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Improvement of the in vitro dissolution of praziquantel by complexation with α-, β- and γ-cyclodextrins

Gordon Becket [a,*], Leo J. Schep [a], Mun Yee Tan [b]

[a] *School of Pharmacy, University of Otago, P.O. Box 913, Dunedin, New Zealand*
[b] *Singapore General Hospital, Outram Park, Singapore, Singapore*

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Abstract

Although praziquantel (PZQ) is the primary drug of choice in the treatment of schistosomiasis, its poor solubility has restricted its delivery via the oral route. In spite of its poor solubility, PZQ is well absorbed across the gastrointestinal tract, but large doses are required to achieve adequate concentrations at the target sites. Improving the solubility would enable the parenteral route to be used, thereby avoiding significant first pass metabolism. The aqueous solubility of PZQ was improved by forming inclusion complexes with α-, β- and γ-cyclodextrins (CDs). These complexes were assessed and confirmed by solubility analysis, Fourier transform infrared analysis, elemental analysis, differential scanning calorimetry and mass spectrometry. Dissolution of PZQ from the α-, β- and γ-CD complexes was 2.6-, 5- and 8-fold greater, respectively, than that of the pure drug. However, only the β-complex had a stability constant in the optimum range for pharmaceutical use, suggesting that the preferred complex for further development would be a water-soluble β-CD derivative.

Keywords: Anthelmintic; Cyclodextrins; Inclusion complexation; Praziquantel

1. Introduction

Praziquantel (PZQ) is recognised worldwide as the primary drug of choice in the treatment of the most common forms of schistosomiasis (bilharzia). It is unusual in being effective against two classes of the Platyhelminths i.e. Trematodes and Cestodes; King and Mahmoud (1989) and is recognised as an essential drug by the World Health Organization. PZQ is a highly lipophilic drug that possesses low aqueous solubility. Although it is poorly water-soluble, PZQ is extensively absorbed from the gastrointestinal tract (Andrews, 1985). High oral doses are necessary, however, to overcome first pass metabolism and thereby achieve sufficient concentrations of PZQ at the larval tissue (Leopold et al., 1978). To our

* Corresponding author. Tel.: +64-3-479-7132; fax: +64-3-479-7132; e-mail: gordon.becket@stonebow.otago.ac.nz.

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knowledge, PZQ is administered to humans by the oral route alone. If solubility can be improved, alternative routes of delivery, such as parenteral formulations, could be considered. Such routes will achieve direct systemic delivery, thereby avoiding first pass hepatic metabolism and reduction in the dose delivered.

The aqueous solubility and dissolution rates of lipophilic drugs have been improved by employing cyclodextrins (CDs) (Hamada et al., 1975; Nambu et al., 1978; Uekama et al., 1983). These agents are water-soluble cyclic oligosaccharides, consisting of six to eight α-1,4-linked glucose monomers. Poorly water-soluble drugs form inclusion complexes within the hydrophobic cavity of the cyclodextrin, thereby increasing their solubility (Loftsson and Brewster, 1996; Rajewski and Stella, 1996).

The aim of this study was to improve the solubility of PZQ to allow parenteral administration of the drug by solubilisation using three different CDs (α-, β- and γ-) and to demonstrate the presence of solid complex formations with these CDs.

2. Materials and methods

2.1. Materials

PZQ and β-cyclodextrin (β-CD) were purchased from Sigma Chemical Company (St. Louis, MO); α- and γ-CDs were purchased from Aldrich Chemical Company (Milwaukee, WI). All other reagents and solvents were of analytical grade and purchased from E. Merck (Darmstadt, Germany). Milli-Q water was used throughout the study. The cyclodextrins were dissolved in water and freeze-dried before use.

2.2. Phase solubility studies

The stability constants for inclusion complex formations between PZQ and α-, β- and γ-CDs were determined using the phase solubility method (Higuchi and Connors, 1965). Briefly, excess amounts of PZQ (50 mg) were added to aqueous solutions (20 ml) containing increasing concentrations of the CDs and shaken at 30 ± 0.5°C at 50 oscillations/min. Following equilibrium (7 days), aliquots were filtered with a 0.45-μm Millipore filter and total PZQ was analysed by spectrophotometry (270 nm). Earlier experiments showed that the CDs did not interfere with the measurements at the concentrations employed. The apparent stability constants (K_f) were calculated according to the method of Higuchi and Connors (1965).

2.3. Preparation of the solid complexes

Solid complexes were obtained by mixing appropriate amounts of CD and PZQ (156, 253, and 208 mg of α-, β- and γ-CD were added to 75, 100 and 75 mg of PZQ, respectively) as previously described (Kurozumi et al., 1975), and samples were shaken in 25 ml of water at 30 ± 0.5°C (50 oscillations/min). Following equilibrium (7 days), the solutions were freeze-dried in ampoules, sealed and stored (-20°C) until further use. Uncomplexed drug was separated from the complex by washing the powder with dried diethyl ether (Kurozumi et al., 1975). The complexes were assayed by dissolving 40 mg in 20 ml of water and the absorbance of PZQ measured at 270 nm. The drug content of the α-, β- and γ-inclusion complexes was found to be 7.6, 9.6 and 12.0% PZQ, respectively.

2.4. Differential scanning calorimetry (DSC)

DSC was performed on a Perkin Elmer DSC-4 calorimeter, using 5-mg samples in crimped aluminium pans at a heating rate of 10°C/min over a 70–200°C range. Indium (m.p. 156.6°C, ΔH_f 28.45 Jg^{-1}; Perkin Elmer) was used to calibrate the instrument.

2.5. Spectroscopic studies

Mass spectra were recorded on a VG Analytical 7035 mass spectrometer at 70 eV.

^{1}H NMR was performed on a Jeol FT NMR (300 MHz) spectrophotometer (Tokyo, Japan) using DMSO-d_6 as solvent for the complexes. D_2O exchange was performed on all samples. The as-

signment of the proton chemical shifts for the CDs was made by referring to the literature (Qi et al., 1994) and confirmed by decoupling experiments.

Infrared spectra were recorded on a Perkin Elmer FTIR spectrophotometer using KBr discs.

2.6. Elemental analysis

Elemental analyses (C, H, N) were obtained on the complexes and CDs after drying under vacuum at room temperature for 3 days.

2.7. Computer modelling

Computer modelling of the CD–PZQ complexes was carried out using PCMODEL™ (Serena Software, Bloomington, IN). The selected low energy conformations of *levo*- and *dextro*-PZQ were docked into each CD and the resulting interactions were minimised.

2.8. Dissolution studies

Dissolution of PZQ from the complexes was measured using a method modified from Helm et al. (1991). Briefly, a 20-mg sample of PZQ or an equivalent amount of the inclusion complex or the physical mixture was enclosed in a gelatine capsule (No. 2), sealed and rotated in a USP basket at 100 rpm in 100 ml of water (37°C). Samples were assayed spectrophotometrically at 270 nm. Each experiment was carried out in triplicate and dissolution rates of PZQ were based on the average of the t_{90} values.

3. Results and discussion

3.1. Solubility studies

Fig. 1 shows the phase solubility profiles between PZQ and α-, β- and γ-CDs. Over the concentration range examined in this study, the α-CD complex showed a linear, type $A_{L,}$ relationship between the solubilised substrate and the concentration of CD in solution, while the γ-CD showed a non-linear type A_N profile. In contrast, the β-CD profile reached a solubility limit at approximately 22 mM, with further additions of CD resulting in the precipitation of insoluble complexes (type B_S diagram) (Higuchi and Connors, 1965). The calculated stability constants for the complexations between PZQ and α-, β- and γ-CDs were 56.18, 396.91 and 15.17 M^{-1}, respectively. The higher stability constant for the β-CD complex (396.91 M^{-1}) may reflect spatial compatibility of the guest PZQ molecule and the strength of the interaction by partial fit into the CD cavity (Szejtli, 1984). The lower stability constants given by the α- and γ-complexes suggest weaker more labile interactions which would result in premature release of the drug. Only the β-CD complex had a stability constant within the limits reported to be suitable for pharmaceutical utilisation (Szejtli, 1984). Computer-aided modelling and ^{1}H NMR data indicated the formation of 1:1 complexes. Elemental analysis of the solid complexes, however, gave a calculated values for the α-, β- and γ-CD complexes of 1:4, 1:1.7 and 1:2, respectively. These values suggest that the solid material contained uncomplexed CD or the presence of complexes of mixed stoichiometry. Stoichiometric calculation from the phase solubility profiles was not possible because of the absence of plateau regions.

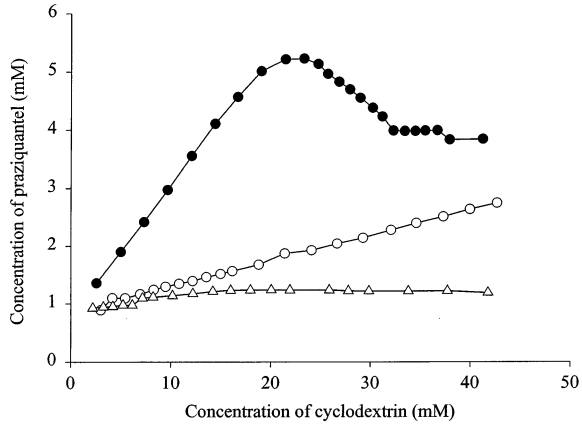

Fig. 1. Phase solubility diagrams of PZQ–α-CD (○), PZQ–β-CD (●) and PZQ–γ-CD (△) systems in water at 30 ± 0.5°C ($n = 3$).

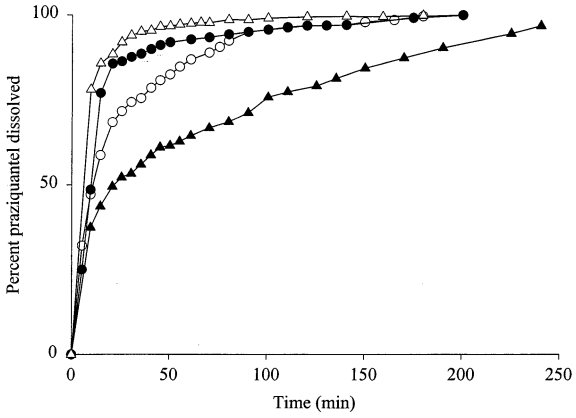

Fig. 2. Dissolution rate profiles of PZQ (▲) and PZQ–α-CD (○), PZQ–β-CD (●) and PZQ–γ-CD (△) complexes at 37 ± 0.5°C ($n = 3$).

3.2. Dissolution rate studies

The dissolution rate profiles of PZQ release from α-, β- and γ-CDs are represented in Fig. 2. Release rates from the inclusion complexes were evidently higher than the dissolution rate of drug alone. Corresponding physical mixtures, as represented by the β-CD profiles (Fig. 3), also demonstrated higher dissolution profiles. Dissolution rates of PZQ complexed with α-, β- and γ-CDs were 2.6-, 5- and 8-fold greater than the pure drug, respectively ($p < 0.05$). At 30 min, the amount of PZQ dissolved was 94%, 88%, 74% and 53% for the γ-, β- and α-complexes and free drug, respectively. Although the γ-CD complex demonstrated the highest solubility, it is the least stable of the complexes ($K_f = 15.17\ M^{-1}$), resulting in the fastest dissolution. Inclusion complexes with small stability constants have also been observed by other investigators (Uekama et al., 1983; Szejtli, 1984; Duchêne and Wouessidjewe, 1990), who have reported faster dissolution rates and extensive dissociation upon dissolution. The low stability constant for the α-CD complex suggests both a high solubility and a fast dissolution rate. These values were lower than anticipated, although other workers have suggested that dissolution rates from cyclodextrin complexes are also dependent on other factors; these include diffusion and dissociation in the dissolution medium (Donbrow and Touitou, 1978; Uekama et al., 1983), decrease in crystallinity and enhanced wettability of the drug by the inclusion complexation (Gandhi and Karara, 1988). Increased dissolution of PZQ when physically mixed with CDs may also be due to an improved wettability of the drug particles at the early stages of the dissolution process (Hassan et al., 1990).

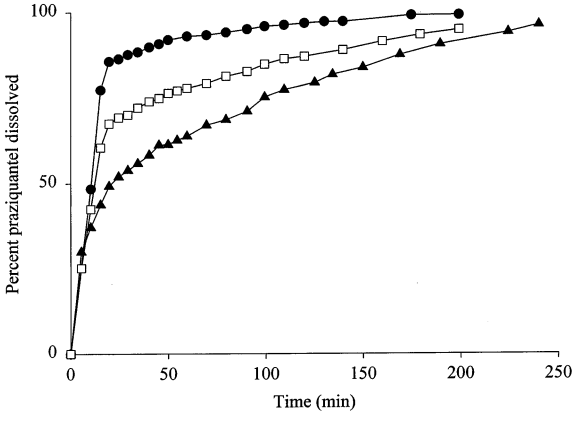

Fig. 3. Dissolution profiles of PZQ (▲), PZQ and β-CD physical mixture (□) and PZQ–β-CD complex (●) at 37 ± 0.5°C ($n = 3$).

3.3. Complex formation

Supporting evidence for complexation of PZQ with α-, β- and γ-CDs was obtained from thermal analysis, mass spectrometry, ^{1}H NMR and elemental analysis.

Representative DSC thermograms for PZQ–α-CD, physical mixture, unwashed complexes and inclusion complexes (α-CD) are shown in Fig. 4 (β- and γ-CD thermograms not shown). DSC thermograms of PZQ alone (i) and the physical mixture (iii) both showed an endothermic T_{max} of 148°C, corresponding to the melting point of PZQ. The unwashed complex (iv) demonstrated a small endothermic peak at 147°C, representing free PZQ. The lower temperature was due to melting point depression by the complex. In contrast, the thermograms of the washed complex (v) lacked the PZQ melting endotherm. Absence of a PZQ endotherm in the washed complex may suggest either complexation of the drug with the CD (Uekama et al., 1983) or removal of the drug by

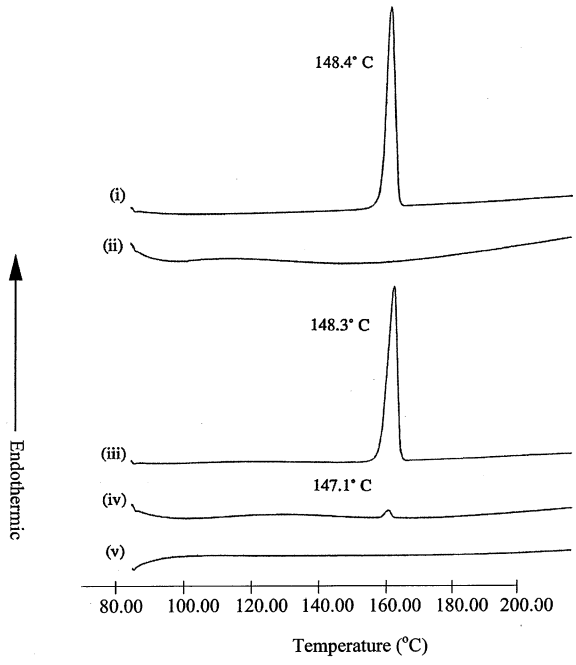

Fig. 4. DSC thermograms of PZQ–β-CD systems. (i) PZQ, (ii) CD, (iii) PZQ and CD physical mixture, (iv) unwashed PZQ–β-CD inclusion complex and (v) PZQ–β-CD inclusion complex.

the washing process. Disruption of the complex, thereby releasing the PZQ from the CD moiety, followed by ether extraction yielded an endothermic peak at 148°C that corresponded to the pure PZQ (similar results were obtained for β- and γ-CD complexes). Furthermore, elemental analysis of the α-, β- and γ-cyclodextrin ether-washed complexes demonstrated the presence of nitrogen, which was not present in the respective pure CDs. It is unlikely that the nitrogen originated from excess PZQ in the complex product since DSC scans of the same samples had shown that all of the free PZQ had been removed by washing with ether.

The mass spectra of the three ether-washed complexes resembled that of pure PZQ. The pseudo-molecular ion in each spectra possessed a m/z ratio of 312, which corresponded to the molecular formula for PZQ ($C_{19}H_{24}N_2O_2$). This confirmed the presence of PZQ in the complexes. The expected molecular ion peak for the PZQ–β-CD complex, if based on a 1:1 stoichiometry, would be m/z 1447, a value greater than the detection limit of the instrument.

The Fourier transform infrared (FTIR) spectra of the three PZQ–CD complexes did not, however, show any significant differences in the region of carbonyl stretching when compared to either the physical mixtures (1659 cm^{-1}) or PZQ alone (1638 cm^{-1}). Others have reported the limited use of this technique in identifying complexation of cyclodextrins (Corrigan and Stanley, 1982; Jones and Parr, 1987; Bekers et al., 1991).

^1H NMR spectroscopy of PZQ, when complexed with the β-CD, demonstrated a downfield shift of the aromatic ring protons (0.015 ppm) (Fig. 5A). This downfield shift suggests that the benzene ring of the PZQ was interacting with the β-CD, and has been attributed to steric perturbation due to inclusion complex formation (Suzuki and Sasaki, 1979; Uekama et al., 1983; Jones and Parr, 1987). In contrast, the chemical shifts associated with the cyclic aliphatic and pyrazino moieties (Fig. 5A) were insignificant (0.004 and 0.002 ppm, respectively). The chemical shifts associated

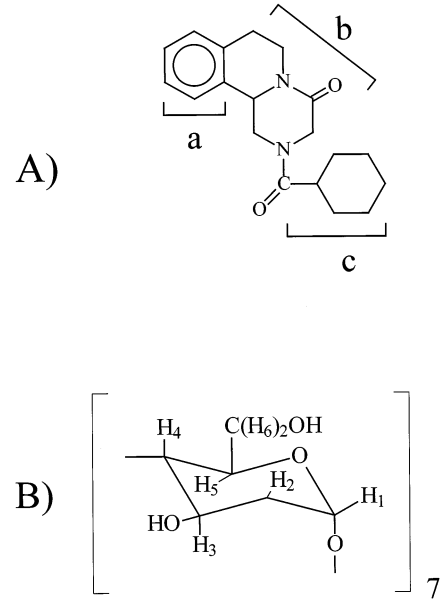

Fig. 5. Chemical structures of praziquantel (A) and β-cyclodextrin (B). Chemical shifts in the praziquantel NMR are grouped into aromatic (a), pyrazino (b) and cyclic aliphatic (c) moieties.

with the β-CD demonstrated upfield movement of the interior dextrin protons H_3 and H_5 (0.041 and 0.166 ppm, respectively) (Fig. 5B). This may be due to an anisotropic shielding effect of the π protons of the aromatic moiety within the CD cavity (Suzuki and Sasaki, 1979; Choi et al., 1992). Furthermore, the greater chemical shift change ($\Delta\delta$) for the H_5 compared to the H_3 protons suggests that β-CD interacts towards PZQ from its primary hydroxyl site in a similar way to chlorhexidine (Qi et al., 1994). The exterior dextrin protons (H_1, H_2, H_4 and H_6) shifted upfield by 0.064, 0.006, 0.003 and 0.013 ppm, respectively. The chemical shifts of the H_1 and H_6 protons (0.064 and 0.013 ppm) suggest that PZQ may also interact with the exterior surface of the β-CD (Suzuki and Sasaki, 1979). Small chemical shift differences were observed for the α- and γ-CD complexes, indicating weaker interactions of PZQ with these CDs.

Computer-aided modelling of PZQ with α-, β- and γ-cyclodextrins, based on low energy conformations and ^{1}H NMR data, suggested inclusion of the benzene moiety of PZQ alone within the cyclodextrin cavity. The depth of penetration was of the order β- > α- > γ-CD, with interaction order consistent with their formation constants. Alternate modelling of the cyclic aliphatic moiety within the cavity led to a less favoured, higher energy conformation after minimisation compared to the proposed type.

3.4. Conclusions

Complexation of PZQ with α-, β- and γ-CDs was confirmed by thermal analysis, NMR, elemental analysis, mass spectrometry and molecular modelling. Furthermore, solubility and dissolution studies suggest that the β-CD is the most suitable of the three complexing agents studied, since it possessed the highest formation complex and acceptable solubility and dissolution profiles. For toxicological reasons, other water-soluble derivatives of β-CD, such as hydroxypropyl-β-cyclodextrin, may be preferred compounds for future development of parenteral formulations.

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