Co-Administration of a Water-Soluble Polymer Increases the Usefulness of Cyclodextrins in Solid Oral Dosage Forms

Jouko Savolainen,1,4 Kristina Järvinen,2 Hannu Taipale,1 Pekka Jarho,1 Thorsteinn Loftsson,3 and Tomi Järvinen1

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Purpose. The aim of this study was to investigate the effect of cyclodextrins (β-CD, HP-β-CD and (SBE)7-β-CD), and co-administration of a water-soluble polymer (HPMC) and cyclodextrins, on the oral bioavailability of glibenclamide in dogs.

Methods. Effects of cyclodextrins on the aqueous solubility of glibenclamide, with and without hydroxypropylmethylcellulose (HPMC), were determined by a phase-solubility method. Solid inclusion complexes were prepared by freeze-drying. Glibenclamide was administered orally and intravenously to beagle dogs.

Results. Aqueous solubility of glibenclamide increased as a function of cyclodextrin concentration, showing an A1-type diagram for β-CD and an A1-type diagrams for both of the β-CD derivatives studied. HPMC enhanced the solubilising effect of cyclodextrins, but did not affect the type of phase-solubility diagram. Orally administered glibenclamide and its physical mixture with HP-β-CD showed poor absolute bioavailability, while orally administered glibenclamide/cyclodextrin complexes significantly enhanced the absolute bioavailability of glibenclamide. Orally administered glibenclamide/β-CD/HPMC and glibenclamide/(SBE)7-β-CD/HPMC complexes showed similar absolute bioavailability compared to formulations not containing HPMC, even though 80% (in the case of (SBE)7-β-CD) or 40% (in the case of β-CD) less cyclodextrin was used.

Conclusions. The oral bioavailability of glibenclamide was significantly increased by cyclodextrin complexation. HPMC increased the solubilising effect of cyclodextrins and, therefore, the amount of cyclodextrin needed in the solid dosage form was significantly reduced by their co-administration. In conclusion, the pharmaceutical usefulness of cyclodextrins in oral administration may be substantially improved by co-administration of a water-soluble polymer.

KEY WORDS: glibenclamide; oral administration; bioavailability; cyclodextrins; hydroxypropylmethylcellulose.

INTRODUCTION

Glibenclamide is an oral hypoglycemic agent of the second generation sulfonylureas, which has been widely used clinically for over 20 years in the treatment of Type-2 (non-insulin dependent) diabetes mellitus. Glibenclamide is practically insoluble in water (1) and, consequently, dissolution of the drug has been considered to be the rate-limiting step for absorption. This limited aqueous solubility may also cause large variations in bioavailabilities between different commercial brands of glibenclamide (2–4) and within one formulation between subjects (3). Absolute bioavailability of oral glibenclamide has rarely been shown due to difficulties in formulating an i.v. solution.

Effects of various cyclodextrins on the solubility of glibenclamide have been studied in vitro (5–7), but only β-CD has been applied in vivo (8). β-CD increased oral absorption of glibenclamide in rabbits, but the poor aqueous solubility of β-CD limits its use in pharmaceutical preparations. Modified β-CD derivatives, such as neutral HP-β-CD and anionic (SBE)7-β-CD might be more suitable than β-CD in pharmaceutical applications because of their safety and pharmaceutical usefulness (9). However, the use of cyclodextrins in solid oral dosage forms is limited to low-dose drugs with large stability constants, due to the mass limitations of oral dosage units (10). The complexation efficiency and solubilising effect of cyclodextrins in aqueous solutions have been increased by addition of water-soluble polymers (11–13), which might be a useful strategy to decrease the amount of cyclodextrin needed in oral dosage forms and, therefore, to increase the pharmaceutical usefulness of cyclodextrins in solid oral dosage forms. The aim of this study was to determine the effects of β-CD and its derivatives, (SBE)7-β-CD and HP-β-CD, and co-administration of cyclodextrin with a water-soluble polymer (HPMC), on the oral bioavailability of glibenclamide.

MATERIALS AND METHODS

Materials

Glibenclamide was purchased from Research Biochemicals (Natick, USA). Sulfoethyl ether β-CD sodium salt (SBE)7-β-CD = Captisol®; average degree of sulfoethyl substitution 7, average MW = 2160 g/mol) was kindly supplied by CyDex, Inc. (Kansas City, USA). Hydroxypropyl-β-cyclodextrin (HP-β-CD = Encapsin®; MW = 1383 g/mol) was obtained from Janssen Biotech N.V (Belgium). β-CD was kindly supplied by Wacker-Chemie (München, Germany). Hydroxypropylmethylcellulose (HPMC; 4000 cp) was purchased from Sigma (Steinheim, Germany). All other materials and solvents used were of analytical reagent grade and used as received.

Phase-Solubility Studies

Effects of cyclodextrins on the solubility of glibenclamide were studied with and without HPMC. In the absence of HPMC, an excess of glibenclamide was added to phosphate buffer solutions (pH 3.0 and 7.4) containing various amounts of (SBE)7-β-CD, HP-β-CD or β-CD. The suspensions were shaken at room temperature for 72 h in order to reach an equilibrium. In the presence of HPMC, an excess of glibenclamide was added to phosphate buffer solutions (pH 7.4) containing various amounts of (SBE)7-β-CD or β-CD and, in same solution, 0.05% (w/v) HPMC-polymer. These suspensions were sonicated at 70°C in an ultrasonic bath for 3 h (glibenclamide remained stable during the sonication) and the samples were...
shaken at room temperature for 72 h. The pH of the suspensions, with and without HPMC, was held constant by adding HCl or NaOH, if necessary. After equilibration, the suspensions were filtered and concentration of glibenclamide was analyzed by HPLC with UV detection.

The HPLC used for determination of in vitro samples contained a Beckman System Gold programmable Solvent Module 116, a Beckman Detector Module 166 with variable wavelength UV detector (set at 203 nm), a System Gold data module (Beckman Instruments Inc., San Ramon, USA), a Marathom autosampler equipped with column thermostat (Spark Holland, Emmen, The Netherlands) and a Rheodyne injection valve with a 20 μl loop. Separations were performed with a Kromasil C8 reverse-phase column (15 cm × 4.6 mm i.d., 5 μm) which was obtained from Higgins Analytical Inc. (CA, USA). The chromatographic conditions were as follows: injection volume, 20 μl; column temperature, 40°C; flow rate, isocratic at 1.0 ml/min. The mobile phase used consisted of 33% (v/v) monobasic potassium phosphate buffer (0.02 M, pH 7.0) in methanol.

Preparation of Dosage Forms

Solid complexes of glibenclamide with (SBE)_{n=7}β-CD and HP-β-CD were prepared by dissolving the maximum amount of glibenclamide in 72.3 mM cyclodextrin solutions (0.05 M phosphate buffer, pH 7.4). In the presence of HPMC, complex was prepared by dissolving the maximum amount of glibenclamide in a 72.3 mM (SBE)_{n=7}β-CD and 0.05% (w/v) HPMC solution. The solid complexes of glibenclamide with β-CD (8.8 mM) were prepared in the presence and absence of 0.05% (w/v) HPMC. HPMC-containing solutions were sonicated 3 h (70°C) and shaken at room temperature for 72 h. The experimental conditions were selected on the basis of earlier studies (11–13). In the absence of HPMC, the solutions were shaken at room temperature until clear solutions were obtained (12–24 h). All solutions mentioned above were freeze-dried and the content of glibenclamide was determined by HPLC. Freeze-dried products were placed in hard gelatin capsules (No. 0; 0.00 or 0.000). For practical reasons (glibenclamide is practically water-insoluble), glibenclamide was used as received when the cyclodextrin-free glibenclamide capsule and the physical mixture of glibenclamide and HP-β-CD were prepared. The physical mixture was prepared by mixing 3.0 mg of glibenclamide and 200 mg of HP-β-CD and the resulting powder then placed into a hard gelatin capsule (No. 00). A pure glibenclamide product was prepared by weighting 3.0 mg of glibenclamide into a hard gelatin capsule (No. 0).

The solution for i.v. administration was prepared by dissolving glibenclamide into a 72.3 mM HP-β-CD solution of 0.05 M phosphate buffer (pH 7.4) (glibenclamide concentration of the i.v. solution was 0.6 mg/ml). This solution was filtered through a sterile membrane (pore size 0.22 μm) before making it isotonic by adding an appropriate amount of NaCl. The concentration of glibenclamide in solution was then analysed by HPLC. Just prior to i.v. administration the solution was again filtered through a sterile membrane filter.

In Vivo Absorption Studies

Four male beagle dogs (weighting 10.5–12.3 kg) were used as experimental animals. The dogs were fasted overnight prior to administration of the drug. During all experiments, water was allowed ad libitum and the dogs were fed 4 hours after dosing. The oral capsules were administered in a randomized crossover design with at least a 2 week wash-out period between doses. The research adhered to the "Principles of Laboratory Animal Care."

Five milliliters of the i.v. solution (equal to 3.0 mg of glibenclamide) was injected directly into the cephalic vein of the conscious dogs in the i.v. study. Gelatin capsules containing various glibenclamide formulations (equal to 3.0 mg of glibenclamide) were administered orally, followed by 20 ml of water.

Blood samples of 3–5 ml were withdrawn from the cephalic saphenous or jugular vein just prior to (blank plasma), and 0.25, 0.5, 1, 2, 4, 6, 8, and 24 hours after, oral administration, and 2, 6, 10, 20, 40 min and 1, 2, 4, 6, 8, and 24 h after i.v. injection of the drug. Blood samples were centrifuged for 15 min, at 3000 rpm. After centrifugation, the plasma was withdrawn and stored at −20°C until analysed.

Analytical Procedure for the In Vivo Samples

The HPLC used for determination of in vivo samples, contained a Merck Hitachi L-6200A Intelligent Pump, a Hewlett Packard HP1046-A Programmable Fluorescence Detector (excitation at 225 nm; emission at 374 nm), Merck Hitachi D-6000 A Interface module, Merck Hitachi AS-2000 Autosampler and a Merck LaChrom column oven L-7350. Separations were performed with a Purospher RP-18 reverse-phase column (12.5 cm × 4.0 mm i.d., 5μm) which was obtained from Merck (Darmstadt, Germany). The chromatographic conditions were as follows: injection volume, 50μl; column temperature, 40°C; flow rate, isocratic at 1.0 ml/min. The mobile phase consisted of a 43% (v/v) monobasic potassium phosphate buffer (0.02 M, pH 5.0) in methanol.

Diazepam (internal standard) was added to plasma. Glibenclamide and the internal standard were extracted from plasma using Bond Elut C18 (6 CC/500 mg) solid phase extraction cartridges (Analytech International, Harbor City CA, USA); acetonitrile was used as an eluent. The samples were then evaporated and the residues dissolved in the mobile phase, before HPLC injection. The results were calculated from peak-area ratios.

A standard curve was prepared by spiking blank plasma with a known amount of glibenclamide (26.1 ± 500 ng/ml) and internal standard (diazepam). The standard curve showed excellent linearity (r² = 0.999) which made one-point calibration feasible. Two separate spiked plasma standard samples were prepared daily for each dog.

Intraday precision (coefficient of variation) of the method was assessed by extracting and analysing plasma samples, containing 26.1 ng/ml and 219.6 ng/ml of glibenclamide, three times in one day. The intraday precision was 2.1% (219 ng/ml) and 3.6% (26.1 ng/ml).

In Vivo Data Analysis

The maximum plasma concentration (Cmax) and the time required to reach the maximum (tmax) were obtained directly from the plasma concentration versus time data. Glibenclamide plasma levels (C) versus time (t) curves were best described by the bi-exponential equation, C = Ae^{−kt1} + Ae^{−kt2}. Results