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Research Paper

Improving glyburide solubility and dissolution by complexation with hydroxybutenyl-β-cyclodextrin

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Abstract

Objectives Glyburide, an important drug for type 2 diabetes, has extremely poor aqueous solubility and resulting low bioavailability. This study describes the ability of hydroxybutenyl-β-cyclodextrin (HBenBCD) to form complexes with glyburide, with enhanced solubility and dissolution rate *in vitro*.

Method Glyburide and glyburide–HBenBCD were evaluated in various test media known to simulate human gastrointestinal conditions in the fasted and fed states, respectively.

Key findings At ~14 wt% drug load, in the presence of HBenBCD, an almost 400-fold increase in glyburide aqueous solubility was observed. In the presence of HBenBCD, glyburide solubility was also significantly improved in all physiologically relevant test media. Subsequent dissolution experiments confirmed the solubility study results; the dissolution rate and total amount of drug released were significantly increased.

Conclusions Complexation with HBenBCD may be an effective way to increase the bioavailability of glyburide.

Keywords bioavailability; β-cyclodextrin; drug complexation; drug solubility; glyburide

Introduction

In order to be bioavailable, orally administered drugs (e.g. tablets or capsules) must enter the systemic circulation, a process that requires dissolution and subsequent permeation through gastrointestinal membranes. Thus, a Biopharmaceutics Classification System (BCS) class I drug,[1] featuring high solubility and high permeability, may be considered as an 'ideal' drug. However, most new chemical entities exhibit poor or very poor solubility and, as a result, they are commonly classified as either BCS class II (good permeability) or BCS class IV (low permeability) drugs. Although they exhibit good permeability, BCS class II drugs are often a challenge to formulate; their intrinsic solubility and dissolution profiles regulate bioavailability. Therefore, developing novel drug delivery systems that mitigate solubility, dissolution and bioavailability issues are important.

Various methods have been described to enhance the solubility of poorly soluble drugs (e.g. the use of pro-drugs, the addition of surfactants, salt selection, solid dispersions and particle size reduction).[2–5] In addition, cyclodextrins, macrocyclic oligosaccharides, represent an important group of excipients used for this purpose. Cyclodextrins typically contain 6, 7 or 8 glucose monomers joined by α-1,4 linkages; these oligomers are commonly called α-, β- and γ-cyclodextrin, respectively. Because of their unique structure, with a hydrophobic cavity and a hydrophilic exterior, they can act as a host and form inclusion complexes with a variety of guest molecules. The cavity of β-cyclodextrin, one of the most common natural cyclodextrins, has an archetypal size to form complexes with many active pharmaceutical ingredients.[6] In general, upon complexation with an active pharmaceutical ingredient, the cyclodextrin will increase the solubility of its sparingly soluble guest molecule. If the drug belongs to BCS class II, complexation should result in increased bioavailability,[7,8] or at least modify the plasma/blood drug concentration profile.[9] In addition, the cyclodextrin component often affords additional beneficial properties (e.g. stabilisation of unstable active

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pharmaceutical ingredients, taste masking).[10–12] However, β-cyclodextrin usage has some negative effects that should be considered, particularly in parenteral administrations where it may result in renal and liver damage.[13,14] Moreover, β-cyclodextrin has limited aqueous solubility and is therefore often not sufficient to overcome various active pharmaceutical ingredient issues.[15]

In order to improve the aqueous solubility and safety profile of β-cyclodextrin, a number of cyclodextrin derivatives ranging from neutral through anionic, and cationic to zwitterionic have been examined.[16–18] Fortunately, the aqueous solubility of unmodified cyclodextrins can be significantly increased by adding a few substituents to the anhydroglucose hydroxyl groups, resulting in disruption of crystallinity. Recently, the synthesis and characterisation of a novel cyclodextrin, hydroxybutenyl-β-cyclodextrin (HBenBCD; Figure 1), were described[6] and various in-vitro and in-vivo studies were conducted.[5,9,19–22] As an amorphous white solid (Tg ~210°C), HBenBCD is highly soluble in water (>500 g/L) and soluble in organic solvents such as polyethylene glycol 400 (>400 g/L).[6] HBenBCD may solubilise a broad spectrum of drugs, including glyburide,[6] letrozole,[9] tamoxifen,[19] itraconazole,[5] raloxifene[22] and saquinavir.[21] However, the majority of the cited studies investigated drug solubility with and without HBenBCD in water, various aqueous buffer systems, ethanol, and/or propylene glycol, media that may be useful in terms of screening drug solubility by means of complexation with HBenBCD but that are not physiologically relevant. Therefore, the objective of the present study was to prepare a glyburide–HBenBCD complex and investigate how the complex may alter glyburide solubility and dissolution performance under physiologically relevant conditions. The aim was to effectively formulate a BCS class II drug[23–25] by complexation with HBenBCD that will behave like a BCS class I drug.

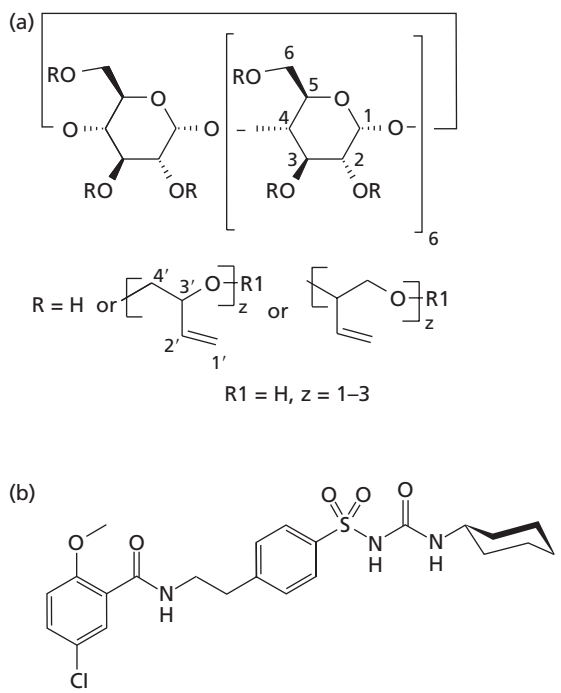

Figure 1 Representative structures of (a) hydroxybutenyl-β-cyclodextrin and (b) glyburide.

Diabetes mellitus is a metabolic disorder characterised by chronic hyperglycaemia with disturbances in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.[26] The World Health Organization estimates that more than 180 million people worldwide have diabetes, a number that is predicted to double by 2030.[27] About 90% of these people have type 2 diabetes. Besides biguanides, current type 2 diabetes therapy employs sulfonylureas. However, most sulfonylureas (e.g. glyburide, glibornuride, gliclazide, glimepiride, glipizide and gliquidon) have low water solubility, which typically translates into low oral bioavailability. Low oral bioavailability can have a negative impact on efficacy, side-effects, inter- and intra-patient variability and, ultimately, drug utility. Glyburide, a weakly acidic (pKa 5.3) second-generation sulfonylurea (Figure 1),[28] has very poor water solubility (intrinsic solubility ~0.06 μg/ml).[29] As a crystalline compound, glyburide has a melting temperature of 172–174°C and a log P value of 4.8 at neutral pH.[30] Glyburide also has limited solubility in organic solvents, with CH_2Cl_2 being the preferred solvent. Due to its poor solubility and high permeability, glyburide has been classified as a BCS class II drug. As is typical in the case of BCS class II compounds, solubilised glyburide will be readily absorbed across the gastrointestinal mucosa. Therefore, glyburide plasma time course profiles reflect the dissolution behaviour of the drug and, consequently, glyburide pharmacokinetic parameters are strongly formulation dependent.[31] The absolute bioavailability of crystalline glyburide has been reported as 14.7% in dogs.[32] The oral bioavailability in humans can be as high as 100% but strongly depends on the crystallinity and particle size distribution of the drug.[33] Thus, to increase the oral bioavailability, some marketed tablet formulations contain the micronised drug.

It is recommended that the typical human glyburide daily dose (2.5–5.0 mg) be consumed with breakfast. However, glyburide may be titrated up to 15 mg daily as necessary. In most countries, glyburide is available as a traditional tablet formulation (e.g. Euglucon N; Aventis Pharma GmbH, Frankfurt, Germany). Additionally, in some countries, micronised glyburide preparations (e.g. Glynase PresTab; Pfizer/Pharmacia and Upjohn Company, New York, NY, US) are available. The latter formulations, intended to enhance bioavailability, have smaller particle size. Since there continues to be a need to develop safe and reliable oral antihyperglycaemic treatments, glyburide had the optimal drug characteristics (i.e. strongly dissolution rate controlled) for the present study, which was designed to determine the impact of HBenBCD on the overall solubility and the dissolution rate of glyburide.

The preparation of a solid glyburide–HBenBCD inclusion complex is described, together with various studies probing glyburide solubility, glyburide dissolution using glyburide, a glyburide–HBenBCD complex, and a commercial formulation (Euglucon N) in various test media including both BCS-conform and biorelevant media known to simulate human gastrointestinal conditions in the fasted and fed states.[34,35]

Materials and Methods

Materials

HBenBCD (molar substitution 4.7) was prepared according to previously described methods.[6] Before use, HBenBCD was dried at 10–15 mmHg at room temperature (22 ± 1°C) for 12–60 h. Crystalline glyburide (lot no. 024K0701) was purchased from Sigma-Aldrich Co. (Steinheim, Germany). Euglucon N (lot no. 40E500) tablets (Aventis Pharma Deutschland GmbH, Frankfurt, Germany) were obtained by prescription and used without modification.

Water used to prepare the complex was filtered through a Milli-Q Water System equipped with a 0.22-μm sterile biofilter (Millipore, Billerica, MA, US); the water had very low total organic and pyrogen content, and low ionic strength. The water was not degassed before use and typically had a pH of 4.7. All glassware and tools used for the preparation of the complex were washed extensively with water and then with absolute EtOH and dried (1–24 h) at 115°C.

White opaque gelatin capsules (Wepa Nr. 35758 according to DAB 10; size 1; 0.5 ml) were purchased from Wepa Apothekenbedarf GmbH & Co KG (Hillscheid, Germany).

Buffers were prepared using Millipore water. Sodium taurocholate (PCA code 2012; lot no. 2003040161) was acquired from Prodotti Chimici e Alimentari S.P.A. (Basaluzzo (AL), Italy). Egg-phosphatidylcholine, Lipoid EPCS (99.1% pure), was kindly donated by Lipoid GmbH (Ludwigshafen, Germany). All other compounds were of analytical grade and were purchased commercially.

Preparation of the glyburide–HBenBCD complex

The exact methods useful for the preparation of a drug–cyclodextrin complex are highly dependent on the drug, cyclodextrin and how the formulation may be used. The following procedure was used to prepare the glyburide–HBenBCD complex described in this study.

An aqueous HBenBCD solution (54.6 wt%) was prepared by dissolving dried HBenBCD (6.00 g) in water (5.00 g). A second solution was prepared by first dissolving ammonium carbonate (300 mg) in purified water (14.7 ml) and mixing this solution with absolute ethanol (22.5 ml). The resulting mixture was added to a vial containing glyburide (1.00 g). This mixture was sonicated (50°C, 2 h; Transsonic Digital T 700 H; Elma, Singen, Germany) until a transparent solution was obtained and then stirred on a magnetic stirrer (MR 2001 K; Heidolph Instruments, Schwabach, Germany), applying a stirring speed of 800 rpm until equilibration to room temperature (22 ± 1°C). The aqueous HBenBCD solution was then slowly added to the glyburide solution, with vigorous mixing, to afford a slightly yellow, homogeneous solution. The resulting solution was filtered (0.45-μm filter) into a freeze-dry flask. Freeze drying at approximately −45°C, 130 mTorr, gave 6.66 g of a white powder with a drug load of 14.2 wt% glyburide determined by high performance liquid chromatography (HPLC).

Thermal analysis

Glyburide and the glyburide–HBenBCD complex were analysed directly after preparation by thermal analysis using a differential scanning calorimeter DSC 7 with a thermal analysis controller TAC 7 (Perkin Elmer, Ueberlingen, Germany). The samples (3–4 mg) were placed directly in perforated aluminium pans and heated to 50–220°C at a rate of 10°C/min under a nitrogen atmosphere.

Selection of test media

It has been shown, particularly for poorly soluble drugs, that dosing conditions are crucial for various pharmacokinetic parameters, particularly the area under the curve and maximum serum concentration.[36] Therefore, it was important to adequately simulate the gastrointestinal environment, assuming different dosing conditions. Another objective was to determine the solubility under BCS-conform conditions, namely at pH 1.2, 4.5 and 6.8, and to determine whether complexation with HBenBCD can shift the dissolution characteristics of glyburide from BCS class II to that of a BCS class I formulation. Thus, compendial and biorelevant test media (Table 1), used in both the solubility and dissolution experiments, were selected to represent different physicochemical conditions that appear in the human upper gastrointestinal tract, conditions that may influence glyburide solubility and formulation dissolution characteristics. All media were prepared according to standard procedures and appropriate precautions were taken to ensure their integrity.[35,37] The pharmacopoeial buffers, simulated gastric fluid without pepsin (SGFsp USP 28 pH 1.2), acetate buffer USP 28 pH 4.5, and simulated intestinal fluid without

Table 1 Biorelevant test media

Test medium	pH	mOsmol/kg	Gastrointestinal segment	Type of medium
Water			Not representative	Compendial
SGFsp USP 28	1.2	182	Stomach	Compendial and BCS-conform
Acetate buffer	5.0	10	Stomach	Biorelevant (pH)
Blank FaSSIF	6.5	266	Upper small intestine, fasted	Biorelevant (pH)
FaSSIF[a]	6.5	273	Upper small intestine, fasted	Biorelevant
Blank FeSSIF	5.0	622	Upper small intestine, fed	Biorelevant (pH)
FeSSIF[a]	5.0	646	Upper small intestine, fed	Biorelevant
Acetate buffer	4.5	291	Duodenum	Compendial and BCS-conform
SIFsp USP 28	6.8	100	Small intestine	Compendial and BCS-conform

Biorelevant test media used for solubility studies and dissolution experiments. SGFsp, simulated gastric fluid without pepsin; FaSSIF and FeSSIF, fasted and fed state simulated intestinal fluid, respectively; SIFsp, simulated intestinal fluid without pancreatin. BCS, Biopharmaceutics Classification System.
[a]Contains physiological concentrations of bile components.

pancreatin (SIFsp USP 28 pH 6.8) represent average pH conditions in the human stomach, duodenum and jejunum, respectively, experimental conditions commonly used to determine drug solubility (the observed solubility in this case) in terms of BCS classification according to the US Food and Drug Administration and World Health Organization guidelines.[38,39] The set of gastric media was completed by an acetate buffer (pH 5.0) to represent conditions that help to address an elevated gastric pH environment typical with elderly patients and/or due to co-therapy with proton pump inhibitors and/or H2 receptor antagonists.[40]

The biorelevant test media, fasted and fed state simulated intestinal fluid (FaSSIF and FeSSIF, respectively),[34,35] and their corresponding blank buffers, were used to determine solubility under upper small intestinal pH conditions in the fasted and the fed state, respectively. These media conditions also help determine whether amphiphilic bile components, including bile salts and lecithin, in concentrations typically present in the fasted state or following a meal, influence in-vitro drug solubility and dissolution rate.

Solubility studies

Solubility measurements ($n = 6$ in each medium) were made using a modified version of the traditional shaking flask method.[41] To each well of a 2-ml 96-well polypropylene mixing plate (Axygen Scientific, Union City, CA, US) an excess of drug (~1–2 mg) or a corresponding amount of glyburide–HBenBCD inclusion complex (14.2 wt% glyburide) was added to ensure a saturation concentration of the drug in all media. To each well of the pre-loaded 96-well mixing plate was then added water, buffer or biorelevant medium (500 μl). After addition of the test media, the plate was sealed using aluminium foil with a non-volatile adhesive on one surface. The plate was then placed on a rotary shaking plate (Titramax 1000; Heidolph Instruments, Schwabach, Germany) and shaken (800–1200 rpm) at 37°C ± 0.5°C for 48–72 h. Preliminary experiments showed that less than 24 h was required to reach equilibrium. For convenience, and to ensure that the duration of this process was well above the time required to reach equilibrium, long mixing times were used. During the mixing period, the plate was inspected to ensure that each well contained un-dissolved, excess drug. Drug was added to maintain an excess as needed.

Following the mixing period, the solutions in each well were transferred to the corresponding wells of a 2-ml 96-well multiscreen filter plate using a multichannel pipette. The bottom of each well was a hydrophilic membrane (AcroPrep 96 filter plate 0.45 μm GHP; Pall, Ann Arbor, MI, US). The filter plate was placed on top of a vacuum manifold and the solutions were filtered at approximately 20 mmHg into the corresponding wells of a 2-ml storage plate. The duration of the filtration period was typically no longer than 60 s. The storage plate was then sealed with a silicon mat (Axymat AM 2 mL-SQ; Axygen Scientific, Union City, CA, US) and samples were removed for analysis as appropriate.

The drug content in each well was determined by UV spectroscopy using a multiwell plate reader (SpectraMax Plus 384; Molecular Devices, Sunnyvale, CA, US). Typically, drug solution (10–20 μl) was transferred to the corresponding well of a 96-well measurement plate (Greiner UV-STAR plates with a spectral range of 190–400 nm) and, if necessary, diluted with 1/1 water and ethanol so that the absorbance was within the linear response range. It is important to note that in these experiments, if the drug has low absorptivity and if the drug concentration is low, it is necessary to subtract absorbance contributions from the measurement plate and/or buffer from the measured absorbance. Absorbance was converted to drug concentration using the appropriate absorptivity for the drug. Additionally, the final pH of each well was measured to ensure that the pH had not changed significantly due to lack of buffering capacity.

Dissolution studies

Dissolution studies were performed with a paddle apparatus (DT 700 HH; Erweka, Heusenstamm, Germany). The test was designed to correspond with current pharmacopoeia (USP, EP) and BCS-conform dissolution tests, particularly with the one recently proposed as a standard test for immediate-release solid oral dosage forms.[42,39] The cited proposal recommends use of the paddle apparatus with a stirring speed of 75 rpm combined with 500 ml of test medium. Experiments were run at 37.0 ± 0.5°C. Before testing, the compounds were exactly weighed into white opaque gelatin capsules. The test dose was 3.5 mg glyburide or the corresponding amount of glyburide–HBenBCD complex (24.7 mg). No further excipients were used to fill the capsules. After filling, to keep the dosage form under the paddle until it dissolved, a helix wire sinker was fixed to the capsule. To investigate whether the in-vitro performance of the glyburide inclusion complex was equal to or superior to that of a marketed formulation, an additional set of experiments was performed using Euglucon N tablets (3.5 mg glyburide).

Experiments were run in triplicate; samples (5.0 ml) were removed after 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180 and 240 min and each was replaced with the same volume of blank, pre-warmed medium. Following appropriate dilution, samples were analysed by HPLC.

HPLC analysis

The HPLC system consisted of a L6220 pump, an AS2000A autosampler, an L4500 diode array detector and a D6000A Interface (Merck Hitachi, Darmstadt, Germany). Before analysis, all samples were diluted 1 : 2 with mobile phase. The analyses were performed on a 125/4 ET Nucleosil 120-5 C18 column (Macherey-Nagel, Dueren, Germany), using a mixture of acetonitrile and purified water (65 : 35) adjusted to pH 3.0 with phosphoric acid as the mobile phase. The flow rate was set at 1.0 ml/min and the amount of released drug was determined using a wavelength of 230 nm. The results were expressed as mean % dissolved at the given sampling time. The HPLC method had been validated before use. For all media, the linearity of this method was shown over a concentration range of 0.288–5.76 μg/ml ($y = 85796x + 1706.8$, $r^2 = 0.9999$). The limits of detection and quantification were 0.144 μg/ml and 0.288 μg/ml, respectively. The coefficient of variation of the intraday precision was less than 1.5% and was less than 0.6% for the accuracy. The recovery of the method was found to be 101.7–104.1%.

Statistical analysis

Prism 5.01TM (GraphPad Software, Inc., San Diego, CA, US) was used for statistical analysis. Statistical analysis of the effects of glyburide and the glyburide–HBenBCD complex in water versus biorelevant media was performed using one-way analysis of variance followed by a Dunn's post-test. Glyburide versus glyburide–HBenBCD complex in biorelevant media was analysed using an unpaired *t*-test with Welch's correction with a two-tailed *P* value test at the 95% confidence level. The effects of time and pH on Euglucon and glyburide–HBenBCD dissolution were compared by conducting repeated measures tests with a Friedman (non-parametric) test followed by a Dunn's post-test at the 95% confidence level. The effects of specific test media after 240 min between Euglucon and glyburide–HBenBCD were compared using paired *t*-tests (two-tailed *P* values). A significance level of $P < 0.05$ denotes significance in all cases.

Results

Thermal analysis

Thermal analysis indicated a true drug complex; the differential scanning calorimeter spectra of glyburide and the glyburide–HBenBCD complex revealed the presence and absence of a melting endotherm, respectively.

Solubility studies

Glyburide and glyburide–HBenBCD complex solubility (37°C) data in BCS-conform and biorelevant media are summarised in Figure 2. In water, HBenBCD significantly increased glyburide solubility (from 4.5 to 1700 μg/ml; $P < 0.001$). In fact, the presence of HBenBCD caused a statistically significant increase in glyburide solubility for all tested media ($P < 0.001$). Since many drug formulations are ingested with a glass of water, water may be thought of as an attractive medium for in-vitro solubility studies. However, aqueous solubility should not be used to predict drug solubility *in vivo*; depending on the source, the purity and pH of water vary and water also lacks an adequate buffer capacity. Particularly for ionisable molecules such as glyburide, water solubility results may therefore be far from those obtained *in vivo*,[43] as the drug particle surface pH may change due to drug dissolution or excipients (if present). This can result in either more or less favourable pH conditions close to the drug surface and may have a huge impact on the corresponding solubility results. To better predict in-vivo drug solubility, BCS-conform, compendial and biorelevant test media (Table 1) were applied. However, in the absence of HBenBCD, acetate buffer (pH 4.5 and 5.0), SGFsp (pH 1.2), blank FaSSIF (pH 6.5) and blank FeSSIF (pH 5.0) offered no significant increases in glyburide solubility relative to water (Figure 2). In fact, in the absence of HBenBCD, only two media produced statistically significant increases in glyburide solubility relative to water: FaSSIF pH 6.5 ($P < 0.01$) and SIFsp pH 6.8 ($P < 0.05$).

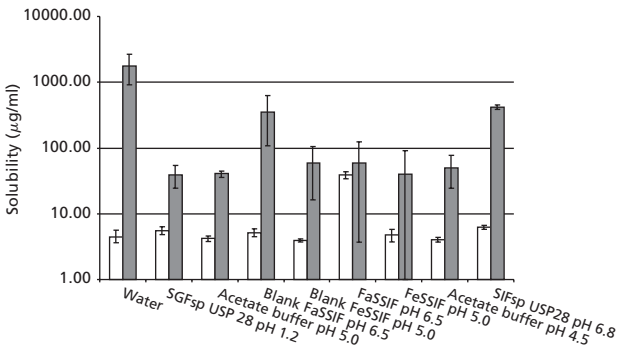

Figure 2 Solubility data for glyburide and glyburide–hydroxybutenyl-β-cyclodextrin complex. Solubility of glyburide (□) and glyburide–hydroxybutenyl-β-cyclodextrin complex (■) in biorelevant, Biopharmaceutics Classification System-conform and compendial media at 37°C ($n = 6 \pm$ SD). SGFsp, simulated gastric fluid without pepsin; FaSSIF and FeSSIF, fasted and fed state simulated intestinal fluid, respectively; SIFsp, simulated intestinal fluid without pancreatin.

The poor solubility of the pure drug in most of the test media is also reflected in the dose–solubility ratios, which, assuming both a 3.5-mg 'standard' dose and the highest dose of 5.0 mg, are higher than 250 ml in all simple buffer media (Table 2); these results reflect the BCS class II drug properties. A dose–solubility ratio less than 250 ml, indicating high drug solubility,[38,39] was only observed in FaSSIF pH 6.5 (90 ml). This result was most likely owing to favourable pH conditions and the presence of bile components, which helped to solubilise glyburide.

The glyburide–HBenBCD complex data (Figure 2; Table 2) illustrate that HBenBCD provides a massive increase in glyburide equilibration solubility. In all test media, the solubility of the glyburide–HBenBCD complex was by far greater than that of the pure drug. In the presence of HBenBCD, all solubility values were statistically increased. The best glyburide–HBenBCD solubility results were obtained in water. However, as already mentioned, water solubility results are typically not representative of the in-vivo solubility of a drug. As expected for a weakly acidic drug, the glyburide–HBenBCD complex solubility in biorelevant media was best under simulated intestinal conditions. As the test medium pH approached the pKa of glyburide (i.e. blank FeSSIF pH 5.0 and FeSSIF pH 5.0), the solubility of glyburide diminished, but it was still far better than in the absence of HBenBCD. Compared with SGFsp pH 1.2, the solubility in blank FaSSIF pH 6.5, FaSSIF pH 6.5 and SIFsp pH 6.8 was statistically increased ($P < 0.01$).

The calculated dose–solubility ratio for the glyburide–HBenBCD complex (Table 2) resulted in values less than 250 ml for both the standard dose (3.5 mg) and the highest dose in all test media, including the BCS-conform media. The latter results indicate a shift in the solubility characteristics from BCS class II to class I after HBenBCD complexation.

Dissolution studies

Glyburide (3.5 mg) dissolution ($n = 3$; 37°C) from gelatin capsules was measured in compendial and biorelevant media. The results confirmed the poor aqueous solubility of glyburide (data not shown). In contrast to the solubility studies, even glyburide in FaSSIF pH 6.5 showed poor dissolution characteristics. In all dissolution media tested, the total amount of glyburide dissolved after 4 h was less than 5%; the greatest

Table 2 Dose–solubility ratios for glyburide and the glyburide–hydroxybutenyl-β-cyclodextrin complex

Test medium	pH	Dose–solubility ratio (ml)			
		For a 3.5-mg dose		For a 5-mg dose	
		Glyburide	Glyburide–HBenBCD complex	Glyburide	Glyburide–HBenBCD complex
Water		788	2	1126	3
SGFsp USP 28[a]	1.2	627	90	896	129
Acetate buffer	5.0	833	86	1190	123
Blank FaSSIF	6.5	676	10	966	14
FaSSIF	6.5	90	59	128	84
Blank FeSSIF	5.0	894	59	1277	85
FeSSIF	5.0	732	88	1046	125
Acetate buffer[a]	4.5	875	70	1250	101
SIFsp USP 28[a]	6.8	556	8	794	12

Glyburide and glyburide–hydroxybutenyl-β-cyclodextrin (HBenBCD) complex dose–solubility ratios calculated for a 3.5- or 5.0-mg dose in different test media. SGFsp, simulated gastric fluid without pepsin; FaSSIF and FeSSIF, fasted and fed state simulated intestinal fluid, respectively (contains physiological concentrations of bile components); SIFsp, simulated intestinal fluid without pancreatin.
[a]Compendial and Biopharmaceutics Classification System-conform test media.

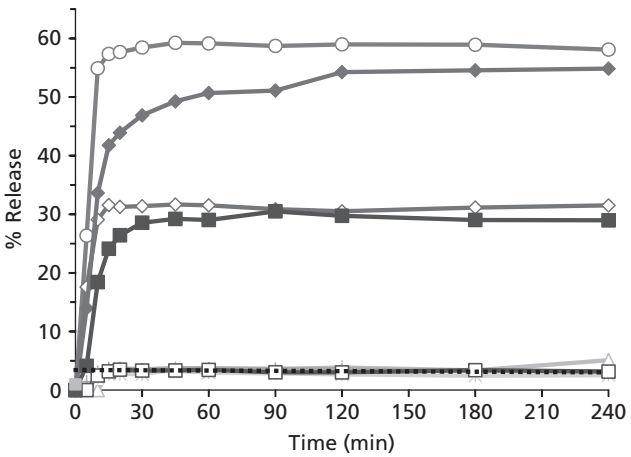

Figure 3 Glyburide dissolution from Euglucon N tablets. Glyburide dissolution (37°C) from Euglucon N 3.5 mg tablets in compendial and biorelevant media (the dotted line indicates the maximum dissolution of the pure drug from a gelatin capsule in all media) (n = 3 ± SD). Simulated gastric fluid without pepsin pH 1.2 (△); acetate buffer pH 4.5 (∗); acetate buffer pH 5.0 (+); simulated intestinal fluid without pancreatin pH 6.8 (○); blank fasted state simulated intestinal fluid (FaSSIF) pH 6.5 (◇); FaSSIF pH 6.5 (◆); blank fed state simulated intestinal fluid (FeSSIF) pH 5.0 (□); FeSSIF pH 5.0 (■).

percentage dissolution, albeit still very low, was observed in blank FeSSIF pH 5.0.

Dissolution experiments using the marketed formulation, Euglucon N (Figure 3), resulted in a significantly greater dissolution rate and total amount of drug released (compared with SGFsp pH 1.2) in certain media (i.e. SIFsp pH 6.8; $P < 0.001$; FaSSIF pH 6.5 $P < 0.001$; blank FaSSIF pH 6.5 $P < 0.01$). Hence, the impact of pH was significant at ≥ 6.5. Dissolution was best under small-intestinal conditions, and the highest total amount released was observed in SIFsp pH 6.8. Small-intestinal pH conditions enable the ionisation of glyburide, which in turn results in greater hydrophilicity of the compound. Furthermore, bile components have a huge impact on the total amount of drug released (FaSSIF pH 6.5 >> blank FaSSIF pH 6.5; FeSSIF pH 5.0 >> blank FeSSIF pH 5.0). Bile components act as solubilising agents that help the drug go into and remain in solution. As observed in the solubility studies of the pure drug, dissolution was better under fasted state conditions since the intraluminal pH of the small intestine is higher and therefore more favourable for glyburide dissolution. Compared with the dissolution behaviour of the pure drug, the increased dissolution rate of the marketed formulation is most likely a result of the presence of the smaller glyburide particles in Euglucon N and/or further excipients used in the formulation. However, while glyburide release was very rapid, even at the highest pH (6.8), the total amount of dissolved drug did not exceed 60% of the dose.

The dissolution profiles obtained from glyburide formulated with HBenBCD in powder-filled gelatin capsules are summarised in Figure 4. It is obvious that the dissolution performance of the glyburide–HBenBCD complex was superior to that of pure drug and the marketed formulation. Dissolution of glyburide from the capsules filled with glyburide–HBenBCD complex was rapid at pH 6.8 (~100% in 30 min) and precipitation of glyburide was not observed over the lifetime of this experiment. Similar drug release results were obtained in blank FaSSIF pH 6.5 and FaSSIF pH 6.5, and suggest that dissolution characteristics will scarcely be influenced by the presence of bile components. As the total amount of glyburide released can significantly affect the bioavailability of this BCS class II drug,[32,44,45] one may predict that complete dissolution in the small intestine from HBenBCD complex will result in a remarkable increase in glyburide bioavailability. Compared with glyburide, drug release from the glyburide–HBenBCD complex in SGFsp pH 1.2, in all of the test media except FeSSIF pH 5.0 and acetate buffer 4.5, was significantly increased ($P < 0.001$ for SIFsp pH 6.8, FaSSIF pH 6.5, blank FaSSIF pH 6.5 and acetate buffer pH 5.0; $P < 0.05$ for blank FeSSIF pH 5.0). In addition, compared with Euglucon N (Figure 3), glyburide

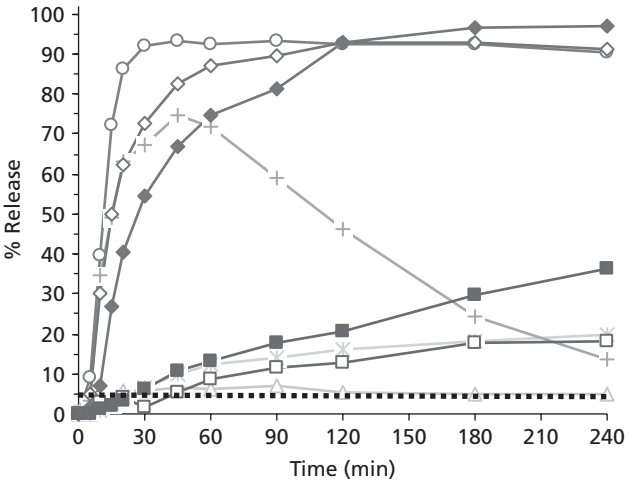

Figure 4 Glyburide dissolution from gelatin capsules filled with solid glyburide–hydroxybutenyl-β-cyclodextrin complex. Glyburide dissolution (37°C) from gelatin capsules filled with 24.7 mg solid glyburide–hydroxybutenyl-β-cyclodextrin complex (≅3.5 mg pure drug) in compendial and biorelevant media (the dotted line indicates the maximum dissolution of the pure drug from a gelatin capsule in all media) (n = 3 ± SD). Simulated gastric fluid without pepsin pH 1.2 (△); acetate buffer pH 4.5 (⁎); acetate buffer pH 5.0 (+); simulated intestinal fluid without pancreatin pH 6.8 (○); blank fasted state simulated intestinal fluid (FaSSIF) pH 6.5 (◇); FaSSIF pH 6.5 (◆); blank fed state simulated intestinal fluid (FeSSIF) pH 5.0 (□); FeSSIF pH 5.0 (■).

dissolution from glyburide–HBenBCD (Figure 4) at 240 min was statistically greater in SIFsp pH 6.8, acetate buffer pH 4.5, blank FeSSIF pH 5.0, FaSSIF pH 6.5, blank FaSSIF pH 6.5 and acetate buffer pH 5.0; all $P < 0.001$ except acetate buffer pH 5.0 ($P < 0.01$) and blank FeSSIF pH 5.0 ($P < 0.05$).

Discussion

Results from the glyburide solubility experiments in both compendial and biorelevant media reflect the poor solubility of this antidiabetic drug. However, the glyburide–HBenBCD complex solubility data illustrate that by using HBenBCD as a complexing agent, it is possible to overcome these solubility issues. Particularly, the solubility results in biorelevant test media predict that independent of the dosing conditions, the entire glyburide–HBenBCD dose should dissolve in the upper gastrointestinal tract.

Results of the dissolution experiments are in good agreement with the observations made in the solubility studies. The pure drug showed poor dissolution characteristics even under the most favourable pH conditions. Under corresponding test conditions, the dissolution performance of a marketed formulation was better, but drug release was also not complete. In contrast, dissolution results obtained from the glyburide–HBenBCD complex suggest that the entire dose will completely dissolve in the fasted small intestine. Simulation of gastric and duodenal conditions resulted in lower, but controlled, release rates, and the resulting drug solutions were stable over the test duration of 4 h; these data indicate that the amount of released drug would not be expected to precipitate in the stomach or in the duodenum. Glyburide began to precipitate (after 45 min) only from acetate buffer pH 5.0, a medium that was used to address a typical gastric environment of patients with elevated gastric pH. However, this observation may be expected: this buffer displays the least favourable pH condition for glyburide, possessing no sufficient buffer capacity and having a pH (5.0) close to the pKa (5.3). Moreover, compared with the dissolution results of the pure drug (values were all < 5%; data not shown) and the marketed formulation, it is impressive that HBenBCD complexation afforded extensive dissolution enhancement. In this context, it is important to mention in-vivo conditions where the dissolved drug can continuously exit the stomach through the pylorus. As the pH conditions become most favourable in the small intestine, the main site of glyburide absorption, it is most likely that the entire glyburide–HBenBCD dose will dissolve and bioavailability problems will not occur; even in the case of an elevated gastric pH, an impact on the bioavailability of the drug complex would not be predicted.

Conclusions

Complexation with HBenBCD significantly improves the solubility and the dissolution behaviour of glyburide. Results from the present study indicate that HBenBCD offers a useful tool in terms of the in-vivo dissolution rate of glyburide and other sulfonylureas with low aqueous solubility, and thus may improve the quality of future oral type 2 diabetes therapies.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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