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Therapeutic equivalence of a low dose artemisinin formulation in falciparum malaria patients

J. W. Wong, K. H. Yuen, S. Nagappan, W. S. Shahul, S. S. David Ho, E. K. Gan and W. T. Toh

Abstract

We have evaluated the therapeutic equivalence of a β-cyclodextrin–artemisinin complex at an artemisinin dose of 150 mg, with a commercial reference preparation, Artemisinin 250 at a recommended dose of 250 mg. One hundred uncomplicated falciparum malarial patients were randomly assigned to orally receive either β-cyclodextrin–artemisinin complex (containing 150 mg artemisinin) twice daily for five days or the active comparator (containing 250 mg artemisinin) twice daily for five days. The patients were hospitalized for seven days and were required to attend follow up assessments on days 14, 21, 28 and 35. All patients in both treatment groups were cured of the infection and achieved therapeutic success. At day seven of treatment, all patient blood was clear of the parasites and the sublingual temperature of all patients was less than 37.5 °C. Moreover, the parasite clearance time in both treatment groups was similar, being approximately three days after initiation of treatment. Comparable plasma artemisinin concentrations were observed between patients in both treatment groups at 1.5 and 3.0 h, although slightly higher levels were obtained with patients in the β-cyclodextrin–artemisinin complex-treated group. The β-cyclodextrin–artemisinin complex at a dose of 150 mg artemisinin was therapeutically equivalent to 250 mg Artemisinin 250. Additionally, patients receiving β-cyclodextrin–artemisinin complex showed less variability in their plasma artemisinin concentrations at 1.5 h post-dosing, which suggested a more consistent rate of drug absorption.

School of Pharmaceutical Sciences, University of Science Malaysia, Penang, Malaysia

J. W. Wong, K. H. Yuen, W. T. Toh

General Hospital Tawau, Sabah, Malaysia

S. Nagappan, W. S. Shahul

Hovid Pte Ltd, Ipoh, Perak, Malaysia

S. S. David Ho

Innovation and Consultancy Center, University of Science Malaysia, Penang, Malaysia

E. K. Gan

Correspondence: K. H. Yuen, School of Pharmaceutical Sciences, University of Science Malaysia, 11800 Penang, Malaysia.
E-mail: khyuen@usm.my

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Introduction

Artemisinin, a unique sesquiterpene lactone endoperoxide, is a potent antimalarial compound isolated from the Chinese medicinal herb, Artemisia annua (Klayman 1985; Luo & Shen 1987). It is one of the few antimalarials that remains effective against multidrug-resistant strains of *Plasmodium falciparum* (World Health Organization 1994). Thus, it has been increasingly used as a first-line therapy to replace other antimalarials in many tropical countries (Li et al 1994; Price et al 1996). The current recommended dose is 500 mg/day, which is divided into two daily administrations for five days (Ashton et al 1998).

However, artemisinin has low aqueous solubility, resulting in poor and erratic absorption upon oral administration. This, together with its short half-life and high first-pass metabolism, might lead to incomplete clearance of the parasites resulting in recrudescence (Titulaer et al 1991). Wong (2001) demonstrated that its solubility could be increased through inclusion complexation with β-cyclodextrin at a molar ratio of 1:1. The β-cyclodextrin–artemisinin complex was shown to display a faster rate and higher extent of dissolution and possessed enhanced bioavailability in-vivo compared with a commercial preparation containing the normal form of the drug (Wong & Yuen 2001). Absorption of artemisinin from the β-cyclodextrin–artemisinin complex was found to increase by 1.7-times compared with the commercial preparation. Therefore, comparable plasma drug level profiles or therapeutic efficacy can be achieved with a lower dose of the complexed form of the drug, being approximately 150 mg. Hence, this study was conducted to determine the therapeutic equivalence of the β-cyclodextrin–artemisinin complex at an artemisinin dose of 150 mg, with a commercial reference preparation at a recommended dose of 250 mg.

Materials and Methods

Materials

Two artemisinin preparations were used. Firstly, an Artemisinin 250 capsule (Mekophar, Vietnam) with batch number VNA 1350-98. Each capsule contained a labelled dose of 250 mg artemisinin. Secondly, a β-cyclodextrin–artemisinin complex, obtained from scale up batches, prepared using a slurry method at the molar ratio of 1:1 (β-cyclodextrin: artemisinin). The weight of the β-cyclodextrin–artemisinin complex containing an equivalent of 150 mg artemisinin was 750 mg. The complex was administered using two size 0 hard gelatin capsules.

Methods

The study was approved by the Permanent Research Committee, Ministry of Health Malaysia with regards to its technical and scientific content as well as ethical issues. Written informed consent was obtained before study inclusion.

The study was carried out according to a randomized single factor parallel group design with two treatment levels and was conducted in Tawau Hospital, located at the southeast of Kota Kinabalu, Sabah, Malaysia from October 1998 to July 2001. The area is endemic for *Plasmodium falciparum* and *P. vivax* malaria with high transmission during the rainy intermonsoon seasons (March–April and October–November). *P. falciparum* malaria accounts for approximately 60% of all the malarial infections.

One hundred male and female patients aged 15–61 years with uncomplicated malaria caused by *P. falciparum* at densities between 200–100 000 asexual parasites μL^{-1} blood were recruited into the study. The exclusion criteria were patients with general danger signs or signs of severe and complicated falciparum malaria according to definition by the World Health Organization, mixed infection with *P. vivax* and pregnant patients. Patients who had taken antimalarial drugs before hospitalization were excluded also.

Patients were randomized to orally receive either two capsules of β-cyclodextrin–artemisinin complex containing 150 mg artemisinin twice daily for five days or one capsule of the commercial preparation (Artemisinin 250) containing 250 mg artemisinin twice daily for five days with the latter acting as the active comparator. Each β-cyclodextrin–artemisinin capsule contained 75 mg artemisinin. Alin et al (1996a, b) demonstrated the efficacy of the active comparator in earlier trials.

Following enrolment into the trial, the patients were admitted into the hospital. The preparations were administered with 100 mL water. Blinding of the ward personnel was not implemented because all parameters investigated could be measured objectively and no subjective measurements were taken.

All patients were hospitalized for seven days during which their parasite density and body temperature were monitored together with complete clinical assessment and drug history. The patients were required to attend weekly follow-up visits on days 14, 21, 28 and 35.

Clinical and parasitological assessments

The parasite density and form of parasites were determined using Giemsa-stained blood film prepared from capillary blood as recommended by the World Health Organization. The parasite density was determined before drug intake and then every 12 h for the first 72 h followed by every 24 h for the subsequent 96 h. The parasite density was determined also during the follow-up visits. The blood was considered negative when no parasite was detected in three consecutive blood films. All blood films were examined by a single microscopist who was blinded to the treatments. Parasite clearance time (PCT) was taken as the time from starting therapy to the first of the three consecutive negative thick films.

The sublingual temperature of the patients was monitored every 6 h until the patients were discharged. Fever subsidence time (FST) was taken as the time required for the sublingual temperature to fall below 37.5 °C and remain so for three consecutive readings. For those afebrile patients, the FST was considered as the time when the first measurement of body temperature was taken. Patients were also interviewed about adverse events associated with the administration of the artemisinin preparations.

In addition, blood samples for determination of the plasma artemisinin concentration were collected at 0 h (predose), 1.5 and 3 h after administration of the first dose to give an estimate of the rate and extent of drug absorption. The former blood sampling time corresponded to when absorption was still actively taking place, while the latter time was when absorption was almost or had been completed (Wong & Yuen 2001). Blood (5 mL) was drawn into a heparinized vacutainer (Becton Dickinson, USA) from a forearm vein, centrifuged at 2500 rev min^{-1} for 20 min and the plasma transferred to a new tube and stored at −20 °C until analysis (within three months). Artemisinin plasma concentrations were measured using a high-performance liquid chromatography method (Chan et al 1997).

Data and statistical analysis

The major parameter monitored was the therapeutic outcome, which was determined by the absence of parasites in the blood and a body temperature of less than 37.5 °C at day 7. Patients meeting both criteria were considered to have been treated successfully and the percentage of therapeutic success or curing rate in each treatment arm was calculated.

The β-cyclodextrin–artemisinin complex was deemed to be therapeutically equivalent to the reference preparation if the 95% confidence interval of the difference in its curing rate with respect to the reference preparation was not more than 5% (Jones et al 1996).

The PCT as well as the FST of the two treatment arms were compared using the Mann-Whitney test. The initial parasitaemia of the patients in the two treatment groups

was compared using an independent Student's t-test. Correlations were sought between initial parasitaemia to PCT and FST as well as PCT to FST by calculating the Pearson correlation coefficient. $P < 0.05$ indicated statistical significance in all data analyses.

The plasma drug concentrations at 1.5 and 3.0 h were compared between the two treatments using an analysis of variance procedure for two-factor repeated measures split-plot design (Kirk 1968) after logarithmic transformation. The 90% confidence interval of the ratio for the concentration value obtained with the β-cyclodextrin–artemisinin complex over that of the Artemisinin 250 at 1.5 and 3.0 h were computed also.

Results and Discussion

The sample size of 100 with 50 patients for each treatment arm was calculated based on the formula recommended by Jones et al (1996), assuming a null hypothesis of non-equivalence and an alternative hypothesis of equivalence (Dunnett & Gent 1977). The sample size was estimated with the α value set at 0.05 and β at 0.2 (80% power). The sample size was large because a small value of δ (range of equivalence for the difference in percentage success rate) in the formula by Jones et al (1996) was usually employed as recommended by Röhmel (1998) to obtain sufficient power to achieve statistical equivalence. Given that δ^2 is inversely proportional to sample size, a large number of patients was thus calculated. Using an unrealistically large value of δ would cause the trial to be of little value (Durrleman & Simon 1990).

Both treatment groups had equal numbers of male and female patients, each consisting of two females and 48males with age ranging from 15- to 61-years old. Seventeen patients (34%) in the commercial preparation (Artemisinin 250) group had previous history of malarial infection compared with sixteen patients (32%) receiving the β-cyclodextrin–artemisinin complex. There was no significant difference in any demographic characteristics between the two groups, namely age, weight and height and ethnicity (Table 1). Moreover, initial parasitaemia of patients in both treatment groups were comparable and no statistically significant difference was detected ($P > 0.05$), thus indicating that the baseline characteristics were comparable.

The mean percentage of parasites remaining in the blood vs time profiles of the two treatments are shown in Figure 1. It was apparent from both plots that there was rapid clearance of parasites from the blood for both treatments, with complete clearance achieved at approximately three days after commencement of therapy. Both profiles were almost superimposable, indicating that the rate of parasite clearance from the blood was essentially similar. The mean PCT for the commercial preparation was 46.6 ± 2.5 h (range: 12–72 h) while that for the β-cyclodextrin–artemisinin complex was 48.7 ± 2.8 h (range: 12–84 h). No statistically significant difference was detected ($P > 0.05$) between the PCT values for both treatment groups.

The mean sublingual temperature vs time profiles of the two treatments are shown in Figure 2. In all cases, the sublingual temperature attained a value of less than 37.5 °C on day 7 and the fever subsidence rates in both treatment groups were quite similar. The mean FST values for the commercial preparation and the β-cyclodextrin–artemisinin complex-treated groups were 21.7 ± 2.6 h (range: 6.0–102.0 h) and 17.6 ± 1.9 h (range: 6.0–54.0 h), respectively. A statistically significant difference was observed between the FST values of the two treatments ($P < 0.05$). However, the interpretation of sublingual temperature is complicated because the pyrexia induced by falciparum malarial infection is periodical, which coincides with the periodical rupture of infected erythrocytes and the release of merozoites into the blood (Cruickshank et al 1977). The periodicity of pyrexia is approximately 48 h and thus may explain the absence of elevated temperature of some patients on admission. Hence, the FST may not be a good parameter for comparing the efficacy of artemisinin.

From the above results, especially with reference to the data on parasitaemia, it can be inferred that all patients in both treatment groups achieved therapeutic success. At day 7, all parasites were cleared from the blood and the sublingual temperature of all patients was less than 37.5 °C. In view of the fact that the therapeutic success achieved was 100% in both treatment arms, the difference in curing rate between the β-cyclodextrin complex and Artemisinin 250 was essentially zero and the curing rate of the test preparation would unequivocally lie entirely within the stipulated equivalence range of 95–100% (difference in curing rate of not more than 5%). As such,

Table 1 Demographic data of patients receiving the commercial preparation (Artemisinin 250) and β-cyclodextrin–artemisinin complex.

	Mean (range) Patients receiving the commercial product	Patients receiving β-cyclodextrin–artemisinin complex
Sex (male/female)	48/2	48/2
Age	33 ± 12 (15–59)	38 ± 14 (16–61)
Weight (kg)	58 ± 11 (35–85)	59 ± 14 (39–100)
Height (cm)	163 ± 5 (150–172)	165 ± 8 (143–181)
Initial parasitaemia	$11581 \pm 15\,688$ (200–64 200)	$7798 \pm 12\,407$ (200–60 000)

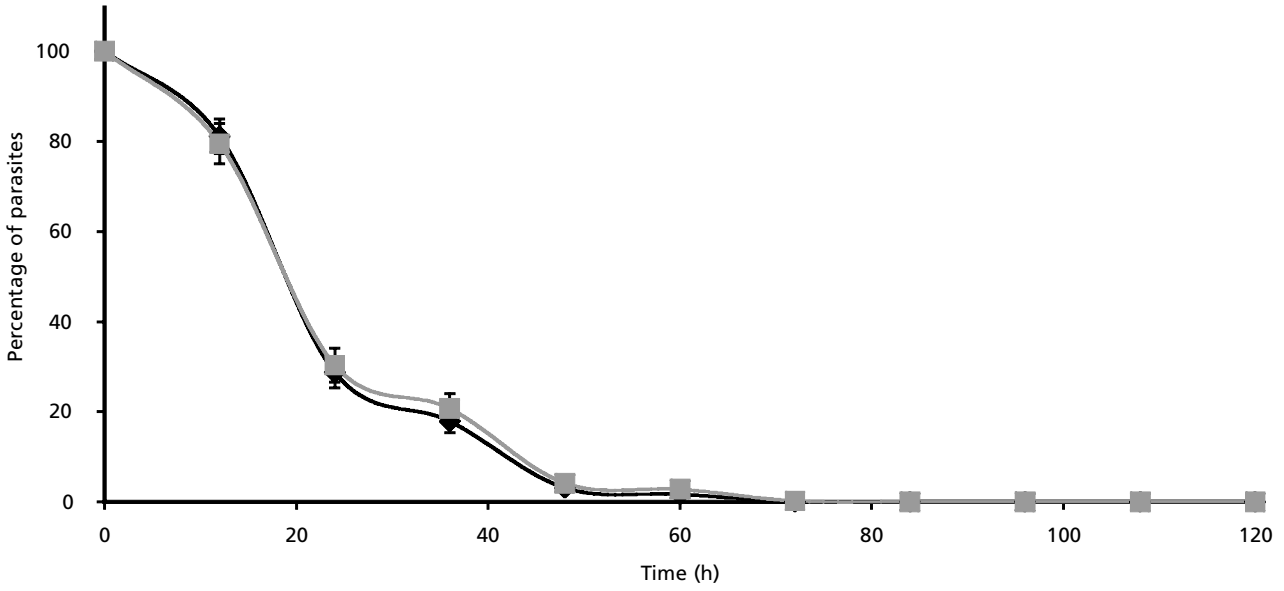

Figure 1 Percentage of parasites remaining in blood vs time. Mean ± s.e.m. n = 50 per treatment arm. Artemisinin 250, ◆; β-cyclodextrin complexes, ■.

therapeutic equivalence between the two treatments could be concluded. Moreover, no adverse drug reaction or toxicity was reported from patients receiving either of the treatments, confirming similar reports that suggested the low risk of adverse drug reactions associated with the administration of artemisinin (White 1994; World Health Organization 1998).

The initial parasitaemia level of the patients was observed to possess a significant positive correlation with the PCT ($r = 0.5670$, $P < 0.05$) as well as with the FST

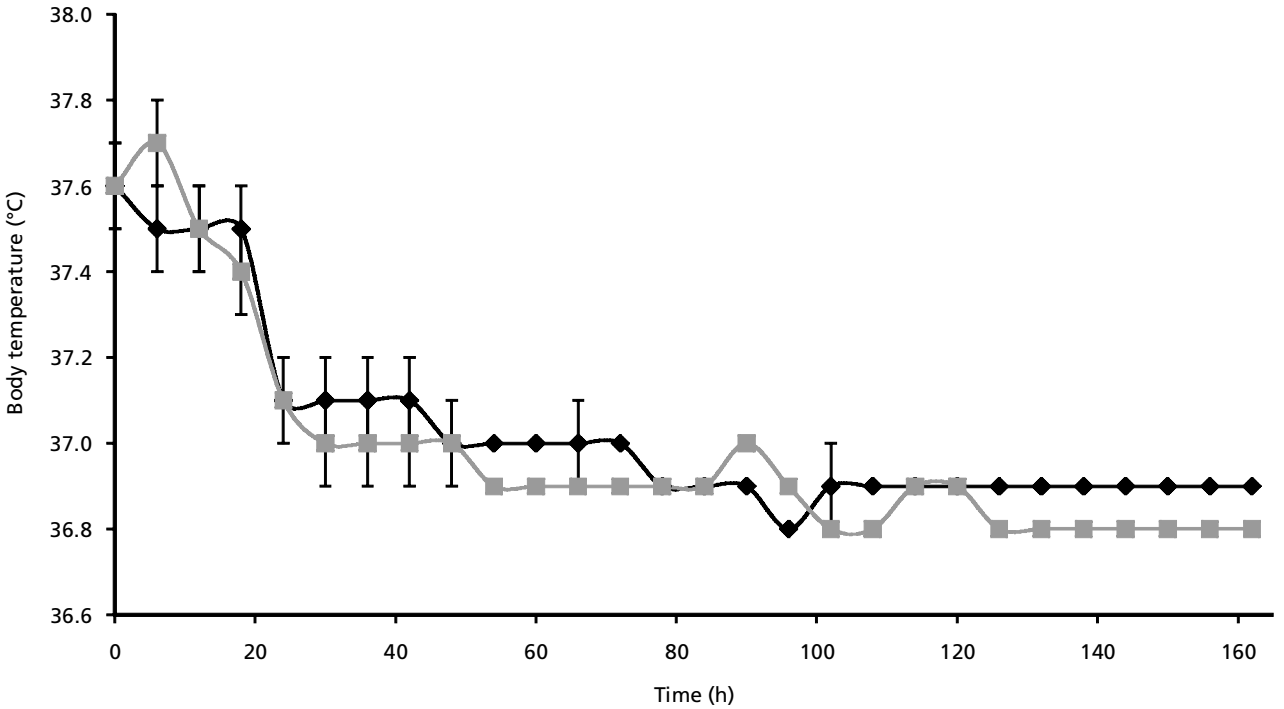

Figure 2 Mean sublingual temperature of patients vs time. Mean ± s.e.m. n = 50 per treatment arm. Artemisinin 250, ◆; β-cyclodextrin complexes, ■.

Table 2 Mean plasma artemisinin concentrations at 1.5 and 3.0 h after administration of the first dose for Artemisinin 250 and β-cyclodextrin–artemisinin complex (mean ± s.e.m.).

Time	Plasma concentration (ng mL^{-1})	
	Artemisinin 250	β-Cyclodextrin–artemisinin complex
1.5 h	234.6 ± 38.4	293.2 ± 27.7
3.0 h	249.3 ± 26.9	284.9 ± 27.3

(r = 0.3904, $P < 0.05$). On the other hand, the FST was poorly correlated with PCT (r = 0.1891, $P > 0.05$), being similar to the observations of Alin et al (1996b). This could be due to the periodicity of pyrexia giving rise to inconsistent FST values and hence the poor correlation. However, it was evident that no elevation of sublingual temperature was detected in the period after PCT.

Table 2 shows the mean plasma artemisinin concentrations at 1.5 and 3.0 h after administration of the first dose. Statistically significant difference was detected between the logarithmic transformed concentration values of the two preparations at both sampling times ($P < 0.05$). Further analysis using the Tukey test showed that the plasma concentration of the test preparation was significantly higher ($P < 0.05$) than that of the reference preparation at 1.5 h. However, no significant difference ($P > 0.05$) was observed between the plasma levels of the two products at 3.0 h. The 90% confidence interval of the ratio for the concentration values of the β-cyclodextrin–artemisinin over those of the commercial preparation at 1.5 h was 1.34–2.22, while at 3.0 h the interval was 0.94–1.57. Thus, it appeared that the test preparation achieved a significantly higher plasma concentration compared with the reference preparation at 1.5 h, suggesting a faster rate of absorption.

The plasma artemisinin concentration of both sampling times showed wide intersubject variations, being consistent with the report by Alin et al (1996a). The coefficient of variation (CV) of the plasma concentrations at 1.5 and 3.0 h obtained with the commercial preparation were 115.7 and 76.3%, while the corresponding values for β-cyclodextrin–artemisinin complex were 66.9 and 67.7%. It is interesting to note that the CV of the test preparation at 1.5 h was markedly smaller than that of the reference preparation. Since at this sampling time, considerable absorption was still occurring, this would tend to suggest that the absorption of the β-cyclodextrin–artemisinin complex was more consistent and hence less variable compared with the reference preparation. On the other hand, at the sampling time of 3.0 h, absorption was deemed minimal or would have ceased (Wong & Yuen 2001), and the CV values of the two preparations were not only smaller (with respect to the reference preparation at 1.5 h) but also quite similar. The variability at this stage could be due more to the variability in disposition of the absorbed drug molecules rather than the absorption process.

From the results obtained, one patient from the commercial preparation-treated group and three patients from the β-cyclodextrin–artemisinin-complex-treated group were found to have parasites in their blood films, either on the second or third week of treatment. However, it was difficult to conclude if these cases were due to reinfection or recrudescence, because of the difficulties in differentiating the two (Alin et al 1996a, b). Most if not all the patients recruited for this study had returned to their place of residence where they were first infected, and hence re-infection could have taken place. The presence of gametocytes in the blood during the course of infection has also been reported as a risk factor for recrudescence (WHO 1998). However, gametocytaemia was recorded for one of the above four patients who had parasitaemia during the follow-up visits. As for the other patients, 38 had gametocytaemia when they were first recruited but none encountered recrudescence during their follow-up visits.

Conclusion

On the basis of the results obtained, it can be inferred that the β-cyclodextrin–artemisinin complex at a dose level of 150 mg artemisinin was therapeutically equivalent to the 250 mg dose of the commercial preparation. Falciparum malarial patients receiving either of the preparations were cleared of parasites and fever within five days, thus showing therapeutic success. In addition, comparable plasma artemisinin concentrations were observed between patients in both groups of treatments at 1.5 and 3.0 h, with slightly higher levels being obtained with patients in the β-cyclodextrin–artemisnin-complex-treated group. Additionally, patients receiving β-cyclodextrin–artemisinin complex showed less variability in their plasma artemisinin concentrations at the sampling time of 1.5 h after dosing, suggesting a more consistent rate of drug absorption. Moreover, there was no adverse drug reaction or toxicity reported with the administration of artemisinin in this study.

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