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Research Article

IN-VIVO BIOAVAILABILITY STUDY OF TELMISARTAN COMPLEX IN WISTAR RATS

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ABSTRACT

The main of the present study is to evaluate *In-Vivo* Bioavailability Study of Telmisartan Complex in Wistar Rats. During study periods, rats were housed singly in polypropylene and stainless steel cages. Two to three groups of rats for optimized formulation and one group of rats for marketed formulation were taken. A blood sample was collected from the retro-orbital plexus of anesthetized rats with diethyl ether. For zero time analysis, a blood sample was withdrawn before administration of the drug and after administration, blood samples were collected at the specific intervals. A High-Performance Liquid Chromatographic System (HPLC) equipped with Shimadzu LC SOLUTION was employed for the present investigation. The system consisted of Shimadzu UFLC 20-AD as a binary solvent delivery system, Shimadzu 7D Rheodyne injector loop injector and Photo Diode Array (PDA) detector as a source of detection. Telmisartan (BCS Class II drug) is an angiotensin II receptor antagonist and these are used in the treatment of hypertension and these are practically insoluble in water. The preliminary studies conducted with quercetin complexes showed that the complex prepared with kneading method and having the drug: complexing agent 1:3 ratios in case of Telmisartan showed better *in vitro* dissolution rate and hence the resulting complex bioavailability was compared with the pure drug. Pharmacokinetic parameters such as absorption rate constant, biological half life, AUC 0-∞ were calculated for pure drug and bioenhancers complexes. These parameters were treated statistically and significant differences in absorption related terms such as extent of absorption (AUC) and rate of absorption (ka) were noticed.

Keywords: Telmisartan, Bioavailability, AUC, HPLC, Cytochrome P450 (CYP), Cmax.

1. INTRODUCTION

Drug absorption is the process whereby drug molecules are transferred from the site of administration across biological membranes into the systemic blood circulation to produce a systemic pharmacological effect. Biological cell membranes have a lipophilic nature due to their phospholipid bilayer structures. Molecules should therefore have sufficient hydrophilic properties to dissolve in the aqueous environments surrounding the biological membranes, but should also have sufficient lipophilic properties to partition into the membranes in order to achieve passive absorption via the transcellular pathway [1]. Adjacent epithelial/endothelial cells are connected by tight junctions, which are traversed by aqueous channels/fenestrae through which only small water-soluble molecules (<600 Da) can pass to get absorbed via the paracellular pathway [2].

Pre-systemic metabolism occurs mainly in the enterocytes of the gastrointestinal epithelium and the hepatocytes of the liver. The Cytochrome P450 (CYP) family of enzymes account for the majority of oxidative metabolic reactions of xenobiotics during pre systemic and systemic metabolism. More than 30 different human CYP enzymes have been identified, of which CYP3A4 appears to be one of the most important drug-metabolizing enzymes in humans [3,4]. Inhibition of CYP enzymes in the intestinal epithelium and liver can significantly impact upon the bioavailability of drugs that are substrates of these enzymes by means of reducing pre-systemic metabolism [4-7].

In the present investigation, In-Vivo bioavailability studies and study of various pharmacokinetic parameters was studied selected anti-hypertensive drug by different ration of herbal bioenhancer.

2. MATERIAL AND METHODS

2.1. Bioavailability study

2.1.1. Selection of animals

Wistar rats of either sex with an average weight of 250 ±10 gm were procured in order to investigate the pharmacokinetic behavior of prepared formulations of Telmisartan with natural bioenhancers. The study was

approved by Institutional Ethics Committee of RKDF College of Pharmacy, Bhopal India (CPCSEA No: RKD FCP/IAEC/2020/28, Dated-24-11-20) and the guidelines were followed throughout the study. All the rats were acclimatized to a temperature (20±2°C) and relative humidity (45±15%RH), with a 12 hr light/dark cycle over a period of 5 days prior to administration of the drug. During this acclimatization period, the animals were carefully observed to ensure their good health and suitability for inclusion in the study. For all rats, a standard laboratory diet (Pranav Agromart Ltd, Baroda, India) and domestic mains tap water were available *ad libitum*. During study periods, rats were housed singly in polypropylene and stainless steel cages [8, 9]. Two to three groups of rats for optimized formulation and one group of rats for marketed formulation were taken.

2.1.2. Collection of blood samples

A blood sample was collected from the retro-orbital plexus of anesthetized rats with diethyl ether. For zero time analysis, a blood sample was withdrawn before administration of the drug and after administration, blood samples were collected at the specific intervals. Samples were collected as per above-mentioned for each group. Blood was collected in such a quantity to get 0.5 ml plasma from it at each time point. The collected sample was analyzed by the suitable analytical method. Maximum blood concentration (Cmax) and time to achieve Cmax (Tmax) was calculated from concentration-time curve data. The area under the concentration-time curve was calculated to the last blood concentration. These parameters and other pharmacokinetic parameters were calculated by Microsoft Excel® version 2013 (Microsoft Corporation, Washington, USA). Reduction of dose to reach Cmax of the marketed formulation was also calculated.

2.1.3. Bioanalytical method for Telmisartan formulations [10]

A High-Performance Liquid Chromatographic System (HPLC) equipped with Shimadzu LC SOLUTION was employed for the present investigation. The system consisted of Shimadzu UFLC 20-AD as a binary solvent delivery system, Shimadzu 7D Rheodyne injector loop injector and Photo Diode Array (PDA) detector as a source of detection.

2.1.4. Chromatographic conditions

Mobile phase: Methanol and Acetonitrile (70:30 %v/v)
Column: Phenomenex Luna® C8 column (300 mm*4.6 mm + pore size 100 Å)
Flow rate: 1 ml/min
Injection volume: 20 µl
Run time: 10 min

2.1.5. Preparation of mobile phase

For preparing a mobile phase, HPLC grade methanol and acetonitrile were filtered through a 0.2 µm membrane filter and subjected to degassing in an ultrasonic bath for a period of 15 mins.

2.1.6. Standard solutions

A primary stock solution (1 mg/ml) was prepared by dissolving 10 mg of Telmisartan in 10 ml of HPLC grade methanol. The stock solution was suitably diluted with HPLC grade methanol to obtain a working range of standard solutions. The working standard solutions were used to prepare a calibration curve. Plasma used in the study was isolated from rat's blood by centrifugation at 10000 RPM for a period of 15 min at 4°C, using a laboratory centrifuge. The calibration curve samples were prepared by spiking 500 µl of drug-free rat plasma with 100 µl of previously diluted working standard solution in order to obtain final concentrations of 10, 25, 50, 75, 100, 250, 500, 750 and 1000 ng/ml. All samples were stored in refrigerated cold conditions (2-8°C) and equilibrated to room temperature prior to use.

2.1.7. Sample preparation

Prior to sample analysis, 100 µl of each solution was extracted using 300 µl of diethyl ether: dichloromethane (60:40% v/v) for protein precipita-tion. Further, each of the mixtures was vortexed for a period of 5 min in a vortex mixer with subsequent centrifugation at 10,000 RPM, for a period of 10 mins at 4°C using a centrifuge. For each sample, an aliquot of a supernatant was isolated and subjected to dryness. The residue was reconstituted in 100 µl of mobile phase and subsequently centrifuged at 10,000 RPM for 10 min at 4°C in a laboratory centrifuge. The supernatant was finally collected and directly injected into the HPLC system.

2.1.8. Construction of calibration curve

The values of peak areas were plotted against their respective concentrations in order to construct the calibration curve for Telmisartan. Linear regression analysis was performed for each set of data using

Microsoft Excel® version 2013 (Microsoft Corporation, Washington, USA).

2.2. Pharmacokinetic studies

The applicability of the developed HPLC method for Telmisartan in rat plasma was demonstrated by the results obtained from pharmacokinetic studies conducted on Wistar rats (n=6). Each rat was treated with oral formulations of Telmisartan at a dose of 4 mg/kg in a single dose by curved gastric gavages tubes directly into the stomach. Serial blood samples were collected from retro-orbital venous plexus with haematocrit over a period 24 h. Blood samples from each group were collected at predetermined time intervals into heparinized plastic tubes. All these samples of blood were kept in refrigerated cold conditions (2-8°C) until separation of plasma. Each sample was processed further by the method as mentioned under sample preparation and subjected to HPLC analysis for the estimation of drug content by a Bioanalytical method. The pharmacokinetic calculations were performed on the basis of plasma concentration-time data [11].

3. RESULTS AND DISCUSSION

3.1. Bioavailability study

Telmisartan (BCS Class II drug) is an angiotensin II receptor antagonist and these are used in the treatment of hypertension and these are practically insoluble in water. The preliminary studies conducted with quercetin complexes showed that the complex prepared with kneading method and having the drug: complexing agent 1:3 ratios in case of Telmisartan showed better *in vitro* dissolution rate and hence the resulting complex bioavailability was compared with the pure drug.

Table 1: Plasma concentration data of telmisartan pure drug and Telmisartan quercetin complex

S. No	Time(hrs)	Concentration (ng/ml)	
		Pure drug	Complex
1	0	0.000	0.000
2	0.5	10.135±0.293	15.42667±0.487
3	1	18.09667±0.538	27.16167±0.563
4	2	30.84167±0.264	43.06333±0.453
5	4	36.71833±0.239	53.495±0.432
6	6	39.695±0.901	56.29±0.213
7	8	44.34667±0.534	63.41667±0.452
8	12	51.69333±0.856	74.445±0.293
9	14	61.71±0.526	86.95333±0.231
10	16	54.62±0.589	78.78833±0.271
11	18	35.88±0.569	50.995±0.234
12	20	28.05333±0.576	38.36333±0.245
13	22	24.45±0.897	34.74333±0.658
14	24	18.46667±0.789	26.705±0.683
15	26	13.08333±0.253	18.77±0.187
16	30	10.18333±0.293	14.74333±0.278
17	36	8.35±0.783	12.05833±0.424

Table 2: Statistical analysis of the pharmacokinetic parameters of telmisartan

Particulars	C max (ng/ml)	Tmax (hrs)	K_a (1/hr)	AUC (ng -hr /ml)	$t_{½}$ (hrs)
Pure drug	61.56±0.293	14	0.7199±0.0254	1067.228±3.753	21.08±0.5
Complex	86.66±1.169	14	0.7815±0.0446	1524.25±17.67	20.52±0.9813
t-value	3.802	-	2.246	1.133	-
Level of significance	<0.0001	NS	<0.002	<0.0001	NS

Telmisartan are anti-hypertensive drugs which are insoluble in water, so these drugs are incompletely dissolved in the gastro-intestinal tract. The rate of dissolution and therefore its bioavailability is less. In the present study, an attempt has been made to prepare binary complexes with luteolin and quercetin and ternary complexes with citric acid by formulating with different techniques and different ratios. It reduces crystallinity of active ingredient by forming complexation. Plasma concentrations of telmisartan pure drug and telmisartan-HP-β-CD complexes were measured from the rabbit plasma and the results are shown in table. Pharmacokinetic parameters such as absorption rate constant, biological half life, AUC 0-∞ were calculated from the plot of time versus plasma concentration and reported. The results were treated statistically. It indicated that the pharmacokinetic parameters of telmisartan-quercetin complexes were differed from the telmisartan pure drug except in biological half life and Tmax. The highest mean Cmax value was observed for Telmisartan. Quercetin complex (88.43}1.169 ng/ml) compared to the maximum plasma concentration observed with telmisartan pure drug (61.56}0.293 ng/ml) and these values were statistically significant. The time taken to reach peak plasma concentration Tmax was 14 hrs in both pure drug and complexes. The mean Ka for pure drug and complex were found to be 0.7198} 0.0252 h-1and, 0.7816}0.0444 h-1 respectively. The AUC0-∞ values observed with telmisartan-luteolin complexes 1524.25} 17.67 ng hr/ml in compared to pure drug values 1067.228}3.753 ng hr/ml. These results were also statistically significant the biological half life for 21.08 0.5 hrs for telmisartan pure drug and 20.52 0.9813 hrs for telmisartan-quercetin complex. However the difference in t1/2 values recorded for pure drug and complexes was statistically insignificant. The pharmacokinetic parameters were treated statistically with paired sample's t-test. Significant differences in absorption related terms such as extent of absorption (AUC) and rate of absorption (ka) were noticed. However no differences in elimination phase were observed. Telmisartan-quercetin complexes showed 1.45 fold increases in bioavailability.

4. CONCLUSION

This investigation is aimed to improve the dissolution rate of Telmisartan by forming inclusion complexes. The drugs telmisartan exhibited pH dependent and particle size dependent solubility. Pharmacokinetic parameters such as absorption rate constant, biological half life, AUC 0-∞ were calculated for pure drug and bioenhancers complexes. These parameters were treated statistically and significant differences in absorption related terms such as extent of absorption (AUC) and rate of absorption (ka) were noticed.

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