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Biopharmaceutical classification of candesartan and candesartan cilexetil

G.N. Darwhekar *, D.K. Jain, Jitendra Chouhan
College of pharmacy, IPS Academy, Rajendra Nagar, A.B. Road, Indore-452012 (M.P.) India
Corresponding Author s Email**:** gdarwhekar@yahoo.com

ABSTRACT

Candesartan, an angiotensin II receptor blocker, due to its inherent low bioavailability, its cilexetil salt (prodrug) is used. The prodrug used shows improved oral bioavailability which still remains to only 15%. The objective of present study is to characterize candesartan through Biopharmaceutical Classification System (BCS) to find out the possible reasons for its poor bioavailability. Candesartan was classified as per BCS according to the available guidelines. The solubility was determined over pH range of 1-7 and permeability was determined using perfused everted intestinal sac of rat at 37ºC. The perfused everted sac of rat intestine was used as models of the intestinal mucosa to assess uptake and transport of candesartan and candesartan cilexetil. The determination of candesartan was done by HPLC. The study indicates low solubility and low permeability of candesartan and therefore it can be classified into BCS class IV.

Key words: *Apparent permeability, candesartan, everted perfused sac, intestinal absorption.*

INTRODUCTION

Candesartan (CS) is a potent and selective angiotensin II type 1 (AT1) receptor blocker, which has widely been used orally in patients with hypertension, kidney disease and heart failure[1,2]. CS lowers blood pressure through blockade of the renin–angiotensin–aldosterone system[3]. The AT1 binding affinity of CS is 80 times greater than that of losartan and 10 times greater than that of EXP 3174, an active metabolite of losartan[1]. The efficacy of CS has been shown to be much higher or at least equivalent to that of many other commonly prescribed antihypertensive agents [4, 5].

Candesartan is also a long-acting angiotensin II receptor antagonist. To overcome a poor oral absorption, a series of ester prodrugs was synthesized, and candesartan cilexetil (CC) was identified as the compound that provided the best angiotensin II antagonistic activity profile after oral administration. CC is rapidly and completely converted to its active compound, CS, during gastrointestinal absorption [6]. The extent of absolute oral bioavailability (F) of CC is 15%–40% [6,8]. It is generally believed that the low and variable F of CC may be explained by physicochemical factors (i.e. solubility, permeability and dissolution) as well as physiological factors (i.e. intestinal absorption, efflux and the first- pass metabolism). However, little is known about the transport characteristics of CS in the human intestine.

The objective of the study has to classify CS according to the biopharmaceutical drug classification scheme. BCS is based on the aqueous solubility and intestinal permeability of drug so once a drug is to be classified according to this system its pH-solubility profile and intestinal permeability should be estabilized. Since the bioavailability of CS following administration of CC prodrug, was estimated to be 15%. The determination of pH-solubility profile and permeability of CS and its prodrug (CC) help to find out the reason for its poor oral bioavailability.

MATERIALS AND METHODS Materials

Candesartan cilexetil was obtained as gift sample from Ranbaxy Laboratories, Dewas, India. All other chemicals were analytical grade.

Isolation of candesartan form candesartan cilexetil

Candesartan cilexetil is an ester of candesartan. Conversion of prodrug into drug was done by hydrolysis of esters by alkali. The hydrolysis of ester prodrug candesartan cilexetil was done by method described Earb D.V. et al. About 610mg of candesartan cilexetil was dissolved in 50 ml a mixture of methanol: tetrahydrofuran: water (40:40:20). 1.83g of lithium hydroxide was added into the solution. The reaction was stirred at room temperature for 3 hours by mechanical stirrer and completion of reaction was confirmed by TLC. The resulted solution was then acidified with 10% aqueous solution of HCl. Upon acidification free drug was precipitated. The solution was diluted with ethyl acetate and water and transferred into the separating funnel. Allowed to stand for few hours. The organic phase was collected and dried over magnesium sulfate, filtered and dried into air to provide the free drug[9].

Determination of pH-Solubility profile of both candesartan and candesartan cilexetil

The solubility of candesartan and candesartan cilexetil shall be determined in aqueous solutions with pH values as in table as recommended in regulatory guidelines[10]. Various selected pH are shown in table 1.

Determination of solubility

pH-solubility profile of test drug in aqueous media with pH range of 1 to 7.5 at $37^0C \pm 1^0C$ was determined by saturation shake flask method. The solubility of candesartan cilexetil was determined in aqueous solutions with pH values of 1.2, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.4, whereas for candesartan pH values were 1.2, 2.4, 3.4, 4.4, 5.4, 6.4 and 7.4 as recommended in regulatory guidelines. The media with pH values of 1.2, 2.0, 2.5, and 3.0 were prepared by diluting HCl, whereas pH 4.5 (0.02 M acetate buffer), 6.5 and 7.5 (both 0.05 M phosphate buffers) was prepared according to USP/NF 19. Saturated solutions of candesartan and candesartan cilexetil in specific medium were prepared by dispersing an excessive amount of the drug and then equilibrating at 37 °C for 24 h in a shaking bath. After equilibrium at room temperature the content was filtered and then diluted to measure the candesartan and candesartan cilexetil concentration by a UV spectrophotometric method. Standard buffers described in USP were considered as appropriate[11].

Determination of permeability by everted intestinal sac method

Male albino wistar rats (average weight 275 ± 35 g) were obtained from animal house collage of pharmacy, IPS academy, Indore. Animals were kept in animal house and fed with pallets with water. The animals were kept for at least 3 days before sacrifice. After an overnight fast, the rat was humanely sacrificed by cervical dislocation and its intestinal segment of around 10 cm was excised, starting at the pylorus. The intestinal segment was washed twice with warm (37ºC) NaCl (0.15 mol/l) using a syringe and was transferred to a trough containing oxygenated krebs-bicarbonate Ringer's (KBR) solution. The intestine was gently everted using glass rod (diameter 3 mm). Plastic connectors were inserted into the two ends of a 10 cm segment and secured with surgical silk. These plastic connectors were then used to connect each everted intestinal segment to saline bottle filled with krebs-bicarbonate Ringer's (KBR) solution. After connection, each everted segment was placed in an organ bath (in a water bath at 37ºC) and immersed in krebs-bicarbonate Ringer's (KBR) solution (100 ml) containing drug with a continuous bubbling with aerator. The final concentration of candesartan was 10µg/ml and candesartan cilexetil was 100 µg/ml. The serosal side of the intestine was perfused with krebs-bicarbonate Ringer's (KBR)

solution (100 ml). The flow rate of perfused solution was 2 ml/min[13]. A simplified diagram of apparatus is shown in Fig. 1.

Samples of 1 ml were collected at 0 min form mucosal medium and at 15, 30, 45, 60, 90, 120, 150, 180, 240 and 300 min form serosal medium using a plastic syringe. The experiments were performed in triplicate for CS and CC.

Quantitative estimation of CS and CC

CS and CC content in sample was determined by an in-house developed high pressure liquid chromatographic (HPLC) method. Quantitative estimation of CS and CC using RP- HPLC was performed by using methanol: acetonitrile: 10 mmol potassium dihydrogen phosphate solution as mobile phase in the ratio of 90:8:2 (v/v/v, pH 4.0) at a flow rate of 0.8 ml/min. 20 µl sample ware injected and chromatograms were recorded at 254 nm. The retention time for CS and CC was 4.051 min and 5.137 min respectively.

Determination of protein contain of Intestine

Protein was assayed in the dissolved tissues using the method reported by Lowry method[14] with bovine serum albumin as the standard. . At the end of experiment, each everted segment was gently washed, blotted dry with tissue paper, weight and dissolved in 100 ml of sodium hydroxide (1 mol/ml) for at least 3 h at 37 °C for determination of protein contain of intestinal segment.

Calculation of the permeability

Drug permeability was calculated as microgram of drug transported into the serosal medium per milligram of tissue protein, and then expressed as apparent permeability (P_{app}) according to the following equation:

$$P_{app} = (dQ/dt) / (Co*A)$$

where, P_{app} is the apparent permeability coefficient (cm/s), dQ/dt is the linear appearance rate of permeability obtained for the transported amount of the substance over time (µg/min), Co is the initial concentration of the drug in the donor compartment (µg/ml), and A is the diffusion area (cm^2) of the mucosa. The diffusion surface area was calculated from the relationship between the crude surface area and the protein content (1 mg protein = 9.9546 mm^2) [13] as previously described. P_{app} is a concentration independent parameter.

Determination of the Maximum absorbable dose (MAD)

The maximum absorbable dose is the maximum amount of a drug that can be absorbed at a certain dose, MAD is defined as follows:

$$MAD = S \times K_a \times SIWV \times SITT$$

Where S = solubility (mg/ml, pH 6.5), K_a = intestinal absorption rate constant (min^{-1}; permeability in rat intestine perfusion experiment, quantitatively similar to human K_a), SIWV= small intestine water volume (250 ml, approximately), and SITT = small intestine transit time (min; 270 min, approximately)

RESULTS

pH-Solubility profile of candesartan and candesartan cilexetil

The solubility of candesartan and candesartan cilexetil was determined in aqueous solutions with pH values as in table 1 as recommended in regulatory guidelines. Solubility data of candesartan and candesartan cilexetil is shown in table 2.

Permeability study by everted intestinal sac method

The absorption rate of CS and CC across the intestine segment was determined for candesartan cilexetil.

The permeation profile (fig 3 and 4) was plotted using sample from the serosal medium at 0, 15, 30, 45, 60, 90, 120, 150, 180, 240 and 300 min. Intestinal segment of 3 different rats were used in study for each drug.

Maximum absorbable dose (MAD) of candesartan and candesartan cilxextil

Maximum absorbable dose was calculated and results are described in table 6

DISCOSSION

The solubility profile of candesartan cilexetil and candesartan was measured in various pH media within the range of the entire gastrointestinal (GI) tract is presented in Fig 2. The lowest value of solubility for candesartan cilexetil (about 37.22 ± 1.07 µg/ml) and for candesartan (about 5.49 ± 0.85 µg/ml) was obtained in the lowest pH media (1.2). The highest value of solubility for candesartan cilexetil (about 126.02 ± 1.29 µg/ml) and for candesartan (about 38.34 ± 0.85 µg/ml) was obtained in the pH 7.4. The solubility changed significantly within the well-accepted pH range of the stomach (fasting/fed state), with a dramatic increase when the pH was increased from 3 to 5, for both drug with a further increase as the pH was increased to 7.4 (fig 2). As per HHS-FDA guideline the solubility of drug should be shown in 250 ml of aqueous media over pH range 1-7.5 at 37ºC. By the pH- solubility profile, the solubility of candesartan and candesartan cilexetil was calculated for 250 ml of aqueous media and results are shown in table 3 and 4 respectively.

For CC, Diffusion area of the intestinal segment was 4.347cm2(based on total protein content). The absorption rate (mean), calculated from the slop was 5.318 × 10-3µg/mg of protein/sec. The Papp (Apparent permeability coefficient) value calculated from rate was 11.986 × 10-6 cm/s, whereas for candesartan diffusion area of the intestinal segment was 4.607cm2. The absorption rate (mean), calculated from the slop was 4.539 × 10-4 µg/mg of protein/sec. The Papp (Apparent permeability coefficient) value calculated from rate was 9.854 × 10-6 cm/s. Diffusion area of the intestinal segment was 4.607cm2. The absorption rate (mean), calculated from the slop (n =3) was 4.539 × 10-4 µg/mg of protein/sec. The Papp (Apparent permeability coefficient) value calculated from rate was 9.854 × 10-6 cm/s.

CONCLUSION

As per US-FDA guideline for biopharmaceutical classification system, a drug is considered highly soluble when the highest dose is soluble in 250 ml or less of water over the pH range of the gastrointestinal tract (pH 1—7.5). Highest dose of candesartan cilexetil (32mg) is not soluble at all pH values in 250 ml of water. It would not meet the definition of ―highly soluble‖. Consequently, candesartan cilexetil with low solubility would be classified as either Class II (high permeability) or IV (low permeability) in the BCS. A drug substance is defined as highly permeable when the extent of absorption in humans is ≥ 90% of an administered dose, based on mass-balance or in comparison to an intravenous reference dose; however, there are no intravenous data available for candesartan cilexetil. Ungell et al[15]. divided the compound, which are thought to be passively transported across the intestinal mucosa can be divided into two groups; low and high permeability drugs with P_{app} values ranging from 0.9-8.3 × 10^{-6} and 11.4-100.3 × 10^{-6} cm/s, respectively. In-vitro permeability study of candesartan and candesartan cilexetil was determined by everted intestinal sac method. Candesartan was found to have low permeability since it shows less than 90% permeation. Candesartan cilexetil was found to have high permeability since it shows more than 90% permeation. Finally on the bases of result CS can be classified into class IV and CC can be classified into class II.

Table 1: Buffers of Different pH selected for solubility study

S.No	pH Condition	Candesartan Cilexetil (pKa=4)[12]	Candesartan (pKa=4.4)[12]
1	Acidic	1.2	1.2
2	pKa - 2	2.0	2.4
3	pKa - 1	3.0	3.4
4	pKa	4.0	4.4
5	pKa + 1	5.0	5.4
6	pKa + 2	6.0	6.4
7	Basic	7.4	7.4

Table 2: pH-solubility profile of candesartan and candesartan cilexetil (n=3)

S.No.	pH	Solubility (µg/ml)	
		Candesartan Cilexetil	Candesartan
1	1.2	37.21 ±1.08	5.49±0.85
2	2.0	41.37±1.11	-
3	2.4	-	6.62±0.24
4	3.0	45.68±3.86	-
5	3.4	-	7.59±1.26
6	4.0	57.54±4.40	-
7	4.4	-	18.73±2.83
8	5.0	95.72±3.06	-
9	5.4	-	32.51± 3.75
10	6.0	112.57±5.59	-
11	6.4	-	36.15±2.88
12	7.4	126.02± 5.32	38.34±3.67

Table 3: Maximum solubility of candesartan in 250 ml of aqueous media over pH range 1-7.5 at 37ºC (n=3)

S.No.	pH	Candesartan	
		Solubility (µg/ml)	Solubility (mg/250 ml)
1	1.2	5.49±0.85	1.37±0.21
2	2.4	6.62±0.74	1.65±0.16
3	3.4	7.59±0.10	1.89±0.16
4	4.4	18.73±1.05	4.68±0.92
5	5.4	32.51±2.65	8.12±1.27
6	6.4	36.15±2.86	9.03±2.28
7	7.4	38.34±4.38	9.58± 2.71

Table 4: Maximum solubility of candesartan cilexetil in 250 ml of aqueous media over pH range 1-7.5 at 37ºC (n=3)

S.No.	pH	Candesartan cilexetil	
		Solubility (µg/ml)	Solubility (mg/250 ml)
1	1.2	37.21±1.08	9.30±0.79
2	2.0	41.37±3.11	10.34±1.67
3	3.0	45.68±3.87	11.42±2.96
4	4.0	57.54±3.12	14.38±2.28
5	5.0	95.72±4.33	23.93±3.08
6	6.0	112.57±3.30	28.14±3.51
7	7.4	126.02±4.81	31.50±3.32

Table 5: In-vitro Intestinal permeability of candesartan cilexetil and candesartan (n=3)

S.No.	Time (Min)	Cumulative % of drug permeated	
		Candesartan Cilexetil	Candesartan
1	0	0	0
2	15	0	0
3	30	15.36±3.78	15.80±4.85
4	45	21.62±4.36	24.53±4.67
5	60	34.46±4.82	28.32±5.27
6	90	43.43±6.08	39.74±5.93
7	120	66.21±5.71	48.92±6.07
8	150	74.53±7.63	64.79±8.94
9	180	92.36±6.62	77.05±5.58
10	240	95.58±6.73	81.36±6.06
11	300	97.64±8.83	85.24±6.24

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Table 6: Maximum absorbable dose (MAD) of candesartan and candesartan cilxextil

S.No.	Drug	Solubility at pH 6.5 (mg/ml)	Rate of absorption (mg /min)	MAD (mg)
1	Candesartan	0.036 ±0.01	$4.53x10^{-3}$	11.03
2	Candesartan Cilexetil	0.112±0.03	$5.38x10^{-3}$	38.84

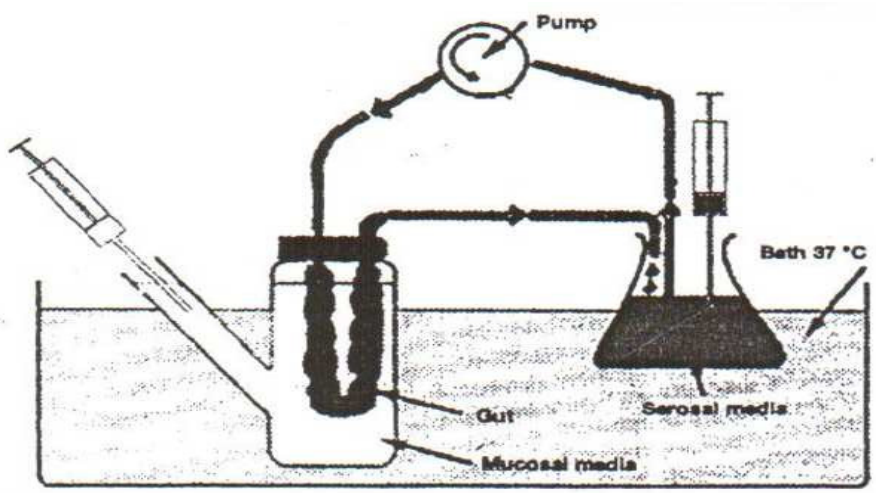

Fig 1: Perfusion of the everted intestinal gut

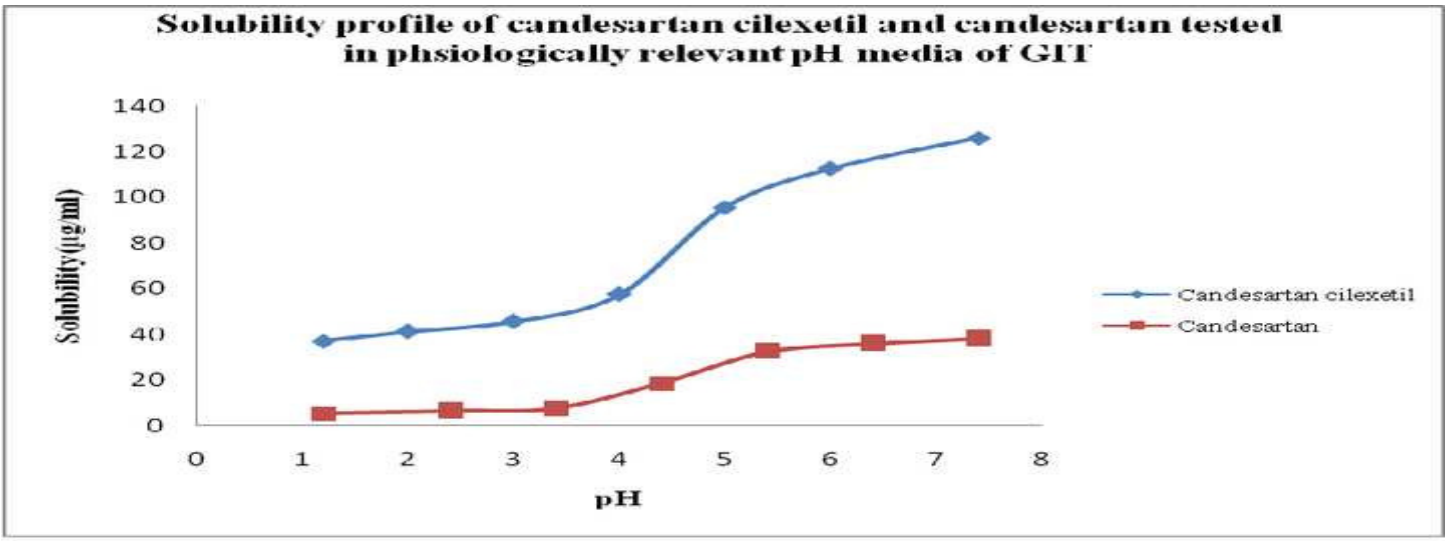

Fig 2: pH-solubility profile of candesartan cilexetil and candesartan

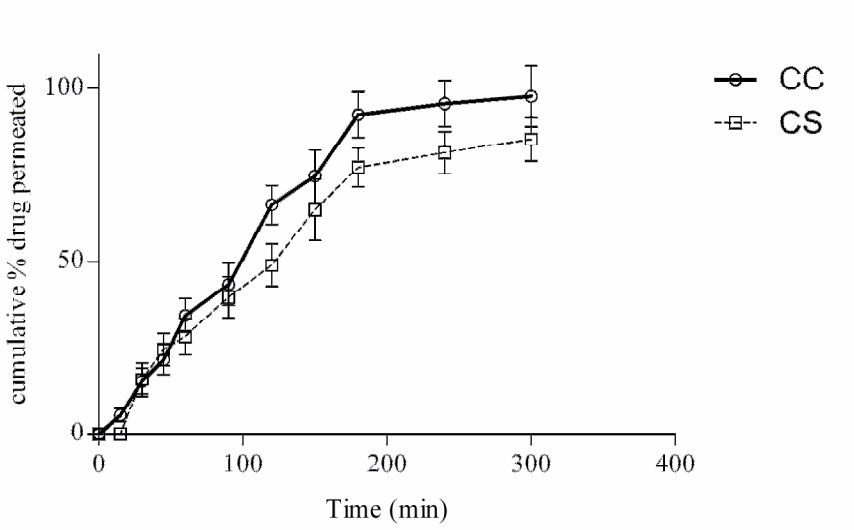

Fig. 3 In-vitro permeation profile of CC and CS

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