Formulation Development and in vivo Characterization of Solubility Enhanced Gliclazide Tablets

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Abstract
The antidiabetic drug gliclazide has very poor aqueous solubility leading to variable bioavailabilities on oral administration thus posing problems in the design of controlled release tablets. Therefore, the aim of the study was to increase the solubility of the drug by making inclusion complex with hydroxypropylbetacyclodextrin in the ratio 1:2 and to incorporate the solubility enhanced drug in matrix forming polymer like sodiumcarboxymethylcellulose for designing oral controlled release tablets. The AUC₀⁻²₄ and AUC₀⁻α obtained from the standardized solubility enhanced gliclazide tablets were 1.98 and 2.09 folds greater than that of the tablets containing plain gliclazide respectively (P<0.05) during the in vivo studies conducted on Newzealand rabbits.

Key words
Gliclazide, Controlled release tablets, Inclusion complex, Hydroxypropylbetacyclodextrin.

Introduction
Gliclazide is a second generation of hypoglycemic sulfonylureas (1). The major drawback in the therapeutic application and efficacy of gliclazide as oral dosage form is its very low aqueous solubility because of its hydrophobic nature. It is characterized by low dissolution rate in water. Because of these reasons, its bioavailability shows interindividual variations (2) and therefore poses problems in the design of controlled release (CR) tablets (3).

Various techniques have been used to improve the solubility/dissolution rate of poorly water-soluble drugs. Among them, complexation with cyclodextrins is most frequently used (4). Therefore, in the present study, it was proposed to prepare inclusion complex of gliclazide in hydroxypropylbetacyclodextrin (HPβCD) and to design CR tablets incorporating solubility enhanced gliclazide to improve the bioavailability of the drug.

Materials and Methods
Gliclazide and HPβCD were obtained as gift samples from Microlabs Ltd., Hosur, India. All other materials used were of analytical grade and were obtained from s.d.fine-chemicals limited, Mumbai-25, India. Animal studies were conducted in accordance to the Institutional Animal Ethics Committee of Al-Ameen College of Pharmacy (Ref. No: AACP/P-05; Date: 07/07/2002 and 16/03/2004).

Experimental Protocol
Preparation of solid complex
Solid complex of gliclazide in HPβCD was prepared by common solvent method (CSM) (5).
minimum quantity of dichloromethane, to which drug was added and allowed to dissolve. Then the solvent was evaporated using vacuum evaporator and the residue obtained was sifted through 100 mesh.

**Formulation of oral controlled release tablets**

Various batches of CR tablets were formulated, containing 30mg of plain and solubility enhanced gliclazide, by conventional wet granulation method by incorporating sodiumcarboxymethylcellulose (NaCMC) (1500-3000cps in a 1% w/v aqueous solution at 25°C) as a sustained release matrix former. Ethyl cellulose, an insoluble and erodable polymer, was also included to investigate its effect on controlling the release rate of the drug from the tablets. In case of tablets containing solubility enhanced gliclazide, all the ingredients were blended together thoroughly after passing through 60 mesh and was granulated using 14 mesh after wetting with a solvent mixture of water and alcohol (3:2). After drying the granules at 50°C± 5°C in an oven till the moisture content decreased to 2% level (moisture content was determined by using HR73 halogen moisture analyzer, Mettler, Toledo), the dried granules were regranulated through 18 mesh and lubricated with magnesium stearate and talc; and compressed into tablets using single punch tablet compression machine (Cadmach, Ahmedabad, India). In case of the tablets containing plain gliclazide, the drug was initially blended with only 30% of NaCMC and ethyl cellulose mixture and granulated. The remaining amount of the blend was mixed with the granules during the lubrication stage and the rest of the procedure followed was same as the above.

**In vitro dissolution studies**

The drug release profiles of the formulated tablets were studied using USP XXIII dissolution apparatus I (Electrolab, TDT-06T) in 900 mL of pH-7.4 phosphate buffer solution (PBS) as per BP 2001 (The British Pharmacopoeia). Aliquot samples were withdrawn every 1 hour and after suitable dilutions with PBS and filtration, the samples were analyzed spectrophotometrically and the amount of the drug released was estimated from the calibration curve.

**Curve fitting**

Drug release data from the most satisfactory formulation was fitted to various mathematical models viz., Korsmeyer-Peppas, Zero-order, Higuchi release models and first-order release models for describing the release mechanism from the formulations.

**In vivo studies**

12 male albino New Zealand rabbits of average weight 2.5 ± 0.010 kg were used for the study. The rabbits were divided into 2 groups of 6 rabbits each (n=6). All the rabbits were fasted overnight with ad libitum access to water. One group received formulation containing plain gliclazide whereas the other group received formulation containing solubility enhanced gliclazide of same dose. The order of administration was randomly selected. The tablets were administered through oral wooden gag with a central opening of 9mm diameter. The tablet was placed deep into the throat through the opening and immediately 20mL of water was administered by syringe to facilitate swallowing of the tablet intact and to prevent it from sticking to the animal’s throat. 1mL of blood samples were collected using 27 gauge needle from the marginal ear vein into heparinized tubes at time intervals of 0, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 hours. Xylene was applied to the shaved marginal ear vein, which causes blood vessel to dilate. The blood was immediately centrifuged at 6000 rpm for 10 minutes to separate the plasma and stored at –20°C until analysis.
Determination of gliclazide in the plasma by micro titer plate reader

A stock solution of drug was made in methanol and various dilutions were made to get working standards from 10 to 1000ng/50mcL. A spectrum of the working standards was obtained by scanning the solutions from 200-400nm to fix absorption maxima. Plasma solutions were prepared by spiking various volumes of the working standards of gliclazide into the healthy plasma.

Drug extraction procedure from plasma

500 mcL of acetonitrile was added to the 500 mcL aliquots of spiked plasma samples and vortexed for 10 seconds to which 4mL of chloroform was added and the mixture was shaken vigorously for 1 minute. The mixture was then centrifuged for 15 minutes at 3000 rpm. The organic layer was transferred to a clean glass tube and was air-dried over night. The residue was redissolved in 500mcL of methanol (6). 50 mcL of samples (n=6) were filled in 384 micro well UV plate and analyzed using micro titer plate reader against the blank and a calibration curve was constructed. Extracted recoveries of gliclazide spiked in plasma matrix were determined by comparing the known concentrations with the spiked concentrations.

Results

Formulation development and in vitro evaluation

Various batches of tablet formulations from F-1 to F-4 containing a dose of 30 mg of gliclazide were developed using NaCMC as the controlled release matrix forming polymer as indicated in Table 1. Formulations F-1 to F-3 contained solid complex of gliclazide in HPâCD in the ratio 1:2. Formulation F-4 was the same as that of formulation F-3 except that it contained plain gliclazide instead of solubility enhanced gliclazide. The tablets were formulated by conventional wet granulation method but a slight difference in the method of formulation was adopted for formulation F-4 to get a comparable hardness between formulations F-3 and F-4. All the formulations were punched using 9.5 mm normal concave punches to a hardness of about 7 kg/cm². The duration of drug release ranged from about 10 to 12 hours in case of formulations F-1 to F-3 whereas it was variable and more than 12 hours in case of formulation F-4 during in vitro dissolution studies, as illustrated in Figure 1.

Table 1: Formulas of the developed controlled release gliclazide tablets.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F-1</th>
<th>F-2</th>
<th>F-3</th>
<th>F-4</th>
</tr>
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<tbody>
<tr>
<td>Plain Gliclazide (%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.52</td>
</tr>
<tr>
<td>Gliclazide solid complex with HP CD (1:2) (%)</td>
<td>28.57</td>
<td>25.71</td>
<td>23.99</td>
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<td>NaCMC (%)</td>
<td>66.67</td>
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<td>Total weight of the tablets (mg)</td>
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<td>350</td>
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Magnesium stearate and talc were added each at 2% concentration of the total weight of the dried granules. All the formulations contained 30mg dose of the drug.
The dissolution data of the most satisfactory formulation F-3 was further subjected to model dependent methods based on different mathematical functions as indicated in Table 2. The diffusional release exponent value indicative of release mechanism obtained (n=0.9965) by Korsmeyer-Peppas release model was (r²=0.9996) greater than 0.89, which indicated that polymer relaxation played an important role in controlling the drug release (7). The formulation also showed a higher R² values for zero-order kinetics.

**In vivo studies**

The plasma levels and the pharmacokinetic parameters of gliclazide obtained with the most satisfactory formulation F-3 containing solubility enhanced gliclazide from the in vivo studies in rabbits were compared to that of the formulation F-4 containing plain gliclazide as indicated in Figure 2 and Table 3 respectively. Since the experimental animals chosen were rabbits, the size and therefore the dose (8) of the tablets were reduced to half of the actual formulations. The method of preparation and the composition of the tablets represented the actual formulations, which were punched using 7mm normal concave punches.

**Table 2:** Pharmacokinetic parameters of gliclazide after oral administration of formulations containing plain and solubility enhanced gliclazide.

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Each value represents the mean ±S.D. for 6 rabbits.

**Fig 1:** Drug Release Profiles from the Formulated Tablets. Data is expressed as a mean ±S.D. of 3 readings.

**Curve fitting - mechanism of drug release**

The dissolution data of the most satisfactory formulation F-3 was further subjected to model dependent methods based on different mathematical functions as indicated in Table 2. The diffusional release exponent value indicative of release mechanism obtained (n=0.9965) by Korsmeyer-Peppas release model was (r²=0.9996) greater than 0.89, which indicated that polymer relaxation played an important role in controlling the drug release (7). The formulation also showed a higher R² values for zero-order kinetics.

**Fig 2:** Gliclazide plasma concentration-time profiles after administration of formulations containing plain and solubility enhanced gliclazide. Each point represents the mean ±S.D. for 6 rabbits
Discussion

The solubility of gliclazide in water at 37°C was only 55 mcg/mL (9). It was reported that a solid-state complex was formed between gliclazide and betacyclodextrin and the molar stoichiometry of the complex was determined as 1:2 by high performance liquid chromatography (10). It was shown that various cyclodextrins bind with drugs similarly (11). Therefore, in the present study, solid complex of gliclazide in HPβCD at 1:2 ratio was prepared by CSM to enhance the solubility of the drug and it was incorporated in the formulation development of CR dosage form of gliclazide.

The duration of drug release from formulation F-1 was only for 10 hours whereas it was for 11 hours in case of formulation F-2 during the in vitro dissolution studies. This could be due to the higher concentration of NaCMC in the formulation F-2 than formulation F-1. The duration of drug release was further prolonged and well controlled in the formulation F-3, which could be due to the presence of ethyl cellulose along with NaCMC. As the duration of drug release was well controlled and uniform from the formulation F-3, it was chosen as the most satisfactory formulation. Drug release was widely varied and very low from the formulation F-4, which could be due to the poor aqueous solubility of the plain drug.

Drug in plasma was estimated using microtiter plate reader. The absorption maxima was fixed at 230nm ($r^2=0.9979$) after scanning the working standards. The average percentage recovery of the drug from plasma was found to be 96.03% ($\pm 0.9310$). During the in vivo studies conducted in the rabbits, the plasma levels of drug from the formulation containing solubility enhanced gliclazide were clearly faster and higher than those achieved with formulation containing plain gliclazide. In particular, the appearance of the drug in plasma was rapid from the former with Tmax at 4 hours ($\pm 0.0$) whereas it was 6.67 hours ($\pm 1.033$) with the latter. Especially, the Cmax value of the former was 1.93 times greater than that of the latter. The AUC0-24 and AUC0-á of the former were 1.98 and 2.09 folds greater than that of the latter respectively. This enhancement in the values of AUCs of the formulation containing solubility enhanced gliclazide can be attributed to the increase in the solubility of gliclazide upon complexation with HPβCD. The derived pharmacokinetic parameters were further subjected to statistical analysis by unpaired two-tailed t-test, which showed that there was significant difference ($p<0.05$) in all the derived parameters including t1/2 and Ke (12) between both the formulations.

Thus, the most satisfactory formulation F-3 satisfied physico-chemical parameters, in vitro and in vivo drug release profile requirements for a CR oral dosage form of gliclazide in which the drug is present in its most soluble form thus leading to maximum bioavailability which results in better management of the disease.

References


