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Investigation of the complexation of albendazole with cyclodextrins for the design of new antiparasitic formulations

Bénédicte Pradines [a,b], Jean-François Gallard [c], Bogdan I. Iorga [c], Claire Gueutin [a], Philippe M. Loiseau [b], Gilles Ponchel [a], Kawthar Bouchemal [a,*]

[a] Institut Galien Paris Sud, UMR CNRS 8612, Université Paris-Sud, Faculté de Pharmacie, 5, rue J-B. Clément, 92296 Châtenay-Malabry cedex, France
[b] BioCis, Biomolécules: conception, isolement, synthèse-Chimiothérapie Antiparasitaire, UMR CNRS 8076, Université Paris-Sud, Faculté de Pharmacie, 5, rue J.B. Clément, 92296 Châtenay-Malabry cedex, France
[c] Institut de Chimie des Substances Naturelles, CNRS UPR 2301, Centre de Recherche de Gif-sur-Yvette, 1 Avenue de la Terrasse, 91198 Gif-sur-Yvette, France

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ABSTRACT

Albendazole (ABZ) exhibits a potent antiparasitic activity against a broad spectrum of parasites. Unfortunately, the very low water solubility of ABZ (0.2 μg mL^{-1}, 0.7 μM) impairs considerably its formulation. Phase solubility diagrams showed that α-cyclodextrin (10% w/w), hydroxypropyl-β-cyclodextrin (40% w/w) and sulfobutylether-β-cyclodextrin (40% w/w) allowed an increase of apparent solubility with enhancement factors of 570, 3970, and 5880, respectively. The apparent aqueous solubility of ABZ was markedly increased from 0.2 μg mL^{-1} (0.7 μM) without cyclodextrins to 1.52 mg mL^{-1} (5.69 mM) with random methyl-β-cyclodextrin (Me-β-CD) (40% w/w). This corresponds to an apparent solubility enhancement factor of 7600 which is the maximal enhancement factor of ABZ apparent aqueous solubility ever reported in the literature using conventional cyclodextrins. The complexation mechanism between ABZ and cyclodextrins has been investigated using phase solubility diagrams, nuclear magnetic resonance (^{1}H NMR) coupled with two-dimensional nuclear Overhauser effect (NOESY) experiments and molecular docking calculations. The results showed that the central bicyclic fragment from ABZ interacts with Me-β-CD according to 1:1 stoichiometry.

1. Introduction

Albendazole (ABZ), (methyl[5-(propylthio)-1*H*-benzimidazol-2-yl]carbamate, Fig. 1), belongs to the group of benzimidazole derivatives. This molecule has a broad spectrum of activity against human and animal helminthic parasites such as nematodes, and metacestodes. It was approved for human use since 1982. ABZ showed antiprotozoal activity against *Trichomonas vaginalis*[1] in vitro. The IC_{50} of ABZ against *T. vaginalis* was estimated in a range of 3–4 μM.[2] ABZ was also used to boost the trichomonacidal activity of metronidazole.[3] However, like other benzimidazole carbamates, ABZ belongs to biopharmaceutical classification system type II (BCS class II) with low aqueous solubility (0.2 μg mL^{-1} at 25 °C) and high permeability through the biological membranes.

Low water solubility of drugs results very generally in low solubility in biological fluids and is likely to considerably impair either attainable local drug concentrations (when local delivery is foreseen) or pharmacokinetic profiles (after systemic administration). Different approaches have been previously investigated to solve the aqueous solubility limitation of ABZ, including the use of surfactants such as polysorbates,[4] bile salts[5] or co-solvent like molecules (diethylene glycol monoethyl ether: Transcutol®).[6] High ABZ apparent solubility (up to 2.226 mg mL^{-1}) was obtained using Transcutol® (40% w/w) at a pH of 1.2.[4] However, the use of many of these agents should be restricted whenever possible because they can be irritants to mucosa membranes.

There is some evidence in the literature that the addition of cyclodextrins (CDs) into ABZ formulations could enhance ABZ oral bioavailability, particularly hydroxypropyl-β-cyclodextrin (HP-β-CD) which significantly increased ABZ absorption in comparison with a surfactant-based formulation.[7] This study conducted on healthy volunteers showed that the relative bioavailability of ABZ formulated in arachid oil-polysorbate 80 or HP-β-CD containing formulations was enhanced by 4.3- and 9.7-folds, respectively, compared to commercial tablets. The interest of combining ABZ to HP-β-CD was further confirmed by Evrard et al.[8] who showed an improvement of the oral and the effectiveness of ABZ/HP-β-CD inclusion complex against encapsulated larvae of *Trichinella spiralis* in a murine model.[9] ABZ complexed to HP-β-CD resulted

* Corresponding author. Tel.: +33 (0)1 46 83 55 81; fax: +33 (0)1 46 61 93 34.
E-mail address: kawthar.bouchemal@u-psud.fr (K. Bouchemal).

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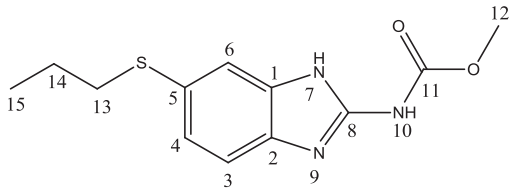


Figure 1. Chemical structure of ABZ.

in an increase of aqueous solubility of the drug, up to 3500 times at 37 °C.[10] More recently, synthetic β-CD citrate derivative showed better ABZ solubility enhancement than native β-CD.[11]

The use of CDs represents thus an interesting strategy for the improvement of ABZ aqueous apparent solubility. It was necessary to screen some pharmaceutically acceptable CDs on rationale bases in order to identify the most adapted one for optimal ABZ bioavailability. In the work to be presented here, phase solubility diagrams were used to investigate ABZ attainable solubility enhancements using conventional cyclodextrins: HP-β-CD, α-cyclodextrin (α-CD), sulfobutylether-β-cyclodextrin (SBE-β-CD), and random methyl-β-cyclodextrin (Me-β-CD) (Table 1) and to identify the best CD derivate capable of improving its aqueous solubility. Then, an in depth physico-chemical characterization including nuclear magnetic resonance (^{1}H NMR) coupled with two-dimensional nuclear Overhauser effect (NOESY) and molecular modeling was used to investigate the mechanism of CD/ABZ complexation and to identify the chemical groups able to interact with the CD.

2. Materials and methods

2.1. Reagents

Albendazole (ABZ) (M_w = 265 g mol^{-1}) was a gift from GlaxoSmithKline (Great Britain). Random methyl-β-cyclodextrin (Me-β-CD Rameb®, M_w = 1331 g mol^{-1}) was from Cyclolab (Budapest, Hungary), hydroxypropyl-β-cyclodextrin (HP-β-CD, M_w = 1375 g mol^{-1}) was from Acros Organics (Geel, Belgium), α-cyclodextrin (α-CD, M_w = 972 g mol^{-1}) Cavamax® W6 was from ISP (Netherlands) and sulfobutylether-β-cyclodextrin (SBE-β-CD, M_w = 2241 g mol^{-1}) Captisol® was a gift from CyDex Pharmaceuticals, Inc (USA). All chemicals were of analytical or reagent grade and were used without further purification. Solutions were prepared by weight using MilliQ® water (Millipore, Molsheim, France). Analytical grade methanol, ethanol, and acetonitrile were from Carlo Erba (Val de Reuil, France). Deuterium oxide (D_2O) (99.96% D) and deuterated acetonitrile (CD_3CN) were from Sigma–Aldrich (Saint-Quentin-Fallavier, France).

2.2. Phase solubility diagrams and apparent aqueous solubility determinations

Phase-solubility studies were carried out according to the method described by Higuchi and Connors.[12] An excess of commercial ABZ was placed in a glass vial (20 mL) with 2 mL of solubilizing agent which was an aqueous solution of α-CD, HP-β-CD, SBE-β-CD, or Me-β-CD at concentrations ranging from 5% to 40% w/w for HP-β-, SBE-β-, or Me-β-CD and from 5% to 10% w/w for α-CD due to its own aqueous solubility limitations at 25 °C.

Vials containing an excess of ABZ powder dispersed in a solution of CD were kept in a shaker for 5 days at 25 °C. This duration was estimated to be long enough for reaching the complexation equilibrium. Then, the samples were filtered through a 0.22 μm membrane filter (Millex, SLAP 0225, Millipore, France).

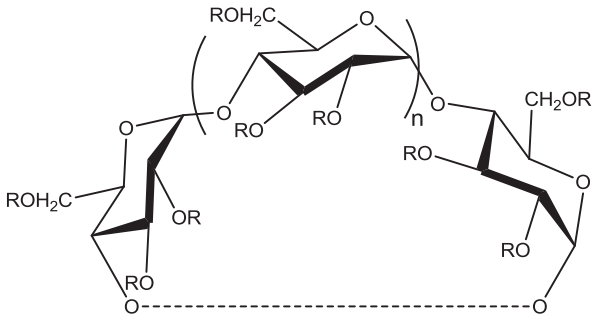
The solubility of ABZ in water was determined according to the same protocol without the presence of CDs.

2.3. HPLC determination of ABZ concentrations

Samples containing ABZ were analyzed by reversed phase high performance liquid chromatography (HPLC) using a symmetry C_{18} column (5 μm, 250 × 4 mm, Waters). Chromatography was performed with a Waters 515 pump and a Waters 717Plus autosampler. UV absorbance at 235 nm was monitored with a Waters 486 absorbance detector. Data were recorded and processed on Azur Software (Datalys). The mobile phase was an isocratic mixture of acetonitrile/water (50:50% v/v). HPLC analyses were performed at a flow rate of 0.5 mL min^{-1}.

A calibration curve was obtained from a stock solution of ABZ/Me-β-CD complexes (250 μg mL^{-1}) prepared according to the method described in the previous section. The concentration of ABZ should be below the solubility of the inclusion complex. The stock solution was diluted with the Me-β-CD solution. Valid concentrations for quantification were in the range 0.25–250 μg mL^{-1}. The equation of the calibration curve was $y = 0.589x + 0.012$ (R^2 = 0.9997).

Table 1
General structures of cyclodextrins used in this study



Type of CD	Abbreviation	R
α-Cyclodextrin, n = 4	α-CD	–H
β-Cyclodextrin, n = 5	β-CD	–H
γ-Cyclodextrin, n = 6	γ-CD	–H
Sulfobutylether-β-cyclodextrin, n = 5	SBE-β-CD	–$(CH_2)_4SO_3Na$ or –H
Hydroxypropyl-β-cyclodextrin, n = 5	HP-β-CD	–$CH_2CHOHCH_3$ or –H
Random methyl-β-cyclodextrin, n = 5	Rameb Me-β-CD	–CH_3 or –H