

Original Research Article

Enhancement of Solubility and Bioavailability of Candesartan Cilexetil using Natural P-Glycoprotein Inhibitors

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Abstract

Purpose: To enhance the otherwise poor solubility and bioavailability of candesartan cilexetil (CDS).

Methods: This study involved enhancing drug solubility by various solid dispersion (SD) methods. The drug: carrier ratio was as follows: for urea (1:2, 1:4 and 1:6; for polyethylene glycol 6000 (PEG, 1:2 and 1:4, 1:8); and mannitol (1:2, 1:4 and 1:6. Piperin and quercetin (natural P-glycoprotein inhibitors) were used as bioavailability enhancers. Bioavailability studies were carried out in a rat model with the SDs formulated in a suspension form and administered by the oral route.

Results: All the carriers enhanced drug dissolution in water 2 to 4-fold depending on drug/carrier ratio. Release kinetics from solid dispersions made with mannitol showed zero order drug release. Urea and PEG 6000-based solid dispersions showed 1st order drug release kinetics. FTIR studies confirmed transformation to an amorphous form of CDS in mannitol solid dispersion; this was buttressed by release kinetic studies. Bioavailability of the drug in the animals was enhanced by 27 and 68 % when quercetine and piperine, respectively, were incorporated.

Conclusion: Formation of solid dispersion enhances the solubility and bioavailability of CDS when natural P-glycoprotein inhibitors such as piperin and quercetin are incorporated as enhancers.

Keywords: Solid dispersion, Candesartan, Cilexetil, Bioavailability, P- Glycoprotein, Piperin, Quercetin

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INTRODUCTION

Candesartan cilexetil (CDS), which is an angiotensin II receptor antagonist, is a prodrug that releases candesartan during absorption. CDS was approved by the US Food and Drug Administration (FDA) in 1998 and patented under the name of 'Tab Atacand' [1]. It has the highest affinity for AT1 receptor, where it suppresses the maximal response to angiotensin compared to any other angiotensin II receptor antagonist [2]. The solubility of CDS in water is lower than 5×10^{-5} g/liter and while its bioavailability in solution form is between 15 and 40 % [18,19].

Researchers have tried different methods [3] to enhance its solubility and bioavailability. CDS undergoes passive absorption, and P-glycoprotein efflux transportation decreases its bioavailability. This has been shown in a study on the transport characteristics [4], intestinal absorptive characteristics and efflux mechanisms (P-gp transportation) in human intestinal Caco-2 cell line. Additionally, the inhibitory effects of angiotensin II type 1 receptor blockers (ARBs) on P-glycoprotein (P-gp) were examined on vectorial transport of digoxin [5]. The enhancement of bioavailability with natural

compounds from medicinal plants depends on mechanisms such as P-gp inhibition or non-specific mechanisms promoting rapid absorption of drugs [6]. CDS is sensitive to pH, light and pressure which cause hydrolysis, disesterification [7] and crystalline changes [8].

The aim of this study was to enhance CDS solubility by achieving a stable amorphous or molecular form of CDS [9] and also enhance its bioavailability with the aid of natural P-glycoprotein inhibitors [5,10,11].

EXPERIMENTAL

Materials

Candesartan Cilexetil BP was received as a gift from Dr. Reddy Laboratories Ltd, Hyderabad, India. Polyethylene glycol (PEG) 6000, mannitol, urea, piperine, quercetin, and microcrystalline cellulose were procured from Sigma Aldrich, Mumbai. All the chemicals used for chromatographic analysis were of high performance liquid chromatography (HPLC) grade. All the other chemicals and solvents used were of analytical grade.

Preparation of physical mixture

The physical mixtures (PM) of CDS, with all the carriers were prepared by mixing the required amount of drug and the carriers in different drug: carrier ratios (for PEG 6000, 1:2, 1:4 and 1:8; for urea, 1:2, 1:4 and 1:6; for mannitol, 1:2, 1:5 and 1:8) in a glass mortar for 5 min. They were sieved through # 60 mesh (250 micron) and stored in a calcium chloride desiccator until used.

Preparation of solid dispersions of candesartan cilexetil (CDSSDs)

CDSSDs were prepared based on drug: carrier ratios of 1:2, 1:5 and 1:8 for mannitol as carrier (co-evaporation method) [12], 1:2, 1:4 and 1:8 for PEG 6000 (melt-fusion) [13], and 1:2, 1:4 and 1:6 for urea as carrier (melting method by mixing of melted drug and carrier).

Assay of total drug content

Known amounts of the drug/carrier (8 mg drug equivalent) binary systems (SDs or PMs) were dissolved in 10 ml of methanol using a vortex mixer. This was appropriately diluted with methanol and drug content evaluated spectrophotometrically (UV-Visible-double beam spectrophotometer, Chemito Instrument Pvt Ltd, India) at 268.2 nm. Drug content was compared

with the theoretical value. The experiments were done in triplicate and the mean values recorded.

Solubility studies

The dynamic solubility of Candesartan Cilexetil Solid Dispersion (CDSSD) was determined by adding an excess of solid (5 mg) to 5 ml of water and the tubes were vortexed at 300 rpm for 10 minutes. Aliquots of samples were withdrawn, filtered, and analyzed for drug content by UV spectroscopy at 268.2 nm.

Dissolution studies

Dissolution studies on pure CDS, physical mixture and solid dispersions were carried out using US Pharmacopoeia type I dissolution apparatus (Electrolab pvt. Ltd India). The dissolution medium was 900 ml of pH 6.5 phosphate buffer, containing 0.35 % Tween 20 at 37 ± 0.5 °C. The stirring speed was 100 rpm. Eight (8) mg equivalent of CDS of solid dispersion in powder form was placed in the basket. A 5.0 ml sample solution was withdrawn at appropriate time intervals and filtered. An equal volume of fresh dissolution medium was immediately replacing the withdrawn sample. The concentration of CDS in the samples was analysed spectrophotometrically at 268.2 nm. The experiments were carried out in triplicate. The mean concentration of the drug was plotted against time. The release data were subjected to first order, zero order, Higuchi and Korsmeyer-Peppas models to ascertain model-dependent kinetics of release.

Fourier transform infra-red (FTIR) compatibility studies

Fourier Transform Infra-red (FTIR) (Shimadzu Corporation Japan) spectra of pure drug or carrier, physical mixture and solid dispersion were recorded in the range $400 - 4000 \text{ cm}^{-1}$ at room temperature.

Preformulation studies

The powder blend was analyzed for bulk density, tapped density, Hausner's ratio, compressibility index, and angle of repose [20].

Bioavailability studies

For the quantification of an active form of CDS in blood, the spiking technique was followed. A known concentration of CDS was added to the Rats' blood and CDS in the blood was evaluated by HPLC method [13]. As CDS (prodrug) is converted to candesartan in the blood, the

amount of candesartan was subtracted from the known amount of CDS added to blood initially, and hence the total amount of candesartan (active drug) was determined in the blood. This was correlated with the peak to peak area obtained from HPLC analysis of the same sample analyzed for candesartan.

Bioavailability studies were carried out in rats, after approval by the institutional ethics committee of G. Pulla Reddy College of Pharmacy, India. Inbred adult albino Wister rats, weighing 90 - 120 g maintained in standard laboratory conditions of temperature, humidity, 12 h/12 h light and dark cycles, normal diet (M/S. Lipton India Ltd) and water ad libitum, were randomly divided into 3 groups of six animals each (3 female and 3 male). The females were nulliparous and not pregnant.

Candesartan cilexetil/PEG 6000 solid dispersion (1:4, 1 mg/kg) was selected as a reference. Group 1 was administered the pure drug. The second group was the reference drug with quercetin (10 mg/kg) as a PGP inhibitor. The third group was the reference drug with piperine (10 mg/kg) as a Pgp modulator. Each group was divided into two subgroups of females and males (Table 2).

Both male and female rats were administered the suspension form of test and reference orally by oral tube and blood samples were collected by retro-orbital method. Samples were collected in 2:45th, 5th, 7th, 27th h. Samples were kept at -17 °C until further studies. The frozen samples were thawed at room temperature and centrifuged at 6000 rpm prior to further studies.

Statistical analysis

Statistical analysis was performed on the dissolution data obtained from dissolution data using Microsoft Excel 2007 and the

pharmacokinetic parameters data obtained from subject were calculated manually.

RESULTS

Drug content

Drug content of solid dispersions made with PEG 6000 showed 100.0 ± 3.5 %, while for those made with mannitol and urea it was 94.0 ± 3.0 , and 84.0 ± 8.5 %, respectively.

Drug solubility

The solubility of CDSSD increased linearly with increase in concentration of the carriers. The rank order of solubility was PEG 6000 (4.3 fold) > mannitol (2.5 fold) > urea (1.8 fold) > physical mixtures (for all carriers).

In vitro drug dissolution

Drug release from physical mixture was greater than that from the pure drug but lower than that of the solid dispersions, [14]. Physical mixture with mannitol, and PEG did not show much increase in the solubility of CDS (Table 1). The rank order for the various carriers was PEG 6000 (4.3 fold) > mannitol (2.5 fold) > urea (1.8 fold) > physical mixtures (for all carriers).

The model dependent methods given in (Table 2) and profiles generated from zero order, first order, Higuchi and Korsmeyer-Peppas derived data suggest urea and PEG SDs followed 1st order kinetics while mannitol SD followed zero order kinetics.

All the other SDs followed Higuchi release model, suggesting that drug release mechanism is by diffusion. The diffusion exponent, 'n', being < 0.5, suggests Fickian release.

Table 1: Dissolution of selected candesartan cilexetil solid dispersion and physical mixtures

Time (min)	Purecandesartan cilexetil (CDS)	CDS: mannitol (1:5)		CDS: urea (1:6)		CDS: PEG 6000 (1:4)	
		PM	SD	PM	SD	PM	SD
5	72.60	77.48	97.96	82.3	82.3	62.6	65.75
10	76.85	78.79	96.14	86.25	86.25	63.82	91.64
15	79.41	80.1	100.4	93.73	95.49	65.49	96.43
30	86.68	91.48	102.4	97.75	97.76	78.54	97.52
45	87.15	93.29	100.6	98.29	100.5	80.28	100.5
60	89.76	95.55	104	98.82	101	82.03	101.8

Crystallinity

The essential features of the three solid dispersion spectra was the strong absorption band at 1755.10 - 1716 cm^{-1} assigned to the carbonyl stretching vibration. The absorption band observed at 1716.53 cm^{-1} in the case of CDS-mannitol solid dispersion was shifted to 1730.03 cm^{-1} , although another carbonyl absorption band was commonly recognized at the same position.

Bioavailability CDS solid dispersion containing ppg inhibitors

The present study was designed to compare single dose bioavailability studies of optimized CDS solid dispersion (PEG-6000) with or without PGP inhibitors in rat. Serum drug concentration of candesartan after oral administration is presented in (Table 3 and Fig 1).

Table 2: Release kinetics of candesartan cilexetil containing various carriers

Formulation	Method	Ratio	Zero order		First Order		Higuchi		Peppas		n	Release type
			R ²	K	R ²	K	R ²	K	R ²	K		
CDS: mannitol	Co-evaporation	1:5	0.679	6.545	0.420	-0.444	0.706	8.691	0.693	2.013	0.025	Fickian release
CDS: urea	Melting	1:6	0.753	18.74	0.881	-1.416	0.853	25.99	0.922	2.012	0.085	Fickian release
CDS: PEG 6000	Fusion	1:4	0.489	26.06	0.788	-1.447	0.604	37.71	0.702	2.030	0.144	Fickian release

Table 3: Pharmacokinetic parameters of candesartan following single dose (12 mg) oral administration

Formulation	Cmax ng/ ml	T _{1/2} (h)	AUC _{0-t} ng h/ ml	AUC _{0-∞} (ng h/ ml)	K _E (h)
CDSSD (female)	134	1.809	859.25	864.211	0.383
CDSSD (male)	150	2.092	1031.3	1037.64	0.331
CDSSDP (female)	212	2.784	1361.125	1370.767	0.2489
CDSSDP (male)	252	2.915	1637.065	1652.07	0.2377
CDSSDQ (female)	171	3.726	1264.065	1304.39	0.0808
CDSSDQ (male)	175	3.72	1251.875	1277.598	0.1866

Note: CDSSD = candesartan solid dispersion with PEG 6000; CDSSDP = candesartan solid dispersion with PEG 6000 and piperin; CDSSDQ = candesartan solid dispersion with PEG 6000 and quercetin

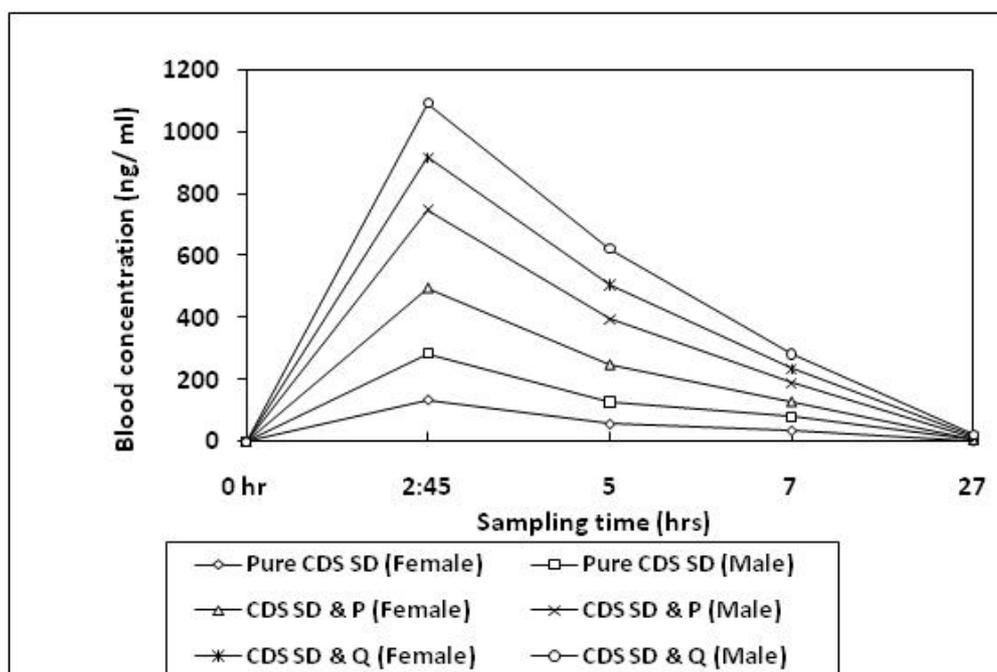


Fig 1: Plasma drug concentration of candesartan after single dose (250 μg equivalent) oral administration

The pharmacokinetic data indicate enhanced bioavailability with marked improvement in absorption rate and extent compared to the CDSSD. The mean area under the serum candesartan curve (AUC) differed significantly from CDSSD/quercetin (CDSSDQ) or candesartan cilexetil/piperin solid dispersion (CDSSDP) and CDSSD. A mean of 58 and 68 % increase in serum candesartan AUC were observed for CDSSDP female and male groups, compared to CDSSD female and male groups respectively. A mean of 27.5 and 17 % increase in serum candesartan AUC was observed in CDSSDQ female and male groups compared with CDSSD female and male groups, respectively. There was a little increase in candesartan $t_{1/2}$, which may be responsible for the decrease in the rate of drug metabolism in the liver due to quercetin's inhibitory effect on CYP3A4. Other pharmacokinetic parameters did not change significantly (Table 3).

DISCUSSION

PEG 6000 gave better solubility and dissolution than the other carriers hence it was chosen as the desired carrier and selected for *in vivo* study. The toxic effect of urea is a drawback for its use as a carrier. Hence urea was not selected for further study.

The differences in the spectra of CDS between physical mixture and the solid dispersion suggest that the conformation of a cyclohexyloxy carbonyloxy ethyl group of CDS in physical mixture is different from that in the solid dispersion. According to Hirokazu *et al* [8] the amorphous of CDS exhibited a broader IR spectrum than the spectra of crystalline forms I and II. The absorption band assigned to the carbonyl stretching vibration was shifted to the middle position (1728 cm^{-1}) of form I (1717 cm^{-1}) and form II (1736 cm^{-1}), indicating the existence of the amorphous form of CDS in CDS-mannitol solid dispersion. In the case of CDS-PEG solid dispersion, the peak at 1724.24 cm^{-1} did not show much difference and thus indicates low level of amorphous CDS. The absorption band at 1716.53 cm^{-1} for CDS-urea solid dispersion was not seen because of the overlap between urea and CDS carbonyl group and therefore it requires further study.

In order to understand the mechanism and kinetics of drug release, the *in vitro* release data were fitted to various kinetic models to ascertain their release mechanisms. The best-fit model was selected based on the highest determination coefficient, smaller difference between each

fitted and actual data and the smaller value of the residual mean square.

Bioavailability enhancement by quercetin was less compared to piperin. Many studies have shown the effect of quercetin. In the present study, 10 mg/kg concentration of quercetin and piperine were used and they showed significant inhibition of intestinal P-gp in rats and enhanced the bioavailability of candesartan cilexetil. Though a little increase in $t_{1/2}$ was observed with quercetin but it requires further study to be done. Promising results were obtained with formulations of PEG solid dispersion containing piperin, a natural P-gp inhibitor as bioavailability enhancer. Despite the fact that the results obtained are promising, further studies are required to optimize the concentration of piperine or quercetin and to formulate them onto herbosomes or use them as a dietary supplement.

The mechanism of increased bioavailability of CDSSDP may be due to various reasons. Piperine, an alkaloid (1-peperoyl piperidine), has been previously evaluated for its ability to increase serum response and lengthen serum half-life of drugs in both experimental animals and human volunteers. These studies suggest that orally administered piperine may significantly increase serum levels, and lengthen the serum half-lives of chemically diverse groups of naturally occurring and synthetic drugs [15,16]. Bioavailability enhancement with piperine may be due to PGP inhibitory effects and the increased micelle formation, hyperemia [17], and epithelial cell wall modification due to the lipophilic nature of piperine, or an increase in the bioenergetics processes of the gastrointestinal epithelium due to the thermogenic properties of piperine [16]. It is also possible that the localized thermogenic effect of piperin on epithelial cells may increase the uptake of drugs from the gut.

CONCLUSION

PEG 6000 was the most efficient solubilising carrier, improving water solubility 4-fold. The bioavailability of candesartan cilexetil was enhanced 22 and 63 % by quercetin and piperine, respectively.

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