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Artemisinin, the Nobel Prize Winner Drug in Cyclodextrin-Enabled Formulations

The 2015 Nobel Prize in Physiology and Medicine was awarded to Prof. Youyou Tu for the discoveries concerning a novel therapy against malaria shared with W.C. Campbell and S. Omura for their discoveries concerning novel therapy against infections caused by roundworm parasites (the next Cyclodextrin News editorial will be dedicated to their discoveries). Prof. Tu, professor at the China Academia of Traditional Chinese Medicine, discovered artemisinin, a drug that has significantly reduced the mortality rates for patients suffering in malaria.

Malaria was traditionally treated with cloroquine or quinine with declining success. Prof. Tu screened various plant extracts, more than 2,000 Chinese herb preparations as traditional Chinese medicines in malaria-infected animals and found the extract of *Artemisia annua* (see the picture of the plant in Fig. 1) to have an effect. At the beginning the results were inconsistent, but later on she worked out the extraction procedure resulting an extract with the active component. This component, later called artemisinin (Fig. 1) was found effective both in infected animals and humans. As dihydroartemisinin has enhanced stability it was also developed as drug. It is now in standard treatment for malaria.

The history of the discovery was described by Y. Tu in a paper published in Nature [1].

Malaria, caused by *Plasmodium falciparum,* infects about 200 million individuals yearly. Combined Artemisinin therapy has saved hundred thousands of lives.

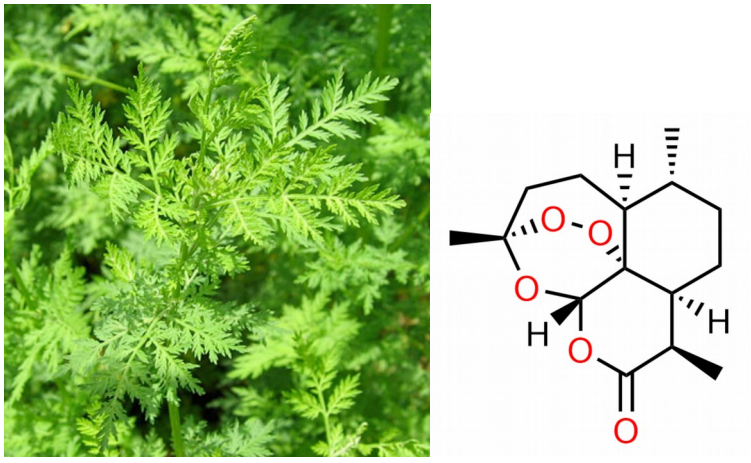

Fig. 1. Artemisia annua and the active anti-malarial component, artemisinin

Artemisinin is a sesquiterpene lactone containing a peroxide bridge. This peroxide is thought to be responsible for the anti-malarial action. It is usually used in combination with other antimalarial drugs to avoid resistance. Artemisinin has low solubility and bioavailability. That is the reason why so many research groups tried to develop cyclodextrin-based drug delivery systems to improve the pharmacokinetic properties.

Complexation with CDs

The very first approach was the phase solubility study of Usuda et al [2]. He compared several kinds of CDs and found A_L type solubility isotherms for all of them. The apparent stability constants for artemisinin-parent CD complexes increased in the order of αCD < γCD ≈ βCD. The constants for artemisinin βCD derivatives increased in the order of G_2-βCD ≈ G_1-βCD ≈ PM-βCD ≈ βCD < HPBCD < SBE_7βCD < DIMEB, where PMβCD, G_1-βCD and G_2-βCD stand for partially methylated βCD, glucosyl and maltosyl βCD, resp. In another study randomly methylated-βCD (RAMEB), Crysmeb and HPγCD were also found to improve the aqueous solubility of artemisinin with the highest effect of Crysmeb. The spectroscopic studies showed a lot of interactions between artemisinin and all the CDs studied, but mainly outside the cavity. Molecular modeling confirmed that artemisinin and CDs formed non-inclusion complexes [3]. Another molecular modeling study proved the host-guest inclusion with βCD [4] and docking studies showed that artemisinin is located inside the ring of HPBCD [5]. The complexes with α-, β- and γCD provided an increased rate and extent of drug dissolution [6]. The DSC study revealed that the highest percentage of the included artemisinin was found in the γCD complex.

Artemisinin/beta-cyclodextrin primary microparticles were prepared by spray-drying a water-methanol solution of artemisinin/βCD. Approximately sixfold increased aqueous solubility of artemisinin was obtained and the flowing properties were also improved. The thermal analysis and XRD evidenced a decrease in drug crystallinity. C-13-NMR analysis indicated the partial complexation with βCD. The *in vitro* dissolution rate determination of artemisinin from the microparticles showed that in 10 min about 70% of drug was released, whereas less than 10% of artemisinin was dissolved from raw material powder [7].

Isothermal titration calorimetry method was used to determine the complexation thermodinamics for HPBCD with artemisinin at varying temperature and pH. It was concluded that the complexation was primarily driven by enthalpy with entropic assistance at all temperatures studied [8].

Light controlled release of artemisinin is possible by using 4,4'-bis(6'-O-cyclomaltoheptaosyl) azobenzene (CD2-AB). This host system in the trans configuration, in neutral aqueous solution, is able to form a 1:1 complex with artemisinin using the collaborative action of the two CD cavities and the hydrophobic linker. Photoirradiation at 363 nm switches the linker from the

trans to the *cis* configuration and the latter shows a loss of interaction with artemisinin (Fig. 2). The difference in the binding features between the two geometrical isomers of CD2-AB suggests that light control is possible for artemisinin binding in aqueous solution through the photoisomerization reaction [9].

The artemisinin derivatives artelinic acid and artesunic acid are also promising candidates for the treatment of multidrug resistant strains of *Plasmodium falciparum* (Fig. 3). The complexation of both compounds with βCD resulted in enhanced solubility and stability in aqueous solution. With the help of multidimensional NMR spectroscopy 2:1 molar ratio was found for artelinic acid and βCD but the presence of 1:1, 2:2, and 3:1 complexes in solution cannot be excluded. The NMR data also indicate selective insertion of artelinic acid into the hydrophobic cavity of βCD *via* the primary face. NMR results indicate that artesunic acid forms a similar complex with βCD in a ratio of 1:1; but the presence of 1:1, 2:2, and 3:1 complexes in solution cannot be ruled out [10].

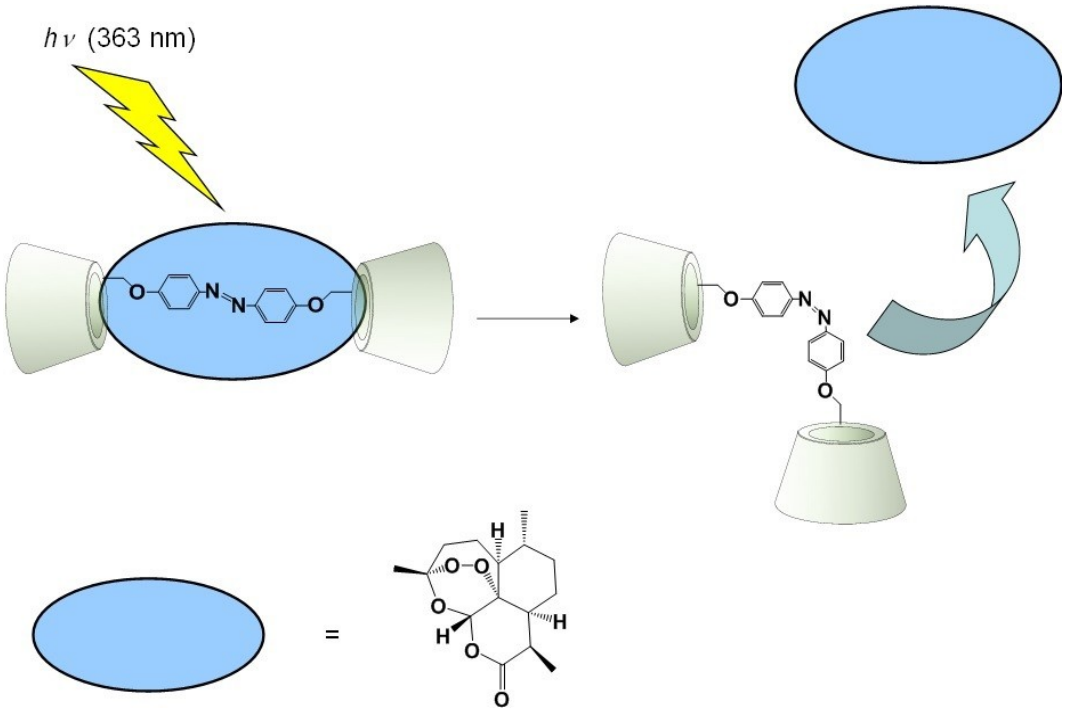

Fig. 2. Schematic representation of the light controlled release of artemisinin using 4,4'-bis(6'-O-cyclomaltoheptaosyl) azobenzene (CD2-AB)

Dihydroartemisinin (DHA) is a major metabolite of artemisinin and its derivatives including arteether, artemether, and artesunate. Pure DHA is crystalline but gets amorphous upon complexation with HPBCD as exhibited by XRD studies. The phase solubility profiles were classified as A_L-type, indicating the formation of a 1:1 stoichiometric inclusion complex. DHA/HPBCD complexes showed a 40% increase in thermal stability (50 ^{o}C) and a 29-fold decrease in hydrolysis rates compared with DHA [11].

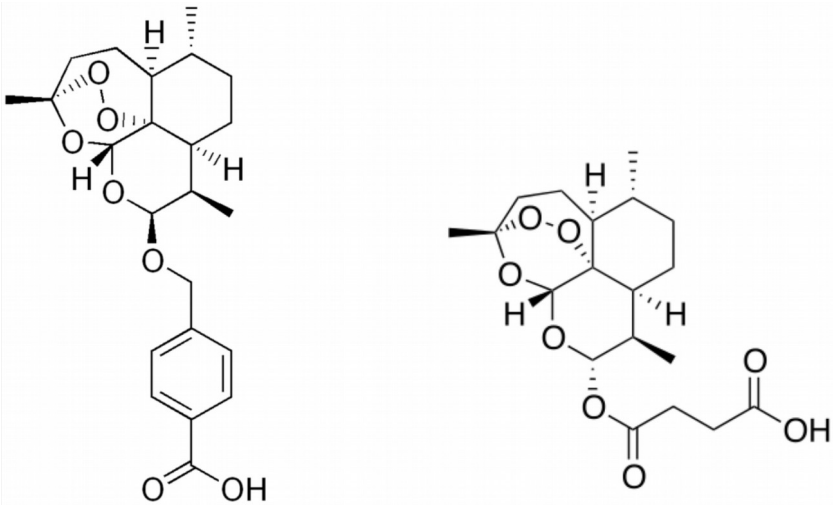

Fig. 3. Artelinic and artesunic acids

The enhanced solubility resulted in enhanced bioavailability of β- and γCD/artemisinin complexes compared to a commercially available preparation, Artemisinin 250. Twelve healthy male volunteers participated in the study conducted according to a three-way crossover design. The pharmacokinetic parameters showed significant improvement [12]. In another study the therapeutic equivalence of a βCD /artemisinin complex at an artemisinin dose of 150 mg was compared with the same 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 βCD/artemisinin complex (containing 150 mg artemisinin) twice daily for 5 days or the active comparator (containing 250 mg artemisinin) twice daily for 5 days. All patients in both treatment groups were cured of the infection and achieved therapeutic success. The parasite clearance time in both treatment groups was similar, being approx. 3 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 βCD/artemisinin complex-treated group. The βCD/artemisinin complex at a dose of 150 mg artemisinin was therapeutically equivalent to 250 mg Artemisinin 250 [13].

Oral administration of Artemisinin/βCD primary microparticles prepared by spray-drying, in rats yielded 3.2-fold higher artemisinin plasma levels compared to those of pure drug [7].

CD-based nanoparticles

The association of artemisinin to a βCD-epichlorohydrin crosslinked polymer (BCDP), organized in nanoparticles of ca. 15 nm size, was investigated in neutral aqueous medium by circular dichroism, UV-VIS absorption and fluorescence. The spectroscopic and photophysical properties of the complexes evidenced an alcohol-like environment for artemisinin in the nanoparticle frame. The data suggest that artemisinin penetrates in the interior of the BCDP nanoparticle, where it is exposed to an alcohol-like environment, which is considerably less

hydrophilic than in the βCD complex where artemisinin is located close to the secondary rim of the cavity and is largely exposed to water [14].

CD derivatives grafted with decanoic alkyl chains (CD-C-10) were prepared by transesterification catalyzed by thermolysin. Nanosphere or nanoreservoir-type systems with a size ranging from 70 to 220 nm able to associate artemisinin were formed. The formulation parameters were optimized to reach stable and high artemisinin dosage corresponding to drug levels of 0.3 and 1.6 mg/mL in the colloidal suspension, for the spherical and reservoir-type nanosystems, respectively. PEG surface-decorated nanoparticles were also prepared by co-nanoprecipitation of PEG fatty acid esters and CD-C-10 molecules. Both types of artemisinin-loaded nanosystems showed a sustained *in vitro* release. The *in vitro* antimalarial activity was evaluated using the lactate dehydrogenase assay. Artemisinin-containing colloidal suspensions inhibited the growth of cultured *Plasmodium falciparum*, both multi-resistant K1 and susceptible 3D7 strains with IC_{50} values (2.8 and 7.0 ng/mL) close to those of reference artemisinin solution [15,16]. The *in vitro* behavior of artemisinin-loaded nanoparticles toward the immune system and their *in vivo* biodistribution in mice were tested. After *iv* injection in mice, the pegylated artemisinin-γCD-C10 NPs showed long residence time (24 h) and no hemolysis. The pharmacokinetic studies carried out on rats using artemisinin-decorated pegylated nanoparticles, (1.5 or 2 mg artemisinin/kg) *vs* artemisinin water/ethanol solution (2 mg artemisinin/kg), showed that the nanospheres exhibited favorable pharmacokinetic parameters: 40 μg.h/mL AUC0-t vs 12 μg.h/mL for artemisinin solution, $t_{1/2}$ of 5 h *vs* 0.8 h, clearance 7.4 L/h *vs* 39 L/h [17].

Artemisinin nanoparticles and artemisinin/βCD complexes were successfully fabricated by means of evaporative precipitation of nanosuspension. Artemisinin nanoparticles and artemisinin/βCD complexes showed significantly faster dissolution than the pure drug due to smaller size (larger surface area), inclusion complex formation and amorphous state [18].

CDs as elicitors

βCD, DIMEB and HPBCD, were added to the culture medium of *Artemisia annua* suspension cultures, and their effects on artemisinin production were analyzed. The effects of a joint cyclodextrin and methyl jasmonate treatment were also investigated. 50 mM DIMEB, as well as a combination of 50 mM DIMEB and 100 mM methyl jasmonate, was highly effective in increasing the artemisinin levels in the culture medium. The observed artemisinin level (27 μmol/g dry wt.) was about 300-fold higher than that observed in untreated suspensions [19].

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Gelatin-cyclodextrin, Nanoencapsulation

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Oleylamine, Hydrogenation of p-nitrophenol

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o-Aminothiophenol, Aryl aldehydes, Catalytic activity, Green catalyst

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Catalyst, Aldehydes, Ammonium acetate, 1,3-Dicarbonyl compounds

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Epichlorohydrin, Liquid-solid phase oxidation, Synergistic effect

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Review, Capillary electrochromatography, Capillary liquid chromatography

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