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Improved oral bioavailability of artemisinin through inclusion complexation with β- and γ-cyclodextrins

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Abstract

The bioavailability of β- and γ-cyclodextrin artemisinin complexes was evaluated in comparison with a normal commercially available preparation, Artemisinin 250®. Twelve healthy male volunteers participated in the study conducted according to a three-way crossover design. The bioavailability was compared using the parameters, total area under the plasma level–time curve ($AUC_{0-\infty}$), peak plasma concentration (C_{max}), and time to reach peak plasma concentration (T_{max}). A statistically significant difference was observed between the values of the complexes and Artemisinin 250® for the three parameters. However, no statistically significant difference was observed between the values of the β- and γ-cyclodextrin complexes. Moreover, the 90% confidence interval for the ratio of the $AUC_{0-\infty}$ values of the β-cyclodextrin complex over those of Artemisinin 250® was estimated to be between 1.51–2.04, while that of C_{max} was between 1.73–2.93. For the γ-cyclodextrin complex, the respective intervals were 1.30–1.76 and 1.43–2.43. These findings indicated that the β- and γ-cyclodextrin complexes had a much higher rate and extent of bioavailability compared to Artemisinin 250®. In addition, the absorption of artemisinin was observed to be poor and negligible when the preparations started to arrive in the colon. This could be attributed to poor dissolution of artemisinin in the semi-solid faecal matter in the lower part of the gastrointestinal tract.

Keywords: Artemisinin; β-cyclodextrins; γ-cyclodextrins; Bioavailability

1. Introduction

Artemisinin is a potent antimalarial drug isolated from the traditional Chinese medicinal herb, *Artemisia annua* (Luo and Shen, 1987; Klayman, 1985). It is a fast acting blood schizonticide with shorter parasite clearance time, leading to rapid symptomatic relief of malarial infections (Qinghaosu Antimalaria Coordinating Research Group, 1979). It is also effective against multidrug resistant strains of *Plasmodium falciparum* and produces rapid recovery even in patients with cerebral malaria (World Health Organization, 1994).

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

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lead to incomplete clearance of the parasites resulting in recrudescence (Titulaer et al., 1991). Recently, Wong (2001) has demonstrated that its solubility could be increased through inclusion complexation with β-cyclodextrin and γ-cyclodextrin at a molar ratio of 1:1. The artemisinin complexes were shown to display faster rate and higher extent of dissolution compared to the normal form of the drug. Also, the saturation solubility of artemisinin in the complexed form was found to increase approximately two-fold. Thus, the present study was conducted to determine the comparative bioavailability of the β- and γ-cyclodextrin complexes of artemisinin in comparison with that of a commercially available product containing the normal form of the drug, using 12 healthy human volunteers.

An attempt was also made to estimate the orocaecal transit time of the three preparations, with the aim of examining the absorption of artemisinin in the upper and lower part of the gastrointestinal tract, namely the small intestine and the colon. The orocaecal transit time was estimated using sulfasalazine as the marker drug. Sulfasalazine, when administered orally, is not absorbed in the small intestine. It is hydrolyzed by the microflora of the large bowel to produce sulfapyridine and 5-aminosalicylic acid. Measurement of the absorbed sulfapyridine in the plasma can then be used to estimate the orocaecal transit time (Kennedy et al., 1979; Staniforth et al., 1987; Kellow et al., 1986; Gramatté and Terhaag, 1991).

2. Materials and methods

2.1. Materials

The artemisinin preparations were:

- Artemisinin 250® capsule (Mekophar, Vietnam) with batch number VNA 1350-98. Each capsule contained a labelled dose of 250 mg artemisinin. Manufacturing date: May 1998, expiry date: May 2001.
- β-cyclodextrin and γ-cyclodextrin complexes were obtained from scale up batches. The weight of β-cyclodextrin complex containing an equivalent of 250 mg artemisinin was 1.25 g. For γ-cyclodextrin complex, the equivalent weight was 1.40 g. Both complexes were administered using four size 0 hard gelatin capsules (Su-Heung, Korea).

Artemisinin standard was obtained from Aldrich, USA, while the sulfasalazine and sulfapyridine standards were obtained from Sigma, USA. Dihydroartemisinin was synthesized by reduction of artemisinin with sodium borohydrate (Brossi et al., 1988).

2.2. Preparation of β- and γ-cyclodextrin complexes

The cyclodextrin complexes were prepared using a slurry method at a molar ratio of 1:1 (cyclodextrins: artemisinin). In the preparation of the β-cyclodextrin complex, 4.0 kg of the cyclodextrin was first mixed with 4.0 kg of water until a homogenous suspension was obtained. One kilogram of artemisinin, which had been previously ground and passed through a sieve size of 300 μm, was then added into the suspension. The mixture was stirred in a gear driven cake mixer for 24 h at ambient temperature (26–28 °C) before being dried in an oven equipped with an extraction fan at 40 °C for approximately 5 h until the moisture content was less than 10% (approximately 9%). The γ-cyclodextrin complex was prepared using the same procedure but the amounts of γ-cyclodextrin, artemisinin and water used were 4.0, 0.9 and 4.0 kg, respectively. The resulting complexes were then sieved through a 250 μm mesh.

2.3. In vivo study design

The study was approved by a Joint School of Pharmaceutical Sciences, USM–General Hospital Penang Committee on Clinical Studies. Twelve healthy human volunteers between 22 and 44 years old (Mean ± SD., 33 ± 8 years) and weighing from 60 to 87 kg (Mean ± SD., 70 ± 8 kg) participated in a three-period, three sequence crossover study after providing written informed consent. All were judged to be healthy with no current medical conditions and were not receiving any medication prior to and during the study period. The volunteers were randomly divided into three groups of four each, and administered the preparations according to the schedule shown below:

Group	Period		
	I	II	III
1	Artemisinin 250	β-Cyclodextrin complex	γ-Cyclodextrin complex
2	β-Cyclodextrin complex	γ-Cyclodextrin complex	Artemisinin 250
3	γ-Cyclodextrin complex	Artemisinin 250	β-Cyclodextrin complex

All three preparations were administered after an overnight fast of at least 10 h with 150 ml of water. In addition, 250 mg of sulfasalazine (Sigma Chemical Co, USA) packed in a size 0 capsule (Su-Heung, Korea) was also administered concurrently to all the volunteers during each phase of the study. Food and drinks were withheld for at least 2 h after dosing. Standardized lunch and dinner comprising chicken with rice were served at 4 and 10 h after dosing. All subjects were ambulatory during the clinical trial but were prohibited from strenuous activity and consuming alcoholic drinks. A washout period of one week was allowed between each study period.

Blood samples of 8 ml volume were collected in vacutainers containing sodium heparin as anticoagulant (Becton Dickinson, USA) at 0 (predose), 0.3, 0.7, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 14 and 18 h after dosing via an in-dwelling cannula (Venofix, BBraun, Germany) placed in the forearm. The blood samples were centrifuged for 20 min at 2500 rpm and the plasma transferred to glass containers to be kept frozen until analysis.

2.4. Analysis for plasma levels of artemisinin and sulfapyridine

Plasma levels of artemisinin were determined using a high performance liquid chromatography (HPLC) method with electrochemical detection reported by Chan et al. (1997) while the sulfapyridine concentrations in the plasma samples were analysed using a method by Yuen et al. (1997). The artemisinin assay method has a limit of quantification of 12.5 ng/ml, while the within day accuracy (denoted by percent error) and precision (denoted by coefficient of variation) were all less than 7%. For between day assay, the respective values were all less than 3%. In the case of the sulfapyridine assay, the limit of quantification was 0.7 μg/ml, while the respective within day and between day percent error and coefficient of variation were all less than 7%.

2.5. Pharmacokinetic analysis

The three preparations were compared using the parameters, total area under the plasma concentration–time curve ($AUC_{0-\infty}$), peak plasma concentration (C_{max}) and time to reach peak plasma concentration (T_{max}). The parameters C_{max} and T_{max} were obtained directly from the plasma concentration data (Weiner, 1981), while $AUC_{0-\infty}$ was obtained by adding the area from time zero to the last sampling time (AUC_{0-t}) and the area from the last sampling to infinity ($AUC_{t-\infty}$). The former was calculated using the trapezoidal formula, and the latter by dividing the last measurable plasma concentration with the elimination rate constant, k_e. The k_e value was estimated from the terminal slope of the plasma concentration versus time plot through logarithmic transformation of the concentration values and application of linear regression (Gibaldi and Perrier, 1982). The apparent volume of distribution, V_d/f, was calculated as dose/($AUC_{0-\infty}.k_e$).

In addition, the in vivo absorption profiles of artemisinin from administration of the two complexes and the reference preparation were estimated from the individual plasma concentration versus time data. For those plasma concentration profiles that were better fitted to a one-compartment pharmacokinetic model, the Wagner–Nelson method (Wagner and Nelson, 1964) was used in the calculation, whereas for those that fitted a two-compartment pharmacokinetic model, the Loo–Riegelman method (Loo and Riegelman, 1968) was employed instead. For the latter method, the microconstants k_{12}, k_{21} and k_{13} were estimated using a nonlinear least square fitting procedure, NONLIN (Argenio & Schumitzky, 1997). The initial estimates required in the NONLIN procedure were calculated using the method of residuals or feathering method (Wagner, 1975). The complexity of the Loo–Riegelman calculation made it necessary to use a computer program (Wagner, 1975) for executing the calculations. The k_eAUC and k_{13}AUC values obtained with the β-cyclodextrin complex were used as the denominator values in the respective Wagner–Nelson and Loo–Riegelman equations during the calculation of the absorption profiles of all three preparations. This is necessary because both methods would yield a final absorption value of 100% absorption even though the relative extent of bioavailability among the three products might be different (Welling et al., 1982).

2.6. Orocaecal transit time

The plasma sulfapyridine levels were used to estimate the time taken for the products to arrive at the caecum. This was carried out by extrapolating the initial portion of the serum sulfapyridine concentration curves to the time axis (Peh and Yuen, 1996).

2.7. Statistical analysis

All the results were expressed as mean ± standard deviation, SD. For each of the parameters, $\text{AUC}_{0-\infty}$, $C_{\max}$, k_e, V_d/f and orocaecal transit time, the values obtained for the three preparations were analyzed statistically using an analysis of variance procedure (ANOVA) which distinguished effects due to group, subjects/group, period and treatment (Wagner, 1975). The $\text{AUC}_{0-\infty}$ and $C_{\max}$ values were logarithmic transformed before analysis. If a statistically significant difference was obtained using the ANOVA, a post-hoc Tukey test was employed to locate the pair of preparations that gave rise to the difference observed. On the other hand, the $T_{\max}$ values were analyzed using the Friedman test, followed by a multiple comparison test if a statistically significant difference was observed (Zar, 1984). A statistically significant difference was considered at $P < 0.05$.

3. Results and discussion

The mean plasma artemisinin concentration versus time profiles obtained with the two complexes and the reference preparation are shown in Fig. 1. All three profiles showed a rapid increase in plasma artemisinin concentration after dosing, being typical of conventional immediate release formulations. It is also apparent from the plots that the two complexes achieved much higher plasma drug levels, with peak plasma concentrations approximately twice that of the reference preparation, thus indicating a higher rate and extent of artemisinin absorption.

The individual numerical values of $\text{AUC}_{0-\infty}$, $C_{\max}$ and $T_{\max}$ obtained with the three preparations are given in Table 1. The parameters $T_{\max}$ and $\text{AUC}_{0-\infty}$ are indicative of the respective rate and extent of drug absorption, whereas $C_{\max}$ is related to both processes (Grahnén, 1984). A statistically significant difference was observed among the logarithmic transformed $C_{\max}$ values ($P < 0.0001$) as well as among the logarithmic transformed $\text{AUC}_{0-\infty}$ values ($P < 0.0001$) of the three preparations. Further analysis using the Tukey test revealed a statistically significant difference ($P < 0.05$) between the values of the β-cyclodextrin complex and Artemisinin 250® for both parameters. Similarly, a statistically significant difference ($P < 0.05$) was also observed between the values of the γ-cyclodextrin complex and the reference preparation in both parameters. Between the two complexes, however, the values of the $C_{\max}$ as well as $\text{AUC}_{0-\infty}$ were not significantly different ($P > 0.05$). In addition, the 90% confidence interval for the ratio of the $\text{AUC}_{0-\infty}$ values of the β-cyclodextrin complex over those of Artemisinin 250® was estimated to be between

Table 1
Individual numerical values of $AUC_{0-\infty}$, C_{max} and T_{max} obtained with the three preparations

Volunteers	Artemisinin 250®			β-Cyclodextrin complex			γ-Cyclodextrin complex		
	$AUC_{0-\infty}$ (ng.h/ml)	C_{max} (ng/ml)	T_{max} (h)	$AUC_{0-\infty}$ (ng.h/ml)	C_{max} (ng/ml)	T_{max} (h)	$AUC_{0-\infty}$ (ng.h/ml)	C_{max} (ng/ml)	T_{max} (h)
1	551.1	240.5	1.0	720.8	353.5	0.7	721.0	326.7	1.0
2	1062.5	385.3	2.0	1323.5	551.8	1.5	1126.4	685.7	1.0
3	1021.7	225.7	3.0	2025.4	577.3	2.5	2143.0	787.4	1.0
4	728.2	255.5	1.5	2199.9	2434.3	0.7	1235.5	455.2	0.7
5	566.1	191.5	2.5	993.1	437.3	2.0	924.7	356.1	2.0
6	528.5	140.8	2.0	612.5	236.0	0.7	657.1	340.7	1.0
7	986.6	641.8	1.5	1763.9	592.3	1.0	1548.2	593.0	1.5
8	404.2	126.6	2.0	895.4	365.9	2.5	721.1	299.8	1.5
9	440.2	150.5	2.5	763.5	262.4	2.5	620.9	237.9	2.5
10	287.9	82.0	2.5	1219.7	473.1	2.0	1004.2	384.9	0.7
11	1226.5	254.1	1.0	1379.2	428.9	2.0	1343.9	341.4	2.5
12	1584.2	565.8	2.5	2055.5	1104.9	1.0	1529.8	687.9	1.5
Mean	782.3	271.7	2.0	1329.4	651.5	1.6	1131.3	458.1	1.4
SD	392.0	174.6	0.6	562.4	604.9	0.8	456.6	182.2	0.6

1.51 and 2.04, while that of C_{max} was between 1.73 and 2.93. For γ-cyclodextrin complex, the respective intervals were 1.30–1.76 and 1.43–2.43. Thus, the results above indicated that both complexes had a much higher rate and extent of bioavailability compared to Artemisinin 250®.

The numerical values of the pharmacokinetic parameters, k_e and V_d/f obtained with the three preparations are given in Table 2. There was no statistically significant difference among the k_e ($P = 0.9653$) values of the three products. Moreover, the values obtained were comparable to those reported in the literature (Ashton, Gordi et al., 1998; Ashton, Sy et al., 1998; Titulaer et al., 1990). While no statistically significant difference was observed between the V_d/f values of the two complexes, they were significantly different ($P = 0.0003$) from those of the reference preparation. This could be explained by the fact that both test preparations were more bioavailable. A larger f value (fraction of dose absorbed) in the term V_d/f obtained with the complexes would result in smaller volume of distribution to be calculated.

The mean in vivo artemisinin absorption versus time profiles of the three preparations are presented in Fig. 2. In accord with the plasma artemisinin versus time profiles shown in Fig. 1, the plots in Fig. 2 show that absorption of artemisinin from both test preparations was faster and considerably higher than that of the reference product, the Artemisinin 250®. Also, while no absorption lag time was observed with the complexes, an absorption lag time of approximately 0.3 h was observed with the reference product. A comparison of the two complexes also revealed that their extents of absorption were essentially comparable, although the initial absorption from the γ-cyclodextrin complex was slightly faster. It can also be inferred from Fig. 2 that all the three plots reached a plateau at approximately 3 h after dosing, suggesting that the duration of absorption of artemisinin from all three products was relatively short.

The individual orocaecal transit time (OTT) estimated from measuring the plasma sulfapyridine levels are shown in Table 3. In general, the intervals between the time of drug administration and the time when sulfapyridine was first detected in the blood were relatively short. The mean values of OTT for the reference product, the β-cyclodextrin complex and the γ-cyclodextrin complex were 3.1, 2.8 and 2.7 h, respectively. No

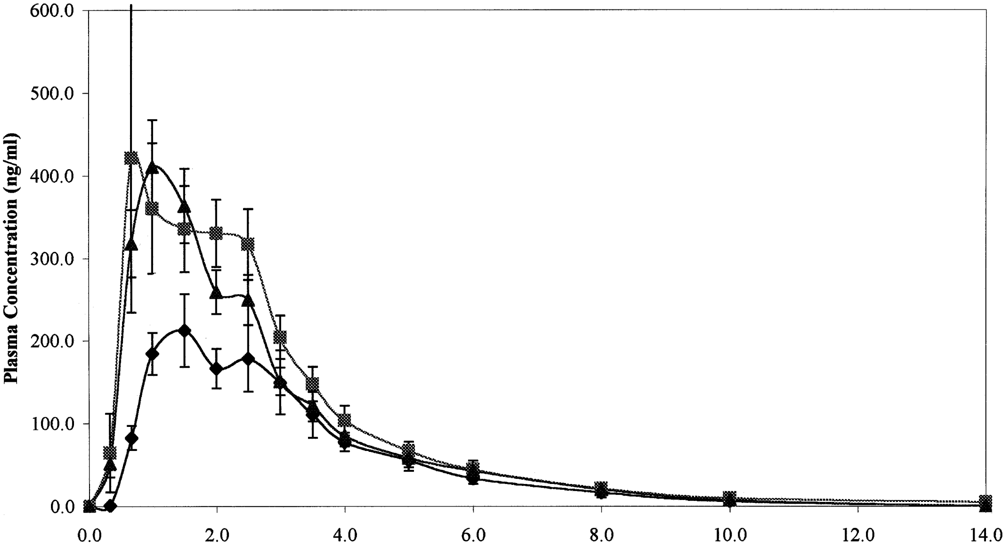

Fig. 1. Mean plasma artemisinin concentration versus time profiles. Mean ± SEM (vertical bar). $N = 12$. ◆ = Artemisinin 250®, ■ = β-cyclodextrin complex, ▲ = γ-cyclodextrin complex.

Table 2
Individual numerical values of k_e and V_d/f obtained with the three preparations

Volunteers	Artemisinin 250®		β-cyclodextrin complex		γ-cyclodextrin complex	
	k_e (h^{-1})	V_d/f (l/kg)	k_e (h^{-1})	V_d/f (l/kg)	k_e (h^{-1})	V_d/f (l/kg)
1	0.489	14.3	0.587	9.1	0.375	14.2
2	0.393	8.0	0.396	6.4	0.315	9.4
3	0.209	16.9	0.335	5.4	0.279	6.1
4	0.299	20.1	0.211	9.4	0.265	13.4
5	0.463	12.2	0.531	6.1	0.468	7.4
6	0.607	9.0	0.499	9.4	0.814	5.4
7	0.303	13.9	0.355	6.7	0.214	12.6
8	0.354	18.2	0.415	7.0	0.623	5.8
9	0.771	9.0	0.941	4.2	0.729	6.7
10	0.726	15.0	0.540	4.7	0.627	5.0
11	0.328	12.4	0.309	11.7	0.338	11.0
12	0.661	4.1	0.427	4.9	0.625	4.5
Mean	0.467	12.8	0.462	7.1	0.473	8.5
SD	0.185	4.6	0.186	2.3	0.203	3.5

statistically significant difference ($P > 0.05$) was observed among the values of the three products. Detection of sulfapyridine in the blood signals the start of the arrival of the preparations in the colon. However, overgrowth of colonic bacteria in the small bowel may underestimate the OTT values (Kennedy et al., 1979). Nevertheless, the method provides a simple and economical method of estimating the orocaecal transit time.

Referring to Fig. 2, it can be observed that the estimated mean orocaecal transit times were quite close to the times when drug absorption started to cease and the plots reached a plateau, suggesting that absorption of artemisinin was poor and neg-

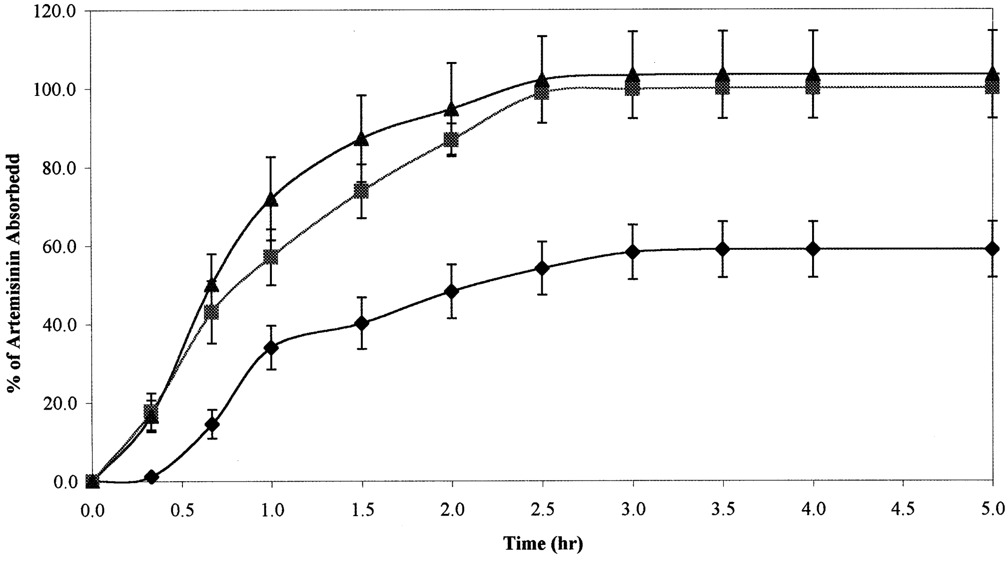

Fig. 2. In-vivo mean percentage of artemisinin absorbed versus time profiles. Mean ± SEM (vertical bar). $N = 12$. ◆ = Artemisinin 250®, ■ = β-cyclodextrin complex, ▲ = γ-cyclodextrin complex.

Table 3
Individual numerical values of orocaecal transit time (OTT) obtained with the three preparations

Volunteers	Artemisinin 250® OTT (h)	β-Cyclodextrin complex OTT (h)	γ-Cyclodextrin complex OTT (h)
1	1.70	2.50	4.00
2	3.50	2.60	3.50
3	5.10	3.30	2.60
4	2.50	2.50	2.50
5	3.20	2.20	4.00
6	4.00	3.50	1.30
7	2.50	2.10	2.30
8	1.30	3.30	1.80
9	3.70	3.20	3.80
10	3.70	1.70	2.00
11	1.80	1.80	1.00
12	3.80	4.80	4.00
Mean	3.10	2.80	2.70
SD	1.10	0.90	1.10

ligible when the preparations reached the lower part of the gastrointestinal tract, such as in the colon, being consistent with the observation of Jinadasa (1996). This could be due to poor dissolution of the drug in the semi-solid contents of the lower part of the gastrointestinal tract (Hirtz, 1984). Thus, increasing the solubility of artemisinin, such as through inclusion complexation with cyclodextrins, as used in the present study, might ensure better drug dissolution in the upper regions of the gastrointestinal tract, leading to higher extent of bioavailability. The increase in oral bioavailability of the artemisinin complexes could also in part be due to the saturable nature of the enzymes involved in the first pass metabolism of artemisinin (Ashton, Gordi et al., 1998). Since the complexes exhibited more rapid dissolution and hence absorption, this could lead to enzyme saturation during first pass in the liver, resulting in a higher fraction of unmetabolized drug entering the systemic circulation, compared to the reference preparation, which has a slower rate of drug dissolution.

4. Conclusion

Both β- and γ-cyclodextrin complexes of artemisinin exhibited a higher rate and extent of artemisinin absorption compared to the reference preparation. Also, the absorption of artemisinin appeared to be poor and negligible when the preparations started to arrive in the colon, which could be attributed to poor dissolution of artemisinin in the semi-solid contents of the lower part of the gastrointestinal tract.

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