Artemether/hydroxypropyl-β-cyclodextrin host–guest system: Characterization, phase-solubility and inclusion mode

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A B S T R A C T

An inclusion complex of the antimalarial artemether (ATM) in hydroxypropyl-β-cyclodextrin (HPβCD) was prepared and characterized. The phase-solubility diagram for the drug showed an increase in water solubility and gave an apparent binding constant of 220 M\textsuperscript{-1}. According to \textsuperscript{1}H NMR and 2D NMR spectroscopy (ROESY), the inclusion mode involves two CH\textsubscript{3} from the drug orientated in the HPβCD cavity. The complex was characterized by Powder X-ray diffraction and thermal analysis. In addition, the complex produces a 1.81-fold enhancement in apparent bioavailability compared to artemether.

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1. Introduction

Malaria has a devastating effect throughout tropical regions. There are approximately 300–500 million clinical cases each year resulting in 1.5–2.7 million deaths. Nearly all fatal cases are caused by \textit{Plasmodium falciparum}\textsuperscript{1}. The problem is compounded by the spread of drug resistant strains of the parasite. As a result, traditional alkaloid drugs such as chloroquine and quinine are now largely ineffective\textsuperscript{2}. The spread of parasite resistance has led the World Health Organization (WHO) to predict that without new antimalarial drug intervention, the number of cases of malaria will have doubled by the year 2010\textsuperscript{3}. Artemisinin (Qinghaosu) is a sesquiterpene 1,2,4-trioxane (sesquiterpene lactone endoperoxide) isolated from the Chinese medicinal herb qinghao (\textit{Artemisia annua} L.). In 1979 it was shown to be an effective antimalarial against chloroquine-resistant strains of \textit{P. falciparum}\textsuperscript{4}. This compound and its derivatives, such as artemether (ATM), dihydroartemisinin, arteether, and artesunate, are effective against both chloroquine-resistant and chloroquine-sensitive strains of \textit{P. falciparum}, as well as against cerebral malaria\textsuperscript{5,6}. Most countries where malaria is endemic have adopted the WHO recommendation of artemisinin combination therapy (ACT) for fast and reliable malaria treatment\textsuperscript{7}. However, artemisinin’s poor solubility in both oil and water, and hydrolytic instability of the lactone function, have led scientists to prepare a series of semisynthetic first generation analogues, such as ATM (Chart 1).\textsuperscript{8} Although ATM is a potent antimalarial, poor bioavailability and rapid clearance are observed with it and the other derivatives in both human and animal models\textsuperscript{9}.

Cyclodextrins (CDs) are truncated-cone polysaccharides mainly composed of six to eight D-glucose monomers linked by \textit{α}-1,4-glucosidic bonds. They have a hydrophobic central cavity and hydrophilic outer surface and can encapsulate model substrates to

![Chart 1. The structure of ATM.](image-url)
form host–guest complexes or supramolecular species. This usually enhances drug solubility in aqueous solution and affects the chemical characteristics of the encapsulated drug.\textsuperscript{10–13} Hydroxypregly-\(\beta\)-cycloextrin (HP\(\beta\)CD, Chart 2) is a hydroxalkylated \(\beta\)CD derivative that combines relatively high water solubility with low toxicity and satisfactory inclusion ability.\textsuperscript{14,15} Several commercial formulations are composed of cycloextrin inclusion complexes, illustrating the usefulness of this approach.\textsuperscript{16–19}

The latest research indicates that HP\(\beta\)CD complexation with dihydroartemisinin increases dihydroartemisinin solubility and concentration.\textsuperscript{14,15} Several commercial formulations are composed of HP\(\beta\)CD/ATM complexes, illustrating the usefulness of this approach.\textsuperscript{16–19}

In order to explore the possible inclusion mode of the HP\(\beta\)CD/ATM complex, we compared the \(\textsuperscript{1}H\) NMR spectra of HP\(\beta\)CD in the absence and presence of ATM (Fig. 2). The \(\textsuperscript{1}H\) resonances of HP\(\beta\)CD were assigned according to the reported method.\textsuperscript{24,25} As illustrated in Figure 2, the majority of ATM chemical shifts were between \(\delta\) 0.5 and 3 ppm and distinct from those of the HP\(\beta\)CD protons. After inclusion complexation with ATM, the H-3 proton of HP\(\beta\)CD shifted 0.010 ppm and the H-5 proton of HP\(\beta\)CD shifted 0.002 ppm (Table 1). Both H-3 and H-5 protons are located in the interior of the CD cavity, with H-3 protons near the wide side of cavity and H-5 protons near the narrow side. These results may indicate that ATM should be included in the HP\(\beta\)CD cavity from the wide side.

Two-dimensional (2D) NMR spectroscopy provides important information about the spatial proximity between host and guest atoms by observation of intermolecular dipolar cross-correlations. Two protons closely located in space can produce a nuclear Overhauser effect (NOE) cross-correlation in NOE spectroscopy (NOESY) or ROESY. The presence of NOE cross-peaks between protons from two species indicates spatial contacts within 0.4 nm.\textsuperscript{20} To gain more conformational information, we obtained 2D ROESY of the inclusion complex of ATM with HP\(\beta\)CD (Fig. 3), including a partial contour plot (Fig. 3, inset). The ROESY spectrum of the HP\(\beta\)CD/ATM complex shows appreciable correlation of H-13 and H-14 protons of ATM with H-3 protons of HP\(\beta\)CD. No correlation is observed between H-5 and H-15 protons of ATM and H-5 or H-3 protons of the cycloextrim. These results indicate that the CH3 of the A and B rings of ATM are included in the HP\(\beta\)CD cavity. In combination with the 1:1 inclusion stoichiometry observed in the phase-solvability diagram, a possible inclusion mode for the HP\(\beta\)CD/ATM complex is proposed (Fig. 4).

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### 2.2. Inclusion mode

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### 2.3. X-ray diffraction of the inclusion complex

Powder XRD patterns allow examination of the medium and long range order of materials.\textsuperscript{26} In contrast to the amorphous character of HP\(\beta\)CD (Fig. 5a), free ATM is a crystalline solid (Fig. 5b). The XRD pattern of the physical mixture confirmed the presence of both species as isolated solids, as the diffractogram showed both ATM peaks and the amorphous halo of HP\(\beta\)CD (Fig. 5c). The lyophilized inclusion complex has an amorphous structure (Fig. 5d), probably due to both the structure of HP\(\beta\)CD and the lyophilization process; this is evidence of the absence of ATM crystalline particles.

### 2.4. Thermal analysis of the inclusion complex

The thermal properties of the HP\(\beta\)CD/ATM complex were investigated by thermogravimetric analysis (TG) and differential scanning calorimetry (DSC). Analysis of the TG curves showed that ATM decomposes at ca. 170 °C (Fig. 6a) and HP\(\beta\)CD at ca. 360 °C (Fig. 6b). However, their inclusion complex had different thermal stability, with a decomposition temperature of ca. 365 °C (Fig. 6d). In contrast, the physical mixture of HP\(\beta\)CD and ATM apparently contains only the free species, as indicated by decomposition temperatures due to ATM at 170 °C and HP\(\beta\)CD at 360 °C (Fig. 6c). These results indicate that ATM's usual thermal properties were altered after inclusion complexation.
The differential scanning calorimetry (DSC) thermogram provides further information about the thermal properties of the HP\(\beta\)CD/ATM complex. The DSC curve of ATM displays an exothermic peak at 170 °C (Fig. 7a). In contrast, the DSC curve of pure HP\(\beta\)CD shows endothermic peaks at 80 and 360 °C (Fig. 7b), indicating HP\(\beta\)CD loses water at temperatures slightly above 80 °C and decomposes above 360 °C. The physical mixture of HP\(\beta\)CD and ATM apparently contains only the free species (Fig. 7c).

Figure 2. \(^1\)H NMR spectra of HP\(\beta\)CD in the absence and presence of ATM in D\(_2\)O and ATM in CDCl\(_3\) at 25 °C. (a) HP\(\beta\)CD, (b) HP\(\beta\)CD/ATM complex, (c) ATM (asterisk highlights the water peak, the window shows the enlarged NMR spectrum from approximately 0.5–3 ppm).
However, in the DSC curve of the HPβCD/ATM complex, the exothermic peak at about 170 °C corresponding to the free ATM disappears, while two new endothermic peaks appear at 80 and 365 °C (Fig. 7d). This suggests that the HPβCD/ATM complex is more stable than ATM. We propose that this result may be related to the complexation of HPβCD with ATM.

2.5. Bioavailability studies in the rats

The plasma concentration–time profiles of artemether are shown in Figure 8. The mean pharmacokinetic parameters derived from a non-compartmental analysis are presented in Table 2. Pharmacokinetic parameters obtained after parenteral administration of artemether suspension (n = 6) show a constant of $C_{\text{max}}$ of 218.78 µg/ml, $T_{\text{max}}$ of 89.32 min, and a $\text{AUC}_{0-480}$ of 62038.65 µg min/ml. $C_{\text{max}}$ of the complex (490.22 µg/ml) is higher than that of the suspension. This difference may be due to fast dissolution and absorption of the complex in the solution, a rapid and quantitative breakdown to yield high circulating concentrations once absorbed. On the other hand, $T_{\text{max}}$ is significantly lower for the complex (29.52 min) in comparison to the artemether suspension. There is some difference between the AUC of complex (112137.89 µg min/ml) and the suspension. The apparent bioavailability of artemether following complex administration was found to be 181% compared to the artemether suspension. Thus, the results above indicated that complex had a much higher rate and extent of bioavailability compared to artemether suspension.

3. Conclusion

In summary, the complexation behavior, characterization and bioavailability of an inclusion complex of ATM with HPβCD were investigated. Results showed that HPβCD could enhance the water-solubility and bioavailability of ATM. Considering the lack of ATM applications, the complex could prove useful in the design of novel medicinal ATM formulations.

4. Experimental

4.1. Materials

ATM (FW = 298, PC >99%) was obtained from Kunming Pharmaceutical Corporation (Yunnan Province, P R China). Hydroxypropyl-
β-cyclodextrin (average FW = 1380) was purchased from Sigma–Aldrich Chemical Corporation (Shanghai, P R China) and used as received. Other reagents and chemicals were of analytical reagent grade. All experiments were carried out using ultrapure water.

4.2. HPLC assay

An Agilent 1100 HPLC system was used to determine the amount of ATM. The Agilent HPLC system was equipped with G1311A pump and controller, G1315B UV absorption detector, and G1313A autosampler. A Lichrospher C18 HPLC column (Hannbon, 5 μm, 150 mm × 4.6 mm, CHA) was used for separation and the mobile phase was water–acetonitrile (40:60, v/v). The injection volume was 20 μl and the effluent with a flow rate of 1.0 ml/min was monitored at an absorption wavelength of 210 nm.

4.3. Preparation of inclusion complexes

The inclusion complex was prepared by the suspension method.27 This involved mixing of ATM and HPβCD in a 1:1 molar

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**Figure 4.** Possible inclusion mode of the HPβCD/ATM complex.

**Figure 5.** Powder X-ray diffractograms (Cu-Kα) for: (a) HPβCD, (b) ATM, (c) HPβCD/ATM 1:1 (mol proportion) physical mixture, (d) HPβCD/ATM inclusion complex collected by lyophilization.

**Figure 6.** TG curves for: (a) ATM, (b) HPβCD, (c) HPβCD/ATM 1:1 (mol proportion) physical mixture, and (d) HPβCD/ATM inclusion complex.

**Figure 7.** DSC curves for: (a) ATM, (b) HPβCD, (c) HPβCD/ATM 1:1 (mol proportion) physical mixture, and (d) HPβCD/ATM inclusion complex.
propotion with stirring at room temperature for 48 h protected from light to prevent degradation. The solid residue was then separated by centrifugation at 15,000 rpm for 15 min and the upper liquid layer was filtered over a 0.45 µm Millipore membrane. The solution was then dried by lyophilization and the resulting solid inclusion complex collected.

A physical mixture, to test for possible inclusion, was prepared by grinding together a 1:1 molar mixture of HP CDs and ATM for 5 min with a small amount of water (the minimum amount to form a slurry) in an agate mortar.

### 4.4. Phase-solubility diagram

The phase-solubility diagram was studied according to the method proposed by Higuchi and Connors. A series of HP CDs solutions were prepared with increasing concentrations: 0.01–0.25 M. A constant mass of ATM, in fivefold molar excess relative to the highest concentration HP CD solution, was added to each solution and the suspensions stirred for 48 h in the dark. Following this, all suspensions were centrifuged and the supernatants were filtered over 0.45 µm Millipore membranes and analyzed by HPLC.

### 4.5. Characterization of the complexes

\[ ^1H \text{ NMR spectra for HP)CD and ATM were obtained on a Bruker Avance DRX500 spectrometer at 298 K in D}_2\text{O and CDC}_{13}, \text{respectively. ROESY experiments were run on a Bruker Avance DRX500 instrument. Samples were equilibrated for at least 24 hrs before measurement. All 2D NMR experiments were carried out in D}_2\text{O. Powder X-ray diffraction (XRD) was measured in a D}^\text{max}-3B diffractometer using Cu-}K\alpha (\lambda = 1.5406 \text{ Å}) with 30 mA, 40 kV, and a scanning rate of 5°/min. Powder samples were mounted on a sample holder and scanned with a step size of 2\(\theta = 0.02^\circ \) between 2\(\theta = 3^\circ \) and 70°.

Thermal analyses (TG and DSC) were recorded using a NETZSCH STA449F3 instrument, with a 10 °C/min heating rate from room temperature to 500 °C and under N2 flow (100 ml/min).

### 4.6. Bioavailability studies in the rats

Bioavailability studies in the rats were performed according to the reported method. Formulation preparation: artemether (60 mg) were suspended in 10 ml of an aqueous solution containing 0.5% of sodium methylcellulose; Oral solution was prepared by dissolving 2460 mg of artemether/hydroxypropyl-β-cyclodextrin complex in 10 ml of ddwater to make a concentration 6 mg/ml of artemether. Rat experiment: Male Sprague–Dawley rats (weight range 280–300 g), after fasting overnight, were randomly treated the oral solution of artemether complex and artemether suspension (n = 6). The dosages were all 10.8 mg/day kg. Before blood sampling, the animals were anesthetized with diethyl ether. Blood samples of 0.4 ml were taken from the ophthalmic venous plexus and put into heparinized tubes at 12, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 420 and 480 min after administration. The blood was immediately centrifuged at 4000 rpm for 10 min and 200 µl of plasma was quickly removed and stored at –20 °C until HPLC analyses.

Treatment of plasma samples: A 200 µl portion of plasma was added 50 µl 1% phosphoric acid solution and was vortexed for 3 min and kept for 5 min. Methanol (0.4 ml) was added and the mixture was vortexed for 3 min, then was centrifuged at 12,000 rpm for 10 min. The organic phase was transferred into new tubes and the contents evaporated to dryness under a stream of air at approximately 40 °C. The dried extracts were reconstituted with 150 µl of mobile phase solution, vortexed at high speed for 3 min, and centrifuged again at 12,000 rpm for 10 min. The entire volume of the reconstituted material (150 µl) was transferred to autosampler vials and 50 µl (sample volume) was injected onto the HPLC.

Pharmacokinetics and statistical analysis: The plasma concentration–time data of artemether were fitted by 3P87 Pharmacokinetics Program (The Section of Mathematical Pharmacology of Chinese Mathematical Pharmacological Society) and the pharmacokinetic parameters were calculated. The area under the concentration–time curve (AUC\text{app}) of the artemether following administration of the complex was calculated by dividing the artemether AUC following complex dosing by that from suspension dosing. Statistical significance was indicated with \(P < 0.01\).

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### References and notes