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Enhancement of dissolution and oral bioavailability of gliquidone with hydroxy propyl-β-cyclodextrin

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The virtual insolubility of gliquidone in water results in poor wettability and dissolution characteristics, which may lead to a variation in bioavailability. To improve these characteristics of gliquidone, binary systems with hydroxypropyl-β-cyclodextrin (HP-β-CD) were prepared by classical methods such as physical mixing, kneading, co-evaporation and co-lyophilization. The solid state interaction between the drug and HP-β-CD was assessed by evaluating the binary systems with X-ray diffraction, differential scanning calorimetry and IR- spectroscopy. The results establish the molecular encapsulation and amorphization of gliquidone. The phase solubility profile of gliquidone in aqueous HP-β-CD vehicle resulted in an A_L type curve with a stability constant of 1625 M^{-1}. The dissolution rate of binary systems was greater than that of pure drug and was significantly higher in the case of co-lyophilized and co-evaporated systems. Upon oral administration, $[AUC]_{0-\alpha}$ was significantly higher in case of co-lyophilized (2 times) and co-evaporated systems (1.5 times) compared to pure drug suspension while other binary systems showed only a marginal improvement. The study ascertained the utility of HP-β-CD in enhancing the oral bioavailability of gliquidone, and points towards a strong influence of the preparation method on the physicochemical properties.

1. Introduction

Gliquidone is a second-generation sulfonylurea used in the treatment of non-insulin dependent diabetes mellitus [1]. It is virtually insoluble in water due to poor wettability, which may lead to erratic absorption with a low and variable bioavailability. Cyclodextrins are known to improve the solubility of lipophilic drugs through molecular encapsulation and thereby improve dissolution and oral bioavailability [2] of a number of sulfonylurea drugs such as tolbutamide [3] and glibenclamide [4]. Cyclodextrin alone or along with a water soluble polymer such as hydroxy propyl methyl cellulose has been reported to enhance the bioavailability of glibenclamide [5] and glicazide [6, 7]. Age related dissolution problems of glibenclamide have been addressed with cyclodextrin complexation [8]. Some of the earlier studies aimed at improving the dissolution characteristics of gliquidone by preparing solid dispersions of the drug with a PVP matrix [9]. The improvement in bioavailability of this short acting drug may enable effective drug delivery and possibly dose reduction. Further, the transdermal bioavailability of gliquidone is enhanced by complexation with hydroxy propyl-β-cyclodextrin (HP-β-CD) in the liquid state [10].

Gliquidone complexes with cyclodextrin were not reported earlier and the present study aims at characterizing such complexes to explore the enhancement in dissolution rate and oral bioavailability. The possibility of preparing stable complexes of gliquidone with HP-β-CD by widely reported techniques like physical mixing, kneading, co-lyophilization and co-evaporation has been addressed. The binary systems were characterized for the molecular interaction between gliquidone and HP-β-CD in liquid state by phase solubility studies and in solid state by differential scanning calorimetry (DSC), X-ray diffraction and IR spectroscopy. The improvement in the dissolution rate of the binary systems *in vitro* and their apparent efficacy in enhancing the oral bioavailability in rats is presented.

2. Investigations, results and discussion

Phase solubility studies of gliquidone in aqueous vehicle containing HP-β-CD (in the concentration range of 0.036 M to 0.181 M) were performed according to the continuous variation method reported by Higuchi and Connors [11]. The solubility of gliquidone increased linearly with an increase in HP-β-CD concentration (Fig. 1). The phase solubility studies showed Higuchi's A_L type curve with an apparent stability constant ($K_{1:1}$) of 1625 M^{-1} This type of curve suggests a 1 : 1 interaction between gliquidone and HP-β-CD at the investigated concentrations of the ligand.

To confirm the association of gliquidone with HP-β-CD in solid state, the binary systems of the drug and the ligand were prepared by co-evaporation, co-lyophilization, kneading and physical mixing, and characterized by differential scanning calorimetry (DSC), X-ray diffraction and IR spectroscopy.

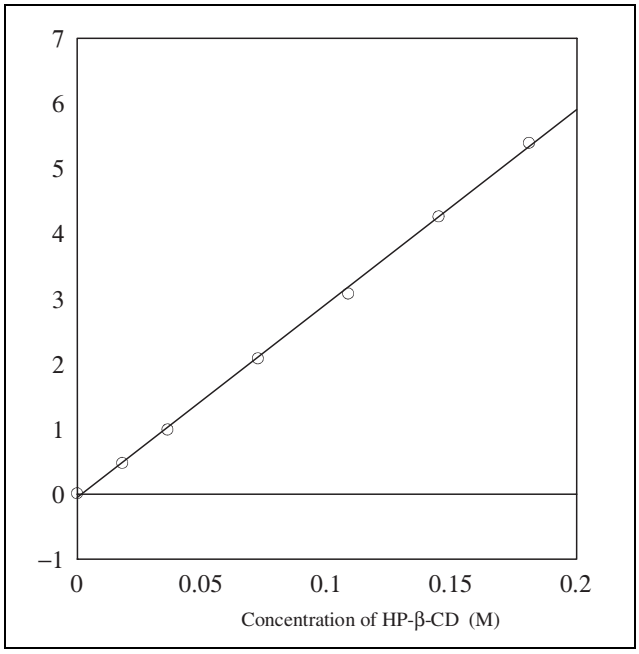

Fig. 1: Phase solubility curve of gliquidone in aqueous HP-β-CD solution (n = 3, RSD < 2%)

The X-ray diffraction patterns of the binary systems showed diffractional peaks relevant to crystalline gliquidone in kneaded and physical systems while hollow patterns, indicative of amorphous state of the sample was observed to co-lyophilized and co-evaporated samples.

DSC curves of pure drug showed a peak at 178 °C as a characteristic melting endothermic peak. The complete disappearance of the drug endothermic effect was found in the curves of the co-lyophilized and co-evaporated systems, indicating the formation of an amorphous product. This endothermic peak was still evident in the samples obtained by kneading and physical mixing.

IR spectra reveal that the SO_2 group of the sulfonylurea portion of the drug shows absorption bands in the region of 1150 to 1600 cm^{-1}. The characteristic SO_2 absorption at 1158 cm^{-1} and the S-N stretching band at 1353 cm^{-1} were not evident in the binary system – prepared by co-evaporation. The N-H bending peak of the urea at 1530 cm^{-1} was not found in the binary system prepared by co-evaporation but appears to a shift of 1522 cm^{-1} with the co-lyophilized system. The spectra obtained from the kneaded and physically mixed samples appeared unchanged.

The study points at a significant influence of the preparation method on the degree of interaction between the drug and the ligand. Co-evaporation and co-lyophilization methods were more suitable for the formation of HP-β-CD molecular complexes of gliquidone.

The dissolution studies of gliquidone and different binary systems of gliquidone-HP-β-CD were performed according to the dispersed amount method [12]. No significant difference in solubility was observed between the lyophilized gliquidone and unlypophilized gliquidone (pure) hence, the formulations were composed of unlyophilized gliquidone. From the dissolution profiles of the binary systems (Fig. 2), it is clear that all the systems containing HP-β-CD exhibited better dissolution properties than the pure gliquidone. At 90 min, where maximum dissolution (22%) of the pure drug sample was observed, the physical and kneaded systems showed a dissolution of 31.9% and 35.4%, respectively. The increased dissolution of the physical and the kneaded systems can be attributed to the

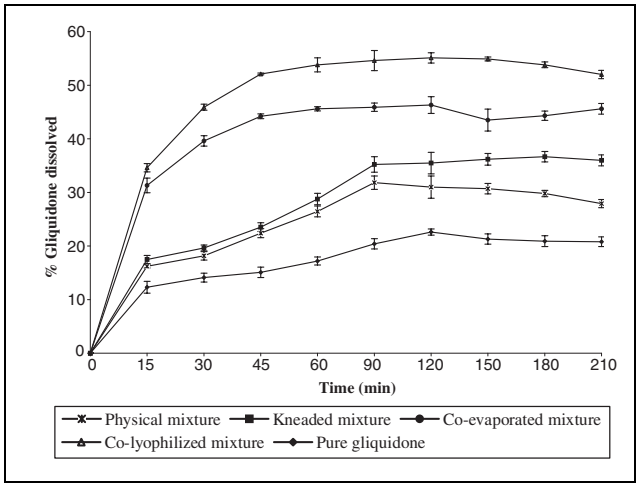

Fig. 2: Dissolution profiles of gliquidone – Hydroxypropyl-β-cyclodextrin binary systems and pure gliquidone in PBS pH 7.4 at 37 °C. $p \leq 0.05$ (n = 3)

increased wettability and an ability of these systems to form a readily soluble complex with the cyclodextrin in the dissolution medium.

However, maximum dissolution of drug was observed in binary systems prepared by co-lyophilized and co-evaporated methods which showed 2.5 and 2 times higher dissolution respectively, compared to pure drug. At 45 min co-lyophilized and co-evaporated systems showed a dissolution of 52% and 44%, respectively, while pure drug showed only 22% at 120 minutes indicating a faster dissolution rate of the former binary systems. This increase in the dissolution rate is presumably a result of the molecular inclusion of the drug in these two binary systems and hence the greater solublization power of HP-β-CD for gliquidone.

Pharmacokinetic profiles of the drug (Fig. 3) showed a trend analogous to the *in vitro* studies. The maximum pharmacokinetic efficacy of gliquidone on oral administration was observed with co-evaporated and co-lyophilized systems (Table). The C_{max} values of these two binary systems were 1.36 and 1.57 times higher and the $[AUC]_{0-\alpha}$ values were 1.6 and 2 times higher respectively, ($p < 0.05$) compared to the pure drug administration. The physical and the kneaded systems showed only a marginal improvement in the $[AUC]_{0-\alpha}$ values, while the C_{max} values did not differ significantly compared to the pure drug administration.

The pharmacodynamic evaluation of the gliquidone – HP-β-CD binary systems was carried out by measuring the

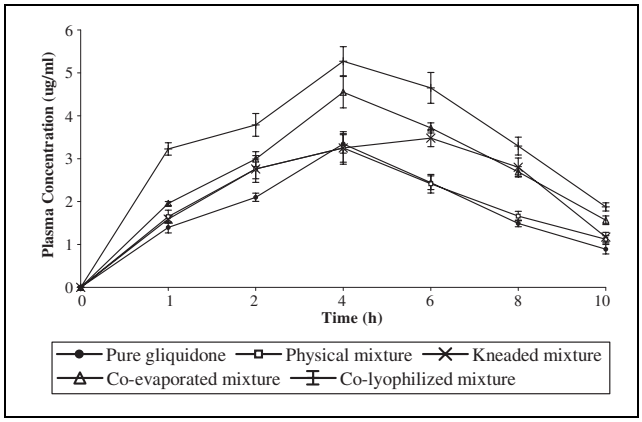

Fig. 3: Plasma concentration vs time profiles of gliquidone – Hydroxypropyl-β-cyclodextrin binary systems and pure gliquidone after oral admistration to male wistar rats. $p \leq 0.05$ (n = 6)

Table: Pharmacokinetic parameters on oral administration of binary systems of gliquidone-hydroxypropyl-β-cyclodextrin and pure gliquidone to male wistar rats (n = 6)

Parameters	Gliquidone	Physical mixture	Kneaded mixture	Co-evaporated mixture	Co-lyophilized mixture
C_{max}(ug/ml)	3.34 ± 0.215	3.25 ± 0.379	3.47 ± 0.198	4.55 ± 0.365	5.27 ± 0.339
T_{max} (hr)	4	4	4	4	4
$[AUC]_{0-\alpha}$ (ug/ml/hr)	23.96 ± 1.53	27.84 ± 2.24	32.86 ± 2.9	38.82 ± 2.1	48.10 ± 2.84
$T_{1/2}$ (h)	3.10 ± 0.043	3.89 ± 0.157	4.05 ± 0.103	3.93 ± 0.035	4.02 ± 0.063

% decrease in blood glucose levels. The pharmacodynamic efficacy of the binary systems did not significantly differ from each other, despite significant differences in the pharmacokinetic parameters, as the plasma drug levels were higher than the minimum effective concentration for all the formulations. Hence, it can be presumed that the threshold in the reduction in blood glucose Levels was reached and hence the formulations did not show significant differences. However, such an enhancement in pharmacodynamic activity may be evident and may prove useful in higher mammals such as humans, where drug levels are not expected to reach such high levels.

3. Experimental

3.1. Materials

Gliquidone was a kind gift from M/s Boehringer Ingelheim, Germany and HP-β-CD was purchased from M/s Fluka Chemicals, Switzerland. All the other chemicals were of HPLC grade.

3.2. Methods

3.2.1. Quantitative analysis [13]

The amount of gliquidone in buffer and in biological samples was determined by means of a HPLC system (Shimadzu 10 Ai, Japan) operated in a binary mode with a photodiode array detector and a communication bus module. The analysis was performed on a shimpack, reverse phase C18, 250 mm × 4.5 mm, 5 μm column maintained at 25 °C (column oven) using a mobile phase of acetonitrile (85%) and 0.1 M acetic acid (15%) pumped at a flow rate of 1.5 ml/min monitored at a wavelength of 229 nm set at Au/Fs −5 to 50 and the retention time of the drug was 2.7 ± 0.1 min. A calibration graph of peak areas Vs concentration followed a linear regression equation: Concentration = [1.6 E-02] area – [1.31 E + 0.2] with correlation coefficient $r^2 = 0.999$ and inter-/intraday variation of RSD ≤ 1.2%.

3.2.2. Phase solubility studies

Phase solubility studies were performed according to the method reported by Higuchi and Connors [11]. An excess of gliquidone was added into screw capped vials containing aqueous buffer (pH 7.4) vehicle with HP-â-CD in the concentration range of 0.036 M to 0.181 M, which, were briefly sonicated and shaken on an orbital shaker at 37 °C for 3 days. After attainment of equilibrium, the samples were filtered through 0.45 μm membranes (Pall- Gelman, USA) and analyzed for gliquidone by HPLC as described previously.

3.2.3. Preparation of solid complex

3.3.2.1. Co-evaporation [4, 14]

Gliquidone and HP-β-CD in equimolar ratios were dissolved in 75% aqueous isopropanol solution. The mixture was gently stirred for 48 h and evaporated rapidly on a rotary vacuum evaporator (Heidolph, Germany) at 70 °C. The product was vacuum dried to a constant weight.

3.3.2.2. Co-lyophilization [12]

Gliquidone and HP-β-CD in 1 : 1 molar ratio were dissolved in aqueous ammonia solution, stirred for 48 h and lyophilized. The absence of ammonia was confirmed qualitatively by Nessler's reagent.

3.3.2.3. Kneading

Gliquidone and HP-β-CD (1 : 1) were thoroughly mixed in a ceramic mortar. Water (3 ml) was added whilst kneading was continued for about 2 hrs. The paste was dried under vacuum.

3.3.2.4. Physical mixture

The physical system was obtained by tumble mixing for 15 min, the 75–150 μm sieve fractions of gliquidone and HP-β-CD (1 : 1).

3.2.4. Characterization of the solid complex

X-ray diffraction patterns of the samples were measured using a Siemens D5000 X-ray diffractometer equipped with graphite monochromator [Cu, K radiation (X = 1.54 Å), 40 KV voltage, 30 mA current] and degree divergent slit at a chart speed of 5 °C/min.
Differential scanning calorimetry was performed using Mettler Toledo DSC apparatus using sealed aluminium crucibles in N_2 atmosphere with a scan speed of 5 °C/min.
IR spectra of KBr discs of the samples were obtained using Perkin-Elmer 882 infrared spectrometer.

3.2.5 In vitro dissolution studies

The *in vitro* dissolution studies of gliquidone and the binary systems prepared by different methods were performed in phosphate buffered saline of pH 7.4 at 37 °C according to the dispersed amount method [12]. Gliquidone and binary systems (equivalent to 50 mg of gliquidone) were added to 100 ml of phosphate buffered saline and stirred at 100 rpm by means of a 3 blade propeller centrally immersed at 20 mm from the bottom of the beaker. Aliquots of the samples were withdrawn at scheduled time intervals and replenished with the similar volume of buffer. After filtration through 0.45 μm membrane, the samples were analyzed by HPLC and the cumulative % dissolution was calculated.

3.2.6. In vivo studies

A group of 6 male wistar rats (150 ± 10 g) were randomly assigned to each formulation (pure drug and the binary systems) for pharmacokinetic and pharmacodynamic evaluation. Blood samples were collected from the tail vein at scheduled intervals after oral administration of 10 mg/kg of the drug or the equivalent amount of the binary system. Plasma was separated immediately and stored at < −20 °C until further analysis.

3.2.6.1. Pharmacokinetic studies

Plasma gliquidone concentration was estimated by HPLC method as described previously. Pharmacokinetic parameters such as maximum plasma concentration (C_{max}) and time to reach the maximum plasma concentration (t_{max}) were read directly from the graphical representation of plasma concentration vs. time curve. Elimination half-life ($t_{1/2}$), area under the curve ($AUC_{0-\alpha}$) were calculated using a model independent pharmacokinetic software "RAMKIN". The model is based on statistical moment analysis and the AUC was calculated by the linear trapezoidal rule, $t_{1/2}$ was measured from the regression of terminal phase of the concentration time curve.

3.2.6.2. Pharmacodynamic studies

The plasma samples obtained periodically after oral administration of the formulations were analyzed for glucose concentration on a Beckman auto analyzer using the Bayer's analytical kit [15]. Percent reduction in blood glucose level was calculated with respect to glucose level at zero time point.

3.2.7. Statistical analysis

The *in vitro* skin permeation studies were an average of three determinations and the results are presented as average ± SD. The statistical analysis of the results obtained was done by t-test assuming unequal variences and a $P \leq 0.05$ was considered significant.
The statistical analysis of the results (n = 6) obtained from *in vivo* studies was performed by ANOVA using a Bonferroni post test for selected pairs of columns and a $P \leq 0.05$ was considered significant.

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