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Comparison of efficacy and safety of different brands of oral artesunate plus mefloquine in uncomplicated falciparum malaria in adults

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The efficacy of artemisinin derivatives in treatment of falciparum malaria is well established and a variety of different brands are now available, assumingly to be identical in composition and efficacy. A hospital-based, double blind randomized, controlled study to find out the efficacy and pharmacokinetics of different brands of artesunate when combined with mefloquine in Myanmar population was carried out in CRU (Malaria) DSGH, to establish the relative merits of the different brands, provide data for consumer choice and for better future utilization of these combinations in the chemotherapy of malaria. A total of 120 adult uncomplicated falciparum malaria patients were randomized, to receive 4 different brands of artesunate 200 mg OD for 3 days. A single brand of mefloquine (Helm-Germany) 500 mg OD for 3 days was used as the combination drug in all four regimens. Artequin TM600/1500 combi-pack (Mepha-Switzerland) was used as the control drug. The Mean Fever Clearance Times (FCT) were 11.15 hrs, 13.69 hrs, 11.8 hrs, 11.68 hrs and 12.88 hrs. The Mean Parasite Clearance Times (PCT) were 46.12 hrs, 50.51 hrs, 46.88 hrs, 42.45 hrs and 51.22 hrs in the Plasmotrim LactabTM 200 mg (Mepha-Switzerland), Falcinate Tab 50 mg (Aurocham-India), Dawnasunate Tab 50 mg (MPF, Myanmar), Artenmed Tab 50 mg (Vietnam) and Control drug. Artequin TM600/1500 combi-pack (Mepha-Switzerland) respectively. The 14 days Adequate Clinical and Parasitological Response (ACPR) was 100 % in all the five groups. There were no adverse, clinical, hematological, biochemical and ECG changes in all the groups. The different brands of artesunate available are comparable in efficacy for the treatment of uncomplicated falciparum malaria in adults.

INRODUCTION

Many previous studies in Myanmar have demonstrated the efficacy of artemisinin derivatives and its great potential to fight against falciparum malaria. National anti-malarial drug policy recommended 3 days course of Artemisinin Combination Therapy (ACT) in uncomplicated falciparum malaria. A variety of oral artesunate are now available in Myanmar, but whether they are identical in composition and efficacy has not yet been confirmed. The present study aimed to test the qualitative and quantitative composition of five locally available commercial brands of artesunate and to compare the efficacy of each when combined with one single brand of mefloquine given in the recommended dosages for three days in adult uncomplicated falciparum malaria patients, using Artequin TM a fixed dose combination pack of artesunate + mefloquine as a control, so as to provide data on the relative merits of the different brands and to provide data for consumer choice.

Objectives

- To compare the therapeutic efficacies, safety & tolerability of 5 commercial tablet formulations of artesunate.
- To verify the composition of active compounds present in them.

PATIENTS AND METHODS

Study design

A hospital-based, double blind randomized controlled study

Acute symptomatic uncomplicated falciparum malaria patients admitted to Clinical Research Unit (Malaria) DSGH were recruited. Patients were categorized as symptomatic if they were febrile or with one of the following symptoms: headache, feeling ill, aches and pains, nausea or vomiting.

Inclusion criteria

- Both sexes
- The age group between 10 - 60 years
- Positive peripheral blood film for trophozoite forms of pure *P. falciparum* with the count ranging from 1000/ul up to 250,000/ul
- Patients who had no evidence of severe and complicated falciparum malaria
- Patients who had not been treated with artemisinin or mefloquine within the past 14 days
- Patients who were willing to give informed consent for treatment and were able to remain hospitalized for 14 days

Exclusion criteria

- Patients with mixed infection (falciparum malaria + vivax malaria)
- Asymptomatic patients
- Patients requiring parenteral treatment
- Patients with fever due to causes other than malaria e.g. TB, etc.
- Patients with other concomitant diseases like diabetes mellitus, etc.
- Pregnant women in 1st trimester of pregnancy

Withdrawal criteria

- Patient's request
- Any serious adverse effects to drugs
- Serious or repeated noncompliance with protocol specifications

Sample size

A total of 120 patients completed the study: 30 patients each for Plasmotrim Lactab (Mepha-Switzerland), Falcinate (India) and Artenmed (Vietnam), 20 patients for Dawnasunate (MPF, Myanmar) and 10 patients for Artequin TM 600/1500 (Mepha-Switzerland) combi-pack.

Study period

14 months

Materials

Study drugs and control drugs

No.	Drug	Manufacture/ Country	Batch no.	Course
1.	Plasmotrim Lactab 200 mg	Mepha - Switzerland	250512	1 tab OD for 3 days
2.	Falcinate Tab 50 mg	Aurocham - India	S-297	4 Tabs OD for 3 days
3.	Dawnasunate Tab 50 mg	MPF - Myanmar		4 tabs OD for 3 days
4.	Artemned Tab 50 mg	Vietnam	30903	4 tabs OD for 3 days
5.	Mefloquine 250 mg	Helm - Germany	165703	2 tabs OD for 3 days
6.	Artequin 600/1500 mg combi-pack (Control drug)	Mepha - Switzerland	390009	(I +2) tabs on Day 1,2,3

Procedure

Eligible patients were subjected to the following procedure; Routine history taking, clinical examination and relevant investigations were done and recorded in the standard proforma. Patients were randomized to 5-drug regimens by means of sealed envelopes. Drug administrations were observed in all patients and if vomiting occurred in less than 30 min, drug administration with full dose were repeated. If vomiting occurred between 30-60 mins, half the dose was repeated. No re-treatment was given if vomiting occurred after 60 minutes.

Clinical assessment

Symptoms review, adverse effect review (according to the checklist) and physical examination were made on Days 0, 1, 2, 3, 4, 7 and 14. Body temperature were recorded 4 hourly until normal for 24 hours and then daily up to Day 14.

Parasitological assessment

The Giemsa stained thick and thin blood smears were examined 6 hourly until negative for 24 hours and daily up to 3 consecutive negatives were resulted, and parasite counts were also noted, then weekly up to Day 14, whenever it is indicated e.g., reappearance of fever.

Haematological measurements

Hb%, PCV, T & DC, platelets were done on Day 0, 3 and 7. Biochemical tests; Blood urea, sugar, serum bilirubin, SGOT, SGPT and Alkaline Phosphatase were checked on Days 0, 3 and 7. ECG was measured on Days 0, 1, 3 and 7, and to be repeated on Day 14 if found abnormal in first week.

Thin Layer Chromatography (TLC)

Quality assurance of artesunate in different brands was done by Thin Layer Chromatography using methanol & ethyl acetate as mobile phase on SiOz TLC plates. Movement of compounds present in different brands was recorded as retention factors on TLC plates. Chromatograms were developed by using iodine as visualizing reagent. Authenticity of *artesunate* in each brand used was checked by observing similarity in R_f values.

Fourier Transform Infrared Spectrometer (FT-IR)

KBr pellet method was used to detect quantitatively the artesunate content in mixed content powder from different brands & blank (KBr) in ratio of 1:200 and checked with specified reference spectrum of a artesunate with regards to the functional group using infrared absorption spectrum. Close resemblance between spectrum of extracted material and specified reference spectrum achieved indicated authenticity of artesunate present in each brand tested.

Therapeutic response

Therapeutic response was assessed according to WHO criteria.

Indicators for drug efficacy were:

- Parasite Clearance Time (PCT)

  Time from initiation of therapy to the first negative blood film that remained negative for 48 hours
- Percent clearance of parasitaemia at 24- 48 hours
- Fever Clearance Time (FCT)

  Time from initiation of therapy to time the temperature reached normal (37°C) and remained so for 24 hours
- Early Treatment Failure (ETF) rate
- Late Treatment Failure (LTF) rate
- Adequate Clinical and Parasitological Response (ACPR)

Definitions (WHO, 2003)

Early treatment failure (ETF)

- Development of danger signs or severe malaria on Day l, Day 2, or Day 3, in the presence of parasitaemia
- Parasitaemia on Day 2 higher than Day 0 count irrespective of auxiliary temperature
- Parasitaemia on Day 3 with axillary temperature +/- >37.5 °C
- Parasitaemia on Day 3 +/- > 25% count on Day 0

Late Treatment Failure (LTF)

It is divided into Late Clinical Failure and Late Parasitological Failure.

Late Clinical Failure (LCF)

- Development of danger signs or severe malaria after Day 3 in the presence of parasitaemia, without previously meeting any of the criteria of Early Treatment Failure
- Presence of parasitaemia and axillary temperature +/- >37.5°C on any day from Day 4 to Day 14 (Day 28*), without previously meeting any of the criteria of Early Treatment Failure

Late Parasitological Failure (LPF)

- Presence of parasitaemia on Day 14 (or Day 28*) and axillary temperature <37.5°C, without previously meeting any of the criteria of Early Treatment Failure or Late Clinical Failure

Adequate Clinical and Parasitological Response (ACPR)

- Absence of parasitaemia on Day 14 (or Day 28*), irrespective of axillary temperature, without previously meeting any of the criteria of Early Treatment Failure, Late Clinical Failure or Late Parasito-logical Failure

(* if followed up for 28 days.)

Retreatment of failure

ETF and LTF cases were retreated with standard seven-day course of oral quinine sulphate (l0 mg of salt/ kg three times/ day) and tetracycline (4 mg/kg four times / day).

Statistical evaluation

Statistical evaluation comparing the regimens was done by computer using EPI-INFO software. Categorical data were compared by calculating the chi-square value with Yate's correction or by Fischer's exact test. Normally distributed continuous data were compared by the Student's *t-test* and analysis of variance. Data not conforming to a normal distribution were compared by the Mann-Whitney U test. PCT, FCT, Symptom clearance times and the resolution of other signs (anemia, Hb%), hepatomegaly, splenomegaly and the risk of treatment failure were evaluated by survival analysis with cumulative incidences and calculated by the product limit method and compared by the Mantel-Haenzellog rank test.

Ethical considerations

The protocol was approved by the Ethical Committee, Research and Development Committee, Directorate of Medical Services, Ministry of Defense. Informed written consent was obtained from all the patients.

RESULTS

Baseline characteristics like age, sex, weight, initial temperature, initial parasite count, Hb% between groups were comparable (Table 1).

Table 1. Baseline characteristics

	Plasmo-trim	Fal-cinate	Dawna-sunate	Arten-med	Arte-quin
No. of subjects	29	29	20	29	9
Age (Year) Mean ± S.D	28.9 ±9.8	24.8 ±8.3	27.1 ±10.8	26.9 ± 7.4	21.2 ±5.1
Height (cm)	165.3 ±6.7	161.2 ±7.3	163.5 ± 4.1	165.9 ± 5.1	160.7 ± 7.9
Weight (Kg)	51.6 ±7.7	47.3 ±6.4	49.7 ±6.2	49.9 ±5.3	47.3 ±10.3
BMI	18.8 ±2.8	18.1 ±2.2	18.7 ±2.3	18.0 ±1. 7	18.1 ±2.4
Initial temperature (°C)	38.5 ±0.8	38.5 ±1.0	38.4 ±1.0	38.5 ±0.8	38.5 ±0.9
Initial parasite count (/cu.mm)	24548.6 ± 49741.3	7503 ± 9643.2	13296.0 ± 26335.7	6814.3 ± 7673.2	3528.9 ± 1909.3
Haemoglobin (g/dl)	10.2 ±1.2	9.8 ±1.5	9.8 ±1.4	9.7 ±1.5	10.4 ±0.7
Total WBC count	5.4 ±1.8	5.3 ±1.6	5.4 ±1.2	5.0 ±1.6	6.0 ±2.1
Bilirubin (mg/dl)	0.4 ±0.2	0.4 ±0.2	0.5 ±0.4	0.4 ±0.2	0.3 ±0.2
Urea (mg/dl)	24.8 ±14.8	25.7 ±5.2	26.5 ±7.1	26.5 ±3.4	25.5 ±5.2
Glucose (mg/dl)	110.5 ±10.8	108.2 ±7.5	115.9 ±11.4	106.6 ±8.4	113.4 ±10.0

Five various commercial tablet formulations of artesunate are all effective equally (Fig. l) and spectrum of each formulation is comparable with standard drug.

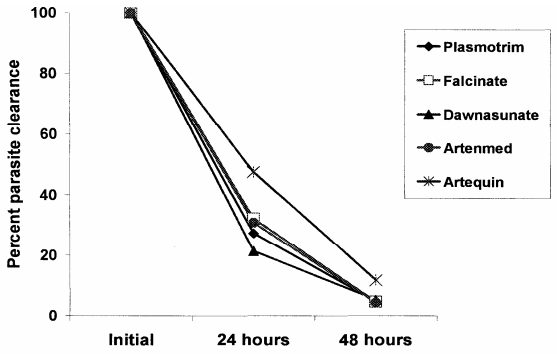

Fig.1. Percentage parasite clearance of five different brands

Authenticity of artesunate in each brand used checked by TLC has similarity in R_f values and by Fourier Transform Infrared Spectrometer (FT-IR) method showed close resemblance (Fig. 2).

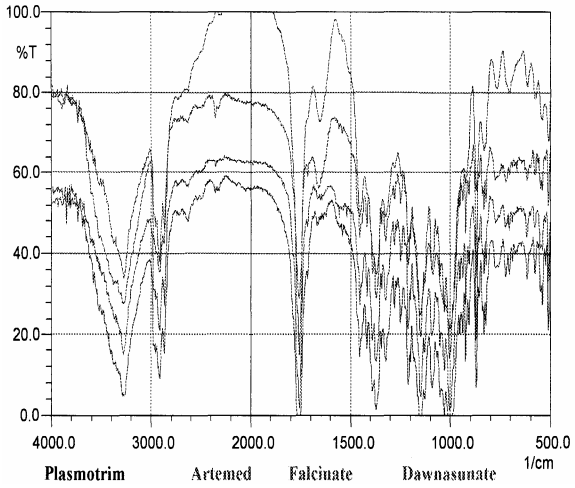

Fig. 2. FTIR spectrum showing the functional groups of artesunate in different brands

There was no early treatment failure (ETF) and adequate clinical and prasitological response (ACPR) was l00% in all groups. There were no adverse clinical effects and laboratory and ECG abnormalities. Drug tolerance was similar in frequency and severity. Dawnasunate from Myanmar Pharmaceutical factory is well tolerated, safe and equally effective compared to that of four other imported brands.

DISCUSSION

The quality of commercially available drugs varies greatly among countries. Due to lack of regulations and poor quality control practices in some countries, the amount of the active ingredient can be inconsistent. Poor formulation techniques can affect the release of active ingredients from a tablet, with some tablets releasing very little amount of drug. Some drugs may be contaminated with other substances. Poor storage conditions, especially in warm and humid tropical environments may contribute to chemical degradation of many pharmaceuticals.

Artesunate is a semi-synthetic derivative of artemisinin, a naturally occuring sesqesterpene endoperoxide. It is difficult to detect and identify by standard spectrophotometric methods. The standard method used to determine artesunate in tablets involves high performance liquid chromatography (HPLC). In many countries, such equipment is not available.

Modern electronic technology is rapidly approaching the state at which it can reliably and affordably provide much greater assurances that a drug product was manufactured safely and distributed under conditions that did not compromise its potency. FDA has concluded that this approach is a much more reliable direction for assuring the legitimacy of a drug than paper record keeping requirements, which are more likely to be incomplete or falsified, and that it is feasible for use by 2007.

Radiofrequency Identification (RFID) tagging of products by manufacturers, wholesalers, and retailers appears to be the most promising approach to reliable product tracking and tracing. Significant feasibility studies and technology improvements are underway to confirm that RFID will provide cost-reducing benefits in areas such as inventory control, while also providing the ability to track and trace the movement of every package of drugs from production to dispensing. Most importantly, reliable RFID technology will make the copying of medications either extremely difficult or unprofitable. FDA is working with RFID product developers, sponsors, and participants of RFID feasibility studies to ensure that FDA's regulations facilitate the development and safe and secure use of this technology. FDA is also working with other governmental agencies to coordinate activities in this area.

In Myanmar, relatively simple and inexpensive methods such as TLC and FT - IR methods are used extensively as quality assurance procedures. These methods, if used together with some form of sample pretreatment such as solvent extraction, can become powerful techniques for identifying and detecting compounds and impurities. The present study is the first time that different brands of artesunate available in the market have been subjected to such tests.

The study supported the high pharmaceutical quality of Dawnasunate from MPF, in attaining similar clinical efficacy on patients with uncomplicated *P. falciparum* malaria.

Conclusion

Different brands of artesunate available in Myanmar are comparable in efficacy and equally effective. All are safe and tolerable. Dawnasunate from (Myanmar Pharmaceutical Factory Myanmar) has equal efficacy like other imported brands. The TLC and FT–IR methods provided a practical and cost-effective means of detecting the artesunate content and can thus, be useful for screening of counterfeit and substandard drugs in the market.

REFERENCES

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