Journal of Molecular Structure 1085 (2015) 90–96

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

Host–guest inclusion system of artesunate with β-cyclodextrin and its derivatives: Characterization and antitumor activity

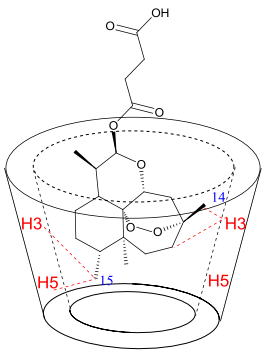
Hudie Xie [a], Bo Yang [a,*], Fen Wang [a], Yulin Zhao [b,*]

[a] Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, PR China
[b] Faculty of Chemical Engineering, Kunming University of Science and Technology, Kunming 650500, PR China

HIGHLIGHTS

- K_s and stoichiometry of inclusion complexes obtained from phase solubility study.
- The inclusion mode of complexes was proposed by NMR analyses.
- Displaying the significant enhancement of water soluble and thermal stability of artesunate.
- Antitumor activity of inclusion complexes was superior to that of artesunate.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 27 December 2014
Accepted 30 December 2014
Available online 6 January 2015

Keywords:
Artesunate
β-Cyclodextrin
Inclusion complex
Solubilization
Thermal stability

ABSTRACT

Inclusion complexes between artesunate (ATS) and three cyclodextrins, namely β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and sulfobutyl ether-β-cyclodextrin (SBE-β-CD), were prepared by a suspension method. The complexes in both liquid and solid were characterized by phase-solubility diagram, nuclear magnetic resonance (NMR), powder X-ray diffraction (XRD) and thermoanalysis. The results suggested that artesunate was partly encapsulated within the cyclodextrin cavity to form a 1:1 stoichiometry host–guest compound. Especially in the SBE-β-CD complex, displayed the greatest stability constant. Significant enhancement of water solubility and thermal stability of ATS in present of β-CDs was shown. The calculated IC_{50} values indicated that the antitumor activities of inclusion complexes were better than that of ATS. Satisfactory aqueous solubility, along with high thermal stability of inclusion complexes will be potentially useful for their application on the formulation design of natural medicine.

Introduction

Malaria is one of the most serious health problems facing the developing world, killing up to 2.5 million people every year (i.e. about 5% of all world-wide deaths are directly caused by malaria) [1]. The vast majority of cases are occurred in African children under five years old (who have frequently been attacked, along with little immunological protection) and pregnant women, estimated at 200–500 million cases per year with 0.7–2.7 million deaths [2]. Moreover, the rapid spread of parasites is aggravated by economic backwardness in many malarious areas. Although the burden of malaria as well as the focus of its control are mainly in Africa, which is still an important public health problem in other tropical areas such as India, Indonesia, Papua New Guinea and the Amazon region of Latin America [3]. It is obvious that research and

* Corresponding authors. Tel.: +86 13064281879.
E-mail addresses: yangbo6910@sina.com (B. Yang), zhaoyulin919@sina.com (Y. Zhao).

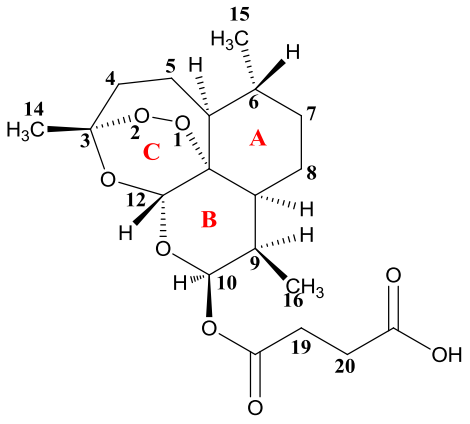
http://dx.doi.org/10.1016/j.molstruc.2014.12.087
0022-2860/

promotion of drug against malaria are a considerable challenge to us.

In response to drug resistance of the widely available antimalarials used as monotherapy, artemisinin-based combination therapy has first been introduced in Asia and now throughout Africa and South America [4]. The artemisinin-based compounds in the recent therapy are either of the parent compound artemisinin, which extracted from the leaves of *Artemisia annua*, and semi-synthetic derivatives such as dihydroartemisinin, artemether, and artesunate. All of these artemisinin derivatives retain the necessary endoperoxide bridge against malaria. Meanwhile derivatization of artemisinin was optimized in terms of the parent properties. For example, sodium artesunate, the more water-soluble derivative, has significant pharmacokinetic advantage over lipid-soluble relatives (such as artemether and arteether) and can be administered by intravenous injection. So the compound is particularly used in the treatment of cerebral malaria and critical to restore the patients' consciousness [5]. However, the utility of sodium artesunate is limited by its poor stability in aqueous solution due to the ease of hydrolysis of the ester linkage [6].

Artesunate (ATS, Scheme 1), is a water soluble derivative of the lactonic sesquiterpenoid compound extracted from artemisinin, a 1,2-α-succinate of dihydro-artemisinin, with a molecular formula of $C_{19}O_8H_{28}$ and molecular weight of 384.43. It is a new anti-malrial drug of the first class innovated in China, also possessing the effect of killing such pathogenic microbes as toxoplasma and *Schistosoma japonicum* [7]. Its anti-tumor effect has been reported in recent years [8], and its effect of antagonizing angiogenesis in tumors has, in particular, raised extensive attention [9]. However, the water-soluble artesunate is rapidly hydrolysis to dihydroartemisinin and the rate of chemical oxidation of artesunate is pH dependent, this explains its rapid conversion to dihydroartemisinin in the stomach, as compared to its greater stability in other compartments at high pH and in plasma [10]. Poor stability and low bioavailability following oral administration were brought about difficulties in formulation, limits in the therapeutic applications as well as bioavailability [11–13]. Under the current situations, designing a novel formulation to improve its biopharmaceuticals properties will be of great significance.

It is well known that cyclodextrins (CDs) are truncated-cone oligosaccharides mainly composed of six to eight D-glucose monomers linked by α-1, 4-glycosidic bonds. They possess the hydrophobic central cavity and hydrophilic outer surface, thus can encapsulate model substrates (such as small molecules, ions, proteins and oligonucleotides) to form inclusion complex or supramolecular system, which is visually called "molecular capsule" [14]. In general, it can enhance drug solubility in aqueous solutions and affect the chemical characteristics of encapsulated drug [15,16]. This feature of cyclodextrin cavity enables them to be successfully utilized as drug carriers, enzyme mimics and so on, but applications of unmodified or unsubstituted β-CD in the pharmaceutical field are limited due to its poor water solubility and nephrotoxicity [17]. Hence, modified and relatively stable β-CDs have been largely synthesized and widely used in parenteral formulations, such as hydroxypropyl-β-cyclodextrin (HP-β-CD) and sulfobutyl ether-β-cyclodextrin (SBE-β-CD) (Scheme 2) [18].



Scheme 1. Structure of artesunate.

In previous study, great effort has been made to improve water solubility, stability and bioavailability of artemisinin drugs, such as artemisinin nanocapsules, inclusion complexes of dihydroartemisinin and artemisinin with cyclodextrins [19–22]. In addition, we have prepared and characterized the inclusion complex between artemether and HP-β-CD, the complex produces a 1.81-fold enhancement in apparent bioavailability compared to artemether [23]. As a continuation of our studies on natural medicines/cyclodextrin inclusion complexes, inclusion complexes of ATS with β-CDs were investigated. In this paper, we have made the evaluation of the inclusion process between ATS and β-CDs by phase-solubility diagram. Furthermore, we have characterized the solid inclusion complexes by means of ^{1}H NMR and 2D NMR spectroscopy, Powder X-ray diffraction and thermal analysis. Moreover, our special interest in exploring the binding behaviors of β-CDs with ATS and solubilization of β-CDs toward ATS, which would provide an effective method to obtain the novel ATS-based formulation with high water solubility and bioavailability.

Materials and methods

Reagents and materials

Artesunate (ATS, MW = 384.43, PC > 98%) was obtained from Kunming Pharmaceutical Corporation in Yunnan Province, PR China. Sulfobutyl ether-β-cyclodextrin (SBE-β-CD) was synthesized according to the literature [24]. Hydroxypropyl-β-cyclodextrin (HP-β-CD, averaged MW ≈ 1541, DS = 7) was commercially available and used without further purification. Other reagents and chemicals were of analytical reagent grade. All experiments were carried out using ultrapure water.

Preparation of inclusion complexes

The solid inclusion complexes between ATS and β-CDs were prepared by a suspension method [23]. Briefly, ATS (0.04 mmol) and β-CDs (0.01 mmol) were added to 15 mL of ultrapure water, and the suspension was stirred for 5 days at room temperature in the dark. Uncomplexed ATS was filtered by 0.45 μm Millipore membrane, the filtrate was evaporated under reduced pressure to remove the solvent and dried in vacuum to give the ATS/β-CDs complexes.

Physical mixtures (to test for possible inclusion), were prepared by grinding together a 1:1 molar mixture of ATS and β-CDs in an agate mortar for 10 min. Finally, the obtained physical mixture was dried in vacuum.

Phase-solubility diagram

The phase-solubility diagram was studied according to the method proposed by Higuchi and Connors [25]. A series of CDs solutions with increasing concentrations: 5–20 mM were prepared in water (unbuffered pH = 7.4). A constant mass of ATS, in 4-fold molar excess relative to the highest concentration of CDs solutions, was added to each solution and the suspensions stirred for 48 h in the dark. Following this, all suspensions were centrifuged and the