Influence of water-soluble polymers, pH and surfactants on mebendazole (MBZ) sulibilization by β-and partially methylated-β-cyclodextrins

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ABSTRACT

The objective of this work was to determine the influence of pH, water-soluble polymers and surfactants on mebendazole solubilization by β- and partialy methylated-β-cyclodextrins (β-CD and PM-β-CD). Mebendazole is an antihelmintic active drug used against a variety of intestinal parasites and potentially active against extra-intestinal parasitic infections. However, its low solubility renders it difficult to use. Cyclodextrins (CD) are able to increase the apparent solubility of poorly water-soluble active drugs by forming complexes. **Methods:** mebendazole was dosed by an HPLC method. To study the influence of pH on mebendazole intrinsic solubility, five aqueous solutions were prepared at pH 1.2, pH 3, pH 5, pH 7 and pH 10. Solubility studies of β-CD and PM-β-CD and mebendazole complexes were carried out according to the method described by Higuchi and Connors. The inclusion complex formation between mebendazole and cyclodextrins was characterized by mass spectrometry (MS), powder X-ray diffractometry (XR) and Fourier transform infrared spectroscopy (FT-IR)**.** To determinate the influence of surfactants and water soluble polymers, poloxamer PE/F68 1%, sodium dodecyl sulphate 1%, benzethonium chlorure 1%, hydroxypropylmethylcellulose 0.1% or polyvinylpyrrolidone 0.25% was added to the range of cyclodextrins. **Results:** Cyclodextrins, and particularly PM-β-CD, are able to significantly increase the mebendazole apparent solubility in biological medium (solubility increased by 4700). Mebendazole seemed to be included in PM-β-CD by its aromatic cycles**.** The addition of polyvinylpyrrolidone or hydroxypropylmethylcellulose, water-soluble polymers, in formulation, does not increase the maximal mebendazole solubility. The use of low cyclodextrin concentrations may produce a slight added advantage but the overall benefit does not justify its inclusion. The addition of surfactants, poloxamer (PE/F68), sodium dodecyl sulfate or benzethonium chloride, also does not enhance solubility. A micelle formation could hamper a synergistic effect between cyclodextrins and surfactant. **Conclusions:** Surfactant use must be excluded in a formulation with cyclodextrins. A formulation with negatively charged cyclodextrins should be considered at acidic pH, where mebendazole is positively charged and therefore more soluble. Increased quantities of mebendazole would in this way be rendered more soluble.

Keywords: Mebendazole, Cyclodextrins, Solubilization, pH, Polymers

1 INTRODUCTION

Mebendazole (MBZ) (Fig.1) is a benzimidazole anthelmintic used in the treatment of ascariasis, oxyuriasis, trichuriasis and more than one worm infection at a time. It's active against intestinal nematodoses and hydatid disease when administered in high doses (2.4 g/day, for 1 to 6 months) [1].

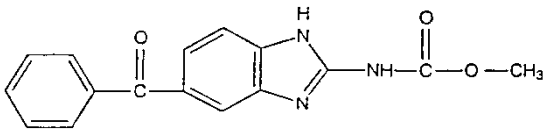

Figure.1

Nevertheless, its poor aqueous solubility limits its bioavailability and in case of a desirable systemic effect, enhancing its solubility could be an alternative to the use of high doses, generally associated to adverse side effects (gastro-intestinal disturbances, alopecia, reversible bone marrow depression …). Currently, many pharmaceutical drugs containing cyclodextrins (CDs) are commercialized and β-cyclodextrin (β-CD), a natural cyclodextrin, is the most frequently used. Partially methylated-β-cyclodextrin (PM-β-CD) would be also an efficient vector due to its high water intrinsic solubility (80 g/100ml). At the same time, some authors have reported that the addition of water-soluble polymers increased the solubilization capacity of CDs [2, 3 and 4] through enhancement of complex stability constant formed between CDs and low water-soluble drug. A pH medium variation can also enhance the drug complexation and solubilization using CDs. This appeared as a result of drug increase water-solubility, which was pH dependent [5]. Similarly, surfactant addition can often

render hydrophobic drugs water-soluble and could enhance MBZ complexation by CDs.

The purpose of this study was to investigate influence of pH, water-soluble polymers and surfactants on the aqueous solubility and complexing capacity of β-CD and PM-β-CD on MBZ.

2 EXPERIMENTAL

2.1 Materials and methods

MBZ (C16H13N3O3; MM: 295.3 g mol-1; melting point 290 °C) was obtained from Janssen Cilag (France). β-CD (MM: 1135 g mol-1, water-solubility 18.5 g/ml) was supplied by Roquette, PM-β-CD (MM: 1330 g mol-1) by Orsan. Polyvinylpyrrolidone K30 (PVP) was obtained by BASF, hydroxypropylmethylcellulose 4000 (HPMC) by Lambert Rivière (France), poloxamer (PE/F68), neutral surfactant by LCI, benzethonium chloride, cationic surfactant by Prolabo (France), sodium dodecyl sulfate, anionic surfactant, hydrochloric acid and sodium hydroxyde, used to adjust pH by Cooper (France).

Mebendazole (MBZ) concentration was evaluated by an HPLC method with a Jasco AS950 pump, equipped with a Cromasil® C18 (18cm) reverse phase column and a Merck multichannel photodetector L3000. Mobile phase consisted in a mixture of acetonitrile and sodium acetate buffer 0.25N (1:1, v/v), pH 5.7. Flow rate was fixed to 1mL/min, detection wavelength at 289 nm. In that case, retention time was comprised between 5 and 6 minutes.

2.2 Mebendazole intrinsic solubility

An excess of MBZ (20 mg) was added to 10ml of five aqueous solutions, buffered to pH 1.2, pH 3, pH 5, pH 7, and pH 10. Suspensions were shaken in a steam room (37°C) for 24 hours, centrifugeated and supernatant filtered on 0.22 μm membrane filter. MBZ in the filtrates was immediately analyzed by HPLC method.

2.3 Phase solubility studies

Phase solubility studies were performed according to the method of Higuchi and Connors [4]. An excess amount of MBZ (2 mg/mL) was added to β-CD and PM-β-CD solutions. After one week equilibrium, in a steam room at 37°C and centrifugation, supernatant was filtered on 0.22 μm membrane filter and analyzed for drug content by HPLC method at 289 nm.

pH, surfactants and water soluble polymers influence were evaluated adjusting MBZ/CDs solution to pH 3 or 10, by addition of 1% PE/F68, 1 % sodium dodecyl sulphate, 1% benzethonium chlorure, 0.1 % HPMC or 0.25 % PVP .

2.4 Preparation of solid inclusion complexes (IC) and corresponding physical mixtures (PM)

PM-β-CDs was dissolved in 5 mL water. To this solution, acetonic solution of MBZ was added, with or without 6% (v/v) formic acid, in a 1:1, 1:2 or 2:1 molar ratio (MBZ over cyclodextrin). The whole solutions were stirred for two weeks in a hood at 37°C±2°C.

The corresponding was obtained by thoroughly mixing the MBZ with PM-β-CD.

3 RESULTS AND DISCUSSION

The phase solubility diagrams of MBZ with β-CD (Fig. 2) or PM-β-CD (Fig. 3) resulted respectively in an A_L and Ap type Higuchi phase solubility diagram. Thus, complexes formed with β-CD were of the first order with regards to the host molecule [6] whereas PM-β-CD led to formation of a higher order than one in the host molecule indicating the formation of 1:1 and 1:2 stoechiometric ratio of MBZ/CD. Apparent solubilities were 35- and 4700-fold increased in presence of β-CD and PM-β-CD, respectively. However, these apparent solubilities were limited to the maximum intrinsic solubility of these CDs: 16 mmol/L and 250 mmol /L in case of β-CD and PM-β-CD respectively. The largest increase of MBZ solubility was obtained by using PM-β-CD, consequently, preparation of inclusion complex was rather done with this derivatized cyclodextrin.
The inclusion complex was characterized by mass spectrometry; powder X-ray diffractometry and Fourier transform infrared spectroscopy [7].

MBZ solubility is pH-dependent due to amino / benzimidazole groups protonation at acidic pH (Fig. 4). A highly acidic pH, pH 1.2, (i.e gastric pH), supported its solubilization with a 117-fold increase versus neutral pH, after 7 days incubation-time at 37°C.

This solubilization was similar to albendazole (a close mebendazole chemical derivative): at pH 1.2, solubility was increased by a factor 23 in comparison to pH 10. Nevertheless, addition of β-CD or PM-β-CD led to a MBZ apparent solubility independent of pH (Fig. 2 and 3). Three explanations can be done: (i) pH do not influence the solubilization capacity of β-CD and PM-β-CD, (ii) an ionized form, more hydrophilic, has less affinity with the CDs hydrophobic cavity (iii) MBZ complexation by PM-β-CD is due to its benzenic cycle and protonation of amine functions at acidic pH does not disturb this interaction[8, 9]. These three effects tend to homogenize apparent solubility in function of pH (Fig. 5 and 6).

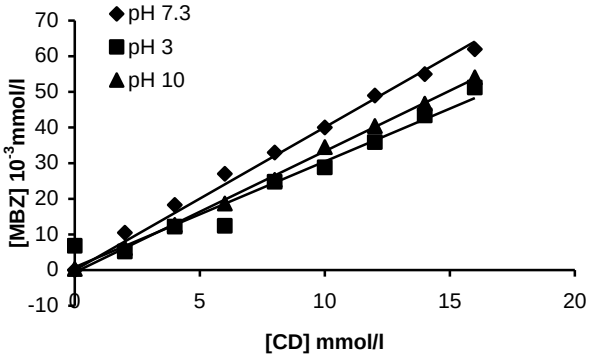

Figure 2: pH influence; Higuchi phase solubility diagrams of MBZ in presence of β-CD.

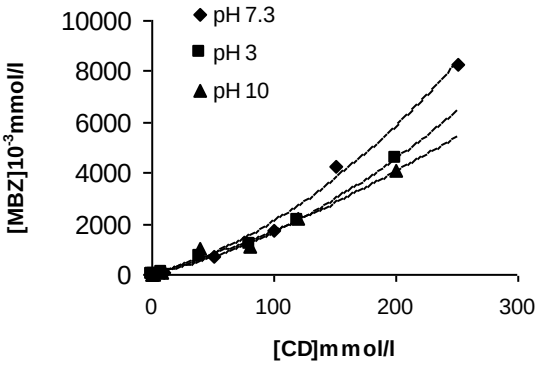

Fig 3: pH influence; Higuchi phase solubility diagrams of MBZ in presence of PM-β-CD.

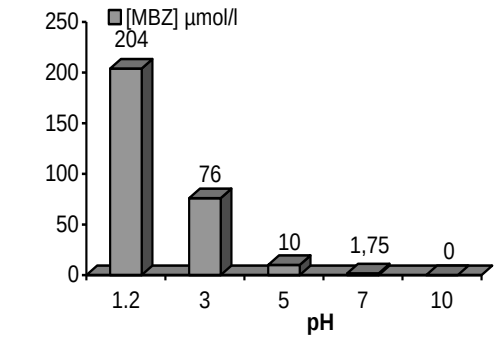

Figure 4: MBZ solubility in function of pH

In case of PM-β-CD, a stronger affinity may exist between PM-β-CD and PE/F68 and SDS than with benzethonium chloride, for which there was no surfactant influence. Several authors have described the use of water-soluble polymers to increase CDs solubilization capacity [4, 10, and 11]. At low β-CD concentrations (Fig. 7), the addition of polyvinylpyrrolidone (PVP) 0.25 % supported the MBZ solubilization, but not significantly. However, at a higher concentration or with HPMC, no benefit was observed. On contrary, addition of these polymers to PM-β-CD (Fig. 8) has a synergic negative effects; due to precipitation of the inclusion complex MBZ/PM-β-CD in presence of polymers.

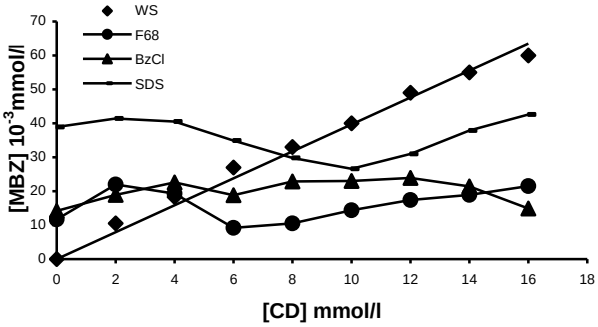

Figure 5: Surfactants influence: Higuchi phase solubility diagrams of MBZ in presence of β-CD with PE/F68, benzethonium chloride (BzCl) or sodium dodecyl sulfate(SDS) or without surfactant (WS)

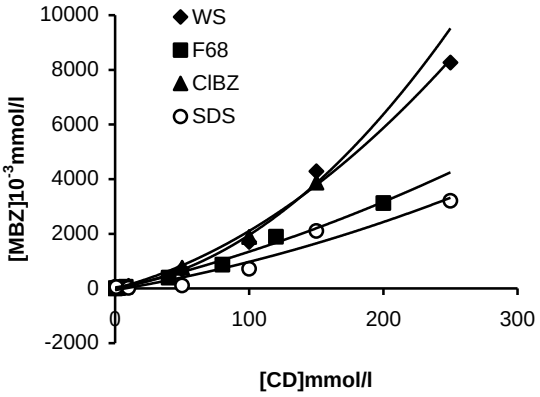

Figure 6: Surfactants influence: Higuchi phase solubility diagrams of MBZ in presence of PM-β-CD with PE/F68, benzethonium chlorure (BzCl) or sodium dodecyl sulphate (SDS) or without surfactant (WS)

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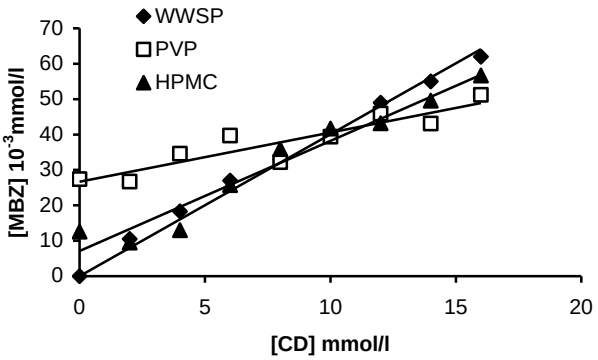

Figure 7: Water-soluble polymers influence: Higuchi phase solubility diagrams of MBZ in presence of β-CD with HPMC or PVP or without water-soluble polymer (WWSP)

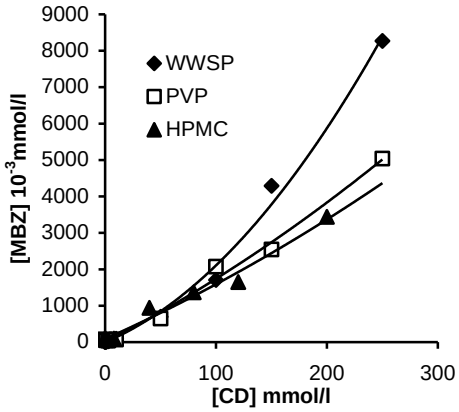

Figure 8: Water-soluble polymers influence: Higuchi phase solubility diagrams of MBZ in presence PM-β-CD with HPMC or PVP or without water-soluble polymer (WWSP)

4 CONCLUSION

Complexation with PM-β-CD resulted in a 4700-fold increase of MBZ apparent solubility in biological medium. In that case, MBZ would be included in PM-β-CD by its aromatic cycles. Neither addition of water-soluble polymers, nor surfactants in formulation produced a significant solubility enhancement, and even should be avoided in certain conditions. Acidic pH was in favour of MBZ solubilization (at pH 1.2, one could observe a 117-fold increase in solubility in comparison with neutral pH). Nevertheless, complexation with β-CD and PM-β-CD tends to reduce this increase. This fact could be explained by MBZ affinity decrease for the hydrophobic cavity, in ionized form, and therefore, more hydrophilic form.

The micelle formation of surfactants may be contributed to reduce the interaction of MBZ with CDs. The interaction between surfactant and β-CD was so major that it removed initial complex between 2-anthraquinone sulfonic acid and β-CD. These formed complexes were 1:1 or 1:2 types. It is possible that, in case of high concentration in β-CD, MBZ solubility was not increased due to previous surfactants inclusion in their cavity

Whatever surfactant type, anionic (sodium dodecyl sulfate, SDS), cationic (benzethonium chlorure, BzCl), or neutral (PE/F68), MBZ solubility was lowered by comparison to its value without surfactant but solubility diagram was still A_p type. In case of β-CD, surfactants contribute to an increase in solubility until a maximum β-CD concentration in the medium (comprised between 5 and 9 mmol/L) and formation of an equimolar complex was not observed any more. Water-soluble polymers or surfactants addition do not bring more benefit on MBZ solubilization than does PM-β-CD alone.

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