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Cyclodextrin solubilization of benzodiazepines: formulation of midazolam nasal spray

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Abstract

The cyclodextrin solubilization of three benzodiazepines, i.e. alprazolam, midazolam and triazolam, was investigated. The cyclodextrin solubilization was enhanced through ring-opening of the benzodiazepine rings and ionization of the ring-open forms. Additional enhancement was obtained through interaction of a water-soluble polymer with the cyclodextrin complexes. The ring-opening was pH-dependent and completely reversible, the ring-open forms dominating at low pH but the ring-closed forms at physiologic pH. The ring-closed forms were rapidly regenerated upon elevation of pH. In freshly collected human serum in vitro at 37°C, the half-life for the first-order rate constant for the ring-closing reaction was estimated to be less than 2 min for both alprazolam and midazolam. Midazolam (17 mg/ml) was solubilized in aqueous pH 4.3 nasal formulation containing 14% (w/v) sulfobutylether β-cyclodextrin, 0.1% (w/v) hydroxypropyl methylcellulose, preservatives and buffer salts. Six healthy volunteers received 0.06 mg/kg midazolam intranasally and 2 mg intravenously, and blood samples were collected up to 360 min after the administration. Midazolam was absorbed rapidly reaching maximum serum concentrations of 54.3 ± 5.0 ng/ml at 15 ± 2 min. The elimination half-life of midazolam was 2.2 ± 0.3 h and the absolute availability was 73 ± 7%. All mean values ± SEM.

Keywords: Solubility; Benzodiazepines; Cyclodextrin; Complexation; Ionization

1. Introduction

Most benzodiazepine drugs are derivatives of 2,3-dihydro-1*H*-1,4-benzodiazepine with sedative, antianxiety, anticonvulsant and muscle relaxant properties. In pharmaceutical formulations benzodiazepines are mainly used as the solid base, and as such they are readily dissolved in lipophilic solvents or in polar organic solvents such as ethanol. Formulation of the benzodiazepine bases in aqueous drug formulation has been hampered by their low aqueous solubility and frequently the only practical means of obtaining pharmaceutically acceptable benzodiazepine solutions is

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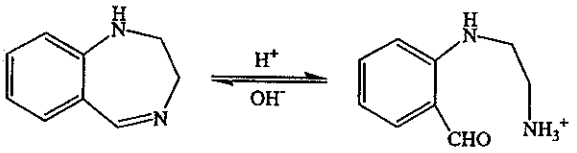
through the use of combinations of cosolvents (Bechgaard et al., 1997; Alvarez Núñes and Yalkowsky, 1998). Unfortunately, administration of non-aqueous drug formulations may result in pain, irritation and drug precipitation upon administration (Yalkowsky and Rubino, 1985; Way and Brazeau, 1999). Replacing the cosolvent formulations with aqueous cyclodextrin containing drug formulations may circumvent these side effects (Brewster et al., 1989; Brewster, 1991; Brewster and Loftsson, 1999). Previously, benzodiazepines have been solubilized through cyclodextrin complexation (Kraus et al., 1991; Loftsson et al., 1994). However, the complexation efficacy is frequently low and, thus, relatively large amounts of cyclodextrin are needed to solubilize small amounts of a given benzodiazepine drug. Increased complexation efficacy can be obtained by increasing either the intrinsic solubility of the drug (S_o) or the apparent stability constant (K_c) of the drug/cyclodextrin complex, or by increasing both simultaneously (Loftsson, 1998; Loftsson et al., 1999). In aqueous solutions, some drugs can exist in more than one structural form, e.g. equilibrium isomers or ionization stages. Although the individual forms are in equilibrium with each other, and thus not totally independent of each other, the overall aqueous solubility (or apparent S_o) of a drug can be enhanced through formation of such multiple structural forms.

Cyclic imines, such as 2,3-dihydro-1*H*-1,4-benzodiazepine, are known to undergo reversible and pH-dependent ring-opening through formation of aldehyde or ketone and a primary amine:



Under certain conditions open and closed forms are both present in aqueous solutions. Coexistence of such forms increases the apparent solubility of the benzodiazepine. Often, the ring-open form is an intermediate which is formed during benzodiazepine degradation in aqueous solutions but in some cases, e.g. in the case of alprazolam, midazolam and triazolam, this form is chemically stable and can contribute to the overall aqueous solubility of the drug (Cho et al., 1983; Kanto, 1985; Kurono et al., 1985; Kuwayama et al., 1986; Sbarbati Nudelman and de Waisbaum, 1995). For example, in the commercial aqueous intravenous (i.v.) solution of midazolam (Dormicum®, Hoffmann–La Roche, Switzerland) the drug is 15–20% in the ring-open form and the pH is approximately 3.3 (Gerecke, 1983). In addition, both forms, i.e. the ring-open and the ring-closed midazolam, can exist in several different ionization forms. In the aqueous i.v. solution, the ring-open form of midazolam can be characterized as a midazolam prodrug since the ring is completely closed when the pH is elevated to 7.4. Previously we have shown that low complexation efficiency can hamper the usage of cyclodextrins in certain pharmaceutical formulations and that both drug ionization and water-soluble polymers can enhance the complexation efficiency (Loftsson, 1998; Loftsson et al., 1999). Ionization of a drug molecule increases the apparent S_o and addition of a water-soluble polymer to the complexation media increases K_c.

Several investigators have attempted to use the commercially available aqueous i.v. solution for intranasal (i.n.) administration of midazolam (Björkman et al., 1997; Burstein et al., 1997). The midazolam concentration in this solution is only 5 mg/ml. Thus, relatively large amounts of the acidic i.v. solution have to be sprayed into the nose in order to induce sedation and anxiolysis. Subsequently midazolam is only partly absorbed from the nasal cavity and partly from the gastrointestinal tract after swallowing. The midazolam bioavailability after i.n. administration of the i.v. solution is frequently about 50% (Burstein et al., 1997). To reduce spilling and swallowing of the i.v. solution after i.n. administration, and to improve the bioavailability, the dosage has to be sprayed in small aliquots into the nasal cavity (Björkman et al., 1997). However, i.n. administration of the acidic i.v. solution can cause severe irritation in the nasal cavity.

The purpose of the present study was to investigate the effects of the reversible ring-opening of the diazepine ring and ionization on the cyclodextrin complexation of benzodiazepines, as well as

formulation and testing of physiologically acceptable aqueous midazolam nasal spray solution.

2. Materials and methods

2.1. Materials

Midazolam base was purchased from Sifa (Shannon, Ireland), and alprazolam and triazolam from Sigma (St Louis, MO). Sulfobutylether-β-cyclodextrin sodium salt with molar substitution of 6.2 (Captisol®, SBEβCD) was kindly donated by CyDex (Kansas City, KS). Randomly methylated β-cyclodextrin with degree of substitution (DS) of 1.8 (RMβCD) and 2-hydroxypropyl-β-cyclodextrin with DS of 0.6 (HPβCD) were kindly donated by Wacker-Chemie (Burghausen, Germany). Hydroxypropyl methylcellulose 4000 (HPMC) was purchased from Mecobenzon (Denmark). All other chemicals used were of pharmaceutical or special analytical grade.

2.2. Solubility studies

An excess amount of the drug to be tested was added to water or aqueous Teorell–Stenhagen buffer system (Bates and Paabo, 1989), or the aqueous nasal formulation, containing various amounts of the different cyclodextrins with or without a polymer. The suspension formed was heated in an autoclave in a sealed container to 130°C for at least 30 min. After cooling to room temperature (22–23°C) a small amount of solid drug was added to the container to promote precipitation. Then the suspension was allowed to equilibrate for at least 3 days at room temperature, protected from light. After equilibration was attained, an aliquot of the suspension was filtered through a 0.45-μm membrane filter (cellulose acetate from Schleicher & Schuell, Germany), diluted with the HPLC mobile phase and analyzed by HPLC. The pH values reported were determined at room temperature at the end of the equilibration period.

The effect of pH on the stability constant (K_c) of the drug/cyclodextrin (1:1) complex was determined as previously described (Loftsson and Petersen, 1998). Briefly the drug solubility was determined in aqueous nasal formulation containing from 0 to 14% (w/v) cyclodextrin. The composition of the nasal formulation was as follows: benzalkonium chloride (0.02% w/v), EDTA (sodium edetate) (0.1% w/v), HPMC (0.1% w/v), phosphoric acid (0.43% v/v) and aqueous sodium hydroxide solution (for pH adjustment) in water. As before, the exact pH of each solution was determined at the end of the equilibration period. Differences in pH were corrected by drawing the pH-solubility profiles at each cyclodextrin concentration and determining the solubilities of the drug from these profiles at selected pH values. The values obtained were used to draw the phase-solubility diagrams, all of which were linear. Finally, K_c was calculated from the equation (Higuchi and Connors, 1965):

$$K_c = \frac{\text{Slope}}{S_o(1 - \text{Slope})}$$

where K_c is the stability constant of the drug–cyclodextrin (1:1) complex, slope is the calculated slope of the linear phase-solubility diagram and S_o is the apparent intrinsic solubility of the free drug determined in the aqueous complexation media, at appropriate pH, when no cyclodextrin or polymer was present.

2.3. Quantitative determinations

The quantitative determination of drugs was carried out on a high performance liquid chromatographic (HPLC) component system consisting of ConstaMetric 3200 isocratic solvent delivery system operated at 1.50 ml/min, a Merck-Hitachi AS4000 autosampler, a Luna C_{18} 5 μm (4.6 × 150 mm) column, a Spectro Monitor 3200 UV/VIS variable-wavelength detector and a Merck-Hitachi D-2500 Chromato-Integrator. The mobile phase for alprazolam and triazolam consisted of methanol and water (68:32). The pH of the mobile phase was adjusted to 2.7 by addition of trifluoroacetic acid. The flow rate was 1.0 ml/min and the detector was operated at 254 nm. For alprazolam, the retention was 2.8 min for the ring-open form and 4.7 min for the ring-closed form. For triazolam the retention was 2.3 min for

the ring-open form and 3.9 min for the ring-closed form. The mobile phase for midazolam consisted of pH 7.2 aqueous 0.004 M phosphate buffer, acetonitrile and triethylamine (55:45:0.1). The flow rate was 1.5 ml/min and the detector was operated at 240 nm. The retention time was 2.6 min for the ring-open form and 4.2 min for the ring-closed form.

When the fraction of ring-open form was determined the concentration of the closed form was determined right after dissolving the benzodiazepine in the aqueous buffer solution, containing either no cyclodextrin or 10% (w/v) cyclodextrin, and again 24 h later (i.e. after equilibration at 23°C). Preliminary experiments had shown that equilibrium between the closed and the open form was attained within 3 h at 23°C and that no degradation of either the ring-open or the ring-closed form occurred during the 24-h experiment.

2.4. Kinetic studies in aqueous buffer solutions

A stock solution (1.0×10^{-3} M) of the drug to be tested was prepared in a 0.1 M aqueous hydrochloric acid solution (pH 1). This solution was equilibrated in a 37°C water bath for 3 h. This was to ensure that only the ring-open form was present in the stock solution. Cyclodextrin, ethanol or dimethyl sulfoxide (DMSO) was dissolved in, or mixed with, pH 7.5 aqueous 0.5 M tris(hydroxymethyl)aminomethane (Tris) buffer solution and the solution equilibrated at 37°C. At time zero, 30 μl of the stock solution was added to 1.5 ml of the buffer solution, mixed for a couple of seconds on a vortex mixer, and placed again in the 37°C water bath. At various time points samples were withdrawn from the reaction media and injected into a HPLC system (see Section 2.3). Both the ring-open and the ring-closed forms could be detected by HPLC and the disappearance of the ring-open form was proportional to the appearance of the ring-closed form. The first-order rate constants (k_{obs}) for the disappearance of the ring-open form was calculated by linear regression of the natural logarithm of the peak height versus time plots.

2.5. Kinetic studies in human serum

The rate constant for the ring-closing reaction was determined in serum. The previously described (Section 2.4) stock solution of the drug (15 μl) was added to 1485 μl of serum which had previously been equilibrated at 37°C. After thorough mixing on a vortex mixer for a couple of seconds the solution was placed in a 37°C water bath. Sample (100 μl) was withdrawn from the solution at various time points and mixed with 900 μl of ice cold methanol and the solution sonicated for 1 min. Then the solution was centrifuged and the clear supernatant analyzed by HPLC.

2.6. Formulation of the aqueous nasal spray solution

The phase solubility of midazolam was determined in a medium which closely resembled the aqueous nasal spray vehicle, i.e. 7–13% (w/v) SBEβCD, 0.10% (w/v) HPMC, 0.02% (w/v) benzalkonium chloride, 0.10% (w/v) EDTA and 0.43% (v/v) concentrated phosphoric acid. Excess midazolam was added to this medium and the pH adjusted to 4.35 with concentrated aqueous sodium hydroxide solution, both before and after heating in an autoclave (121°C for 40 min). Then the samples were allowed to equilibrate for at least 4 days at room temperature and analyzed as before (Section 2.2). The exact composition of the nasal spray was based on this study. The viscosity of the nasal spray was determined with a Brookfield viscometer (UK) fitted with a ULADIN spindle and an UL sample holder with water-circulation jacket (25°C). The osmolarity of the nasal spray was measured by the freezing point depression method using a Knauer Osmometer Automatic (Netherlands). The buffer capacity of the nasal spray was estimated by the titration method using an aqueous 0.1 N sodium hydroxide solution. The preliminary evaluation of the chemical stability of midazolam in the nasal formulation was performed by determining the midazolam concentration after successive heating cycles in an autoclave (Midmark M7 SpeedClave). Each heating cycle consisted of heating to 121°C,

maintaining this temperature for 20 min, and cooling to room temperature. The midazolam concentration was determined after each heating cycle. The total number of heating cycles was six. Finally the midazolam nasal spray was stored at room temperature (22–23°C) and samples collected at 0, 3, 4 and 12 months and analyzed.

2.7. Evaluation in humans

The study was approved by both the ethics committee of the National University Hospital and the State Committee on Pharmaceuticals in Iceland. Six healthy volunteers (two females and four men) were recruited in a non-blind, crossover study. After obtaining informed consent and 8-h overnight fast, each participant received either intranasal (i.n.) or intravenous (i.v.) application of midazolam. The other application was carried out 7 days later. The participants continued to fast until 2 h after administration of the study formulation. For i.n. administration, the participants received 0.06 mg of midazolam per kg body weight (D_{in}), or 200–300 μl, of the aqueous nasal solution (Unit Dose closed spray system from Pfeiffer). For i.v. administration the participants received 2 mg of midazolam (D_{iv}) in an i.v. solution (Dormicum® from Hoffmann–La Roche). Blood samples (5 ml) were collected from an intravenous catheter at 5, 10, 15, 20, 30, 60, 120, 180, 240 and 360 min. Samples were centrifuged and serum collected and kept frozen until analyzed by reversed phase HPLC method (performed by Medicinsk Laboratorium A/S, Denmark). The serum concentration of midazolam after i.n. and i.v. administration was compared in each participant and the maximum serum concentration (C_{max}) and time to reach C_{max} (t_{max}) determined. In each participant the area under the serum–time curve from 0 to 6 h (AUC) was calculated after both i.n. and i.v. administration using the linear trapezoidal method, and the absolute availability determined from the AUC_{in}/D_{in} over AUC_{iv}/D_{iv} ratio.

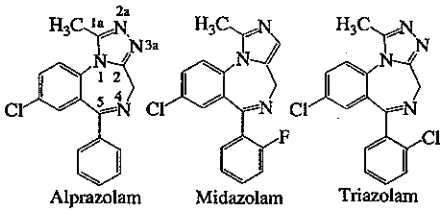


Fig. 1. The chemical structures of the benzodiazepine bases studied.

3. Theoretical background

All the benzodiazepine drugs studied, i.e. alprazolam, midazolam and triazolam, contained 2,3-dihydro-1*H*-1,4-benzodiazepine structure (Fig. 1). Alprazolam and triazolam have a 1*H*-1,2,4-triazole ring fused on the 1,2-carbon–nitrogen bond of the diazepine nucleus (i.e. a triazolo [4,3-*a*][1,4]benzodiazepine structure), where as midazolam has a imidazole ring fused on the 1,2-carbon–nitrogen bond (i.e. an imidazo [1,5-*a*][1,4]benzodiazepine structure). Imidazole is relatively basic (pK_a 6.9) compared to 1*H*-1,2,4-triazole. Thus, in midazolam the protonated nitrogen in position 2 on the imidazole ring (i.e. N-2a) has a pK_a of 6.15 whereas in alprazolam and triazolam the protonated N-2a on the triazole ring has $pK_a \leq 1.5$ (Walser et al., 1978). In the diazepine nucleus the protonated nitrogen in position 4 (i.e. N-4) has been estimated to be about 2.4 (Cho et al., 1983). In aqueous solutions the benzodiazepines undergo a reversible and pH-dependent ring-opening reaction (Fig. 2) (Han et al., 1976, 1977a,b; Cho et al., 1983). The pK_a of the primary nitrogen formed has been estimated to be about 7.0 (Cho et al., 1983). There are some indications that the ring-opening should be pH-independent (Cho et al., 1983) in which case the ring-opening rate constant (k_1) can be described by

$$k_1 = k_{H_2O} f_{HB+} \quad (1)$$

where k_{H2O} is the pH-independent rate constant and f_{HB+} is the fraction of benzodiazepine which is protonated in position N-4. However, Eq. (1) is kinetically equivalent to Eq. (2)

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