria.

Efficacy of short course regimen (4 x 4 tablets of 20 mg artemether plus 120 mg Lumefantrine over 48 h) was demonstrated in India in the year 2000. However, low cure rates in Thailand and better plasma lumefantrine concentration profile with a six-dose regimen over three days, led to the recommendation of higher dose globally. This is the first report on the therapeutic efficacy of the six-dose regimen of AL in Indian uncomplicated falciparum malaria patients. The data generated will help in keeping the alternative ACT ready for use in the National program (Programme) as and when required.

**Fig. 3: Pyronaridine-artesunate**

Pyronaridine and Artesunate/Artemether

This regimen has been approved by the EMA in 2012 for the treatment of uncomplicated plasmodium falciparum malaria. This regimen has the edge over the artemether and Lumefantrine in the terms of the resistance. Pyronaridine-artesunate demonstrated repeatedly high efficacy in the treatment of vivax and falciparum malaria and non-inferiority (noninferiority) was established compared to standard comparator regimens. An innovative pediatric (paediatric) drug formulation was developed in parallel to the tablet formulation for the treatment of young children. Pharmacokinetic analysis showed dose linearity, low inter-individual (individual) variation and absence of a clinically important effect of food on the bioavailability of this drug combination. Overall tolerability and safety data are reassuring; however, further surveillance of safety in special patient populations including young children is warranted.

Various Analytical Methods used to detect the antimalarial drugs used in treatment of falciparum.

Various methods are available for the detection of antimalarial drugs from their pharmaceutical formulations which helps in the estimation of the active products, impurities and the active pharmaceutical ingredients.

However literature survey revealed that that there is no method available which can separate and identify the degradation product (Impurity profiling).

The methods can be selected for the quantitation of the drug based upon its cost effectiveness, its running time, ease of operating and its suitability.

Here from the literature survey various methods have been shown along with their characteristic so from the given data one can produce better methods with short analysis time, and low running cost.

HPLC methods for the estimation of various antimalarial drugs used in *P. falciparum* form various matrices are as follows:
<table>
<thead>
<tr>
<th>Matrix</th>
<th>API</th>
<th>Chromatographic conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pharmaceutical Formulations</strong></td>
<td>Lumefantrine</td>
<td>Column: RP spherisorb C18, Water (pH 3 adjusted with OPA): ACN(4:5:1 v/v/v), PDA detector set at 266nm. Linearity range: 0.15 - 20 µg/ml LOD and LOQ: 0.050 and 0.15 µg/ml</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Artemether + Lumefantrine</td>
<td>Symmetry C18, 250 x 4.6 mm, i.d, 5µm Buffer and acetonitrile in the ratio of 40:60 (v/v), pH 3 ± 0.5 The flow rate was 1.5 ml/min Detection at 210 and 303 nm Stability indicating method</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Artemether + Lumefantrine</td>
<td>Hypersil BDS C18, 250 x 4.6 mm, i.d, 5µm Methanol: 0.05% TFA with TEA buffer pH 2.8 with OPA (80:20 v/v) Flow rate: 1.5 ml/min Detection wavelength: 210 nm Retention time: 6.15 min(A) 11.31 min(L) Linearity range: 20-120 mcg/ml and 120-720 mcg/ml</td>
<td>33</td>
</tr>
<tr>
<td>Solid dosage forms</td>
<td>Lumefantrine</td>
<td>ODS C18, 250 x 4.6 mm, i.d, 5µm Methanol: Acetonitrile (50:50 v/v), Flow rate: 2ml/min detection wavelength 235nm. Linearity range: 50-150 mcg/ml</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Artemether + Lumefantrine</td>
<td>ODS C18, 250 x 4.6 mm, i.d, 5µm Acetonitrile: Buffer (0.1% v/v OPA, pH -3) v/v Flow rate: 1.5 ml/min Detection wavelength: 303 nm Retention time: 13.88 min(A) and 7.207 min(L) Linearity range: 20-60 mcg/ml - Artemether 120-360 mcg/ml - Lumefantrine</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Artemether</td>
<td>ODS C18, 250 x 4.6 mm, i.d, 5µm ACN:Buffer pH6.5 adjusted with TEA (65:35 v/v) Flow rate: 1.5ml/min Detection wavelength: 210 nm Linearity range: 250-750 mcg/ml Stability indicating method: Supelco L1 C18 (4.6 mm x 250 mm, 5 µ) Methanol: Water (80:20 v/v) Flow rate: 1.5 ml/min Detection wavelength: 216 nm Retention time: 12 min</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Artemether</td>
<td>ODS C18, 250 x 4.6 mm, i.d, 5µm Acetonitrile: Buffer (0.1% v/v OPA, pH -3) v/v Flow rate: 1.5 ml/min Detection wavelength: 303 nm Retention time: 13.88 min(A) and 7.207 min(L) Linearity range: 20-60 mcg/ml - Artemether 120-360 mcg/ml - Lumefantrine</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Artemether</td>
<td>ODS C18, 250 x 4.6 mm, i.d, 5µm Symmetry shield RP -18, 4.6 x 150mm, 3.5µm Water:ACN: MeOH(30:35:35 v/v/v) Flow rate: 1ml/min Detection wavelength: 216 nm Retention time: 12 min Linearity Range: 2-6 mcg/ml Stability indicating method: ACN: Buffer pH 6.5 adjusted with TEA (65:35 v/v) Flow rate: 1.5ml/min Detection wavelength: 210 nm Linearity range: 250-750 mcg/ml</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Artemether &amp;dihydro-artemisinine</td>
<td>ODS C18, 250 x 4.6 mm, i.d, 5µm ACN: Water (50:50 v/v) Flow rate: 1.5 ml/min Detection wavelength: 210 nm Stability indicating method: Spherisorb C18 column, 4.6 x 150mm ACN: Water (50:50 v/v) Flow rate: 1ml/min Detection wavelength: 216 nm Retention time: 12 min Linearity Range: 2-6 mcg/ml</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Artemether</td>
<td>Zorbax SB-CN column (3.0 × 150 mm, 3.5 µm water/methanol (0.1% TFA) detection wavelength: 335 nm linearity range:50-10,000 ng/ml extraction with 0.2% Perchloric acid</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Lumefantrine</td>
<td>Zorbax Eclipse XB-R phenyl column (4.6 mm × 150 mm, particle size 5 µm Flow rate of 1 mL/min in acetonitrile – 0.1 ammonium acetate buffer, 0.01 M acetic acid, pH 6.5) (10:90 v/v) Detection wavelength: 335 nm</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Lumefantrine</td>
<td>RP-Spherisorb C18 waters, 250 x 4.6 mm, i.d, 5µm Water (pH 3 with OPA): ACN: MeOH (4:5:1 v/v/v) Detection wavelength: 226nm Linearity Range: 0.15-20 mcg/ml Stability indicating method: major degradation observed with oxidative and alkaline hydrolysis studies</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Lumefantrine</td>
<td>Synergy C18 column 250 x 3 mm, 5µm Acetonitrile: Ammonium acetate buffer pH 4.9 (85:15 v/v) Flow rate: 1.5 ml/min Detection wavelength: 335nm</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Artemether</td>
<td>C18 column 250 x 3 mm, 5µm</td>
<td>44</td>
</tr>
</tbody>
</table>
Pyronaridine which is a potential target for treatment of falciparum and approved by EMA consist of combination of artesunate and Lumefantrine. Other mention drugs are now outdated and resistance have been observed with them.

There is an alternate therapy, which has passed phase 4 clinical trial and approved by EMA consist of combination of artesunate and Pyronaridine which is a potential target for treatment of falciparum

**CONCLUSION**

From literature review we can say that the proposed methods are expensive and usually require longer running times, apart from that there is a strong requirement of a method which can detect both the compounds economically with short analysis time. There is also a need of a method which can detect and identify the degradation product when drugs are exposed to the forced degradation studies along with their degradation kinetics.

**REFERENCE**


### Table 2: Only one spectrophotometric method has been reported for determination of Artemether and Lumefantrine

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Drug</th>
<th>Chromatographic conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablets</td>
<td>Lumefantrine</td>
<td>Stationary phase: aluminum foil plates precoated with silica gel 60 F 254</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Artemether</td>
<td>Linearity range: 8-16μg/ml limit of quantification to be 13.2 ×10−2</td>
<td></td>
</tr>
<tr>
<td>Human Plasma</td>
<td>Pyronaridine</td>
<td>Mobile phase: n-hecane: Ethyl acetate (8:2, %v/v) Detection wavelength: 357 nm</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Linear range: 20-150 ng/spot - Artemether</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>300-900ng/spot - Lumefantrine</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Detection wavelength: 234 nm</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: High performance thin layer chromatographic method for detection of Artemether and Lumefantrine.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Drug</th>
<th>Chromatographic conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablets</td>
<td>Lumenfantrine and</td>
<td>Stationary phase: aluminum foil plates precoated with silica gel 60 F 254</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Artemether</td>
<td>Mobile phase: n-hecane: Ethyl acetate (8:2, %v/v) Detection wavelength: 357 nm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Linearity range: 50-150 ng/spot - Artemether</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>300-900ng/spot - Lumefantrine</td>
<td></td>
</tr>
<tr>
<td>Pharmaceutical Formulation</td>
<td>Artemether</td>
<td>Stationary phase: aluminum foil plates precoated with silica gel 60 F 254</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Toluene- Ethylacetate-formic acid (8:2:0.3, v/v) Detection in Reflectance at 565 nm.</td>
<td></td>
</tr>
</tbody>
</table>
22. European medicinal agencies, Committee for Medicinal Products for Human Use (CHMP), 16 February 2012 (EMA/CHMP/95965/2012).