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Effect of hydrochlorothiazide on pharmacodynamics and pharmacokinetics of losartan in human patient volunteers

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Abstract
In this study, the influence of hydrochlorothiazide on pharmacodynamics and pharmacokinetics of losartan was studied using human hypertensive patients. A total of 135 hypertensive patients were included in this study. The reduction in the blood pressure in patients receiving the Losartan alone and in patients receiving the both Losartan and hydrochlorothiazide was estimated. The pharmacokinetic parameters also estimated using the plasma levels of Losartan in both group of patients. The findings of present investigation have concluded that the efficiency of Losartan along with the Hydrochlorothiazide was high when compared to that of Losartan alone, and preliminary pharmacokinetic data also revealed that there were no interactions with the usage of combination of drugs.

Keywords: losartan, hydrochlorothiazide, drug interaction, pharmacodynamics

1. Introduction
Raised blood pressure or hypertension is one of the most critical risk factors in the development and progressive cardiovascular disease (Weber *et al*., 2004; Yusuf *et al*., 2004) [10, 12]. Hypertension is an hemodynamics disorder in which the blood pressure values are $140/90 mm Hg for systolic/diastolic blood pressures and it is classified in different grades in function of the value measured (WHO., 2011; Stephan *et al*., 2015) [11, 8]. This disorder leads to an increase in morbidity and mortality of patients not adequately controlled (James *et al*., 2014) [4]. There are several classes of drugs designed to maintain blood pressure values lower. In order to arrive to this goal, it has been suggested to start with an antihypertensive drug, if the goal is not reached, addition of another antihypertensive drug is recommended and if the blood pressure is not controlled with the use of two antihypertensive drugs, three agents should be used (Amar *et al*., 2002; James *et al*., 2014) [1, 4]. A rationale combination may include an angiotensin II receptor antagonist like Losartan and a diuretic like Hydrochlorothiazide (Waeber *et al*., 2009).
Losartan is a non-peptide angiotensin II receptor antagonist used as an antihypertensive agent, that block the vasoconstrictor and aldosterone-secreting effects of angiotensin II by type AT1 receptor blockage (Oliveira *et al*., 2006; Tamimi *et al*., 2005) [6, 9]. Losartan may reduce cardiovascular events more significantly than β-blockers in patients with hypertension and left ventricular hypertrophy (Zaiken *et al*., 2013) [13]. Losartan decreased platelet aggregation by a thromboxane A2-dependent mechanism (Guerra-Cuesta *et al*., 1999) [3]. Hydrochlorothiazide (HCT), a benzo thiazide diuretic, by its sodium-depletion effect results in renin-angiotensin-system (RAS) activation, which explains its beneficial therapeutic interaction with drugs acting through blockade of the RAS, including angiotensin II (Ang II) AT1-receptor blockers (ARBs) (Puschett *et al*., 2000) [7]. In view of its combination, it is a need to study the effect of Hydrochlorothiazide on pharmacokinetics and pharmacodynamics of losartan in human patient volunteers. So far no reports have been submitted on this study from India, hence we have undertaken this study. In this present work, patients suffering from Stage I Hypertension from different areas of Karimnagar, southern India, using hypertensive therapies were included. The present work had been aimed to evaluate the effect of Hydrochlorothiazide on pharmacodynamics and pharmacokinetics of Losartan in human patient volunteers.

2. Materials and Methods
Study Population
A total of 135 patients prescriptions were analysed and it was found that 29% of hypertensive patients treating with Losartan (50mg) alone (LOS) or in combination with Hydrochlorothiazide (12.5mg) (LOS and HCT). Among them 39 patients either of the sex patients of age between 38-70 yrs participated in the study. These patients were analyzed from out-patient wards of few hospitals Karimnagar town. Twelve healthy volunteers from karimnagar town were included in this study as controls. Before enrollment all patients were informed about the possible risks and discomfort involved in participating in the study. A voluntary consent form was taken from all the patients. The study was approved by the Institutional Human Ethical committee.

Table 1: Details of subjects

S. No.	Subjects	Number
1	Patients receiving Losartan 50mg alone (LOS)	20
2	Patients receiving combination of Losartan (50mg) and Hydrochlorothiazide (12.5mg) (LOS +HCT)	19
3	Control (Healthy volunteers)	12

Inclusion criteria

- Patients, regardless of gender, at least 30 years of age and diagnosed with high blood pressure based on the average of two or more properly measured, seated, blood pressure readings were included for the study.
- The patients with Systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg are considered in to study (Chobanian *et al*., 2003)
- The prescriptions of patients receiving therapies were analyzed for the diagnosis of Stage I hypertension, and cardiac atherosclerosis disease using Hydrochlorothiazide and Losartan for the first time usage of the drugs.

Exclusion criteria

- Patients were excluded if they had a history of kidney disease; diabetes; acute liver injury (e.g., hepatitis) or severe cirrhosis; pregnancy or breast-feeding; history of drug or alcohol abuse; or participation in a study of an investigational medication within the past 30 days.

Estimation of Pharmacodynamic Parameters

The pharmacodynamics of Losartan i.e., reduction of the blood pressure was recorded. The blood pressure was measured by using traditional sphygmomanometer with help of paramedical staff on first day of treatment before starting of therapy which was considered as zero day, and after four hours of treatment considered as 1st day, and consecutive 15th day, 30th day of treatment.

Estimation of Pharmacokinetic Parameters

Blood Samples were collected from the patients treating with Losartan alone and in combination with Hydrochlorothiazide at 30th day after start of therapy at different intervals. Plasma samples were collected by centrifugation of blood samples and were stored at -20°c until analysis. The stored samples are analyzed for plasma Losartan concentration using High performance liquid chromatographic (HPLC) using method reported by Oliveira *et al*., (2006) [6], Tamimi *et al*., (2005) [9] and Mandes *et al*., (2015).

Estimation for Losartan plasma concentrations using HPLC

i) HPLC Conditions used

Phenomenex (250×4.6mm) C18 5µm reverse phase analytical column was used. The mobile phase consist of 55: 45 (v/v) mixture of orthophosphoric acid (0.1% v/v) and Acetonitrile at a flow rate 1ml/min. Before Use, the mobile phase was filtered by using it through a 0.45µm filter and the filtrate is degassed by using bath sonicator. The mobile phase was pumped at an isocratic flow of 1ml/min and the column temperature was controlled at 25 ^{0}C and the injection volume was 20 µl. The peaks were determined using a UV detector set at a wavelength of 225nm.All the procedures were performed at ambient temperature.

Table 2: HPLC descriptions

HPLC model	Shimadzu SPD 10 UV Detector
Pump model	LC 10AD
Syringe	Reodine
Injector capacity	20µl
Detector	Dual wavelength UV-Visible.
Data analyzer	N 2000 software

ii) Chromatography conditions

Table 3: Mobile phase composition

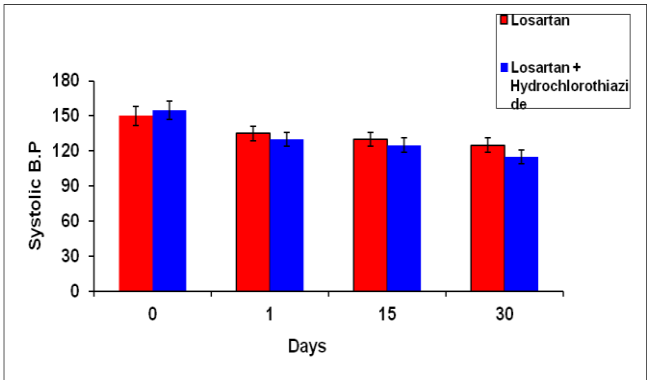
Solvent A + Solvent B	Orthophosphoric acid (0.1% v/v) + Acetonitrile
Proportion	55 : 45 V/V
Flow rate	1ml/min
Wave length	225nm
Injection volume	20 µl
Column	Athena C-18 reverse phase 250x4.6mm and 5µm particle size
Total run time	10 min's
Drug	Losartan
Retention time	2.6 min

Pharmacokinetic Parameters and Statistical Analysis

On 30th day of treatment or study period, blood samples were collected at various intervals (0.5, 1.0, 1.5,2.0,2.5, 4.0, 8.0, 12.0 hrs) from the both treatment groups and based on the estimated plasma Losartan concentrations, various pharmacokinetic parameters were estimated. Pharmacokinetic parameters like area under the curve (AUC), Half life ($t_{1/2}$), C_{max}, T_{max}, Clearance (Cl), Volume of distribution (Vd) are estimated by using RAMKIN Software. All the values are expressed as mean ± S.D. All the results were compared with zero day patients. T-test was used to analyse the results and $p < 0.05$ was considered as statistically significant.

3. Results

The results of interaction between hydrochlorothiazide and losartan on Pharmacodynamics in Hypertensive patients were showed in Figures 1&2.



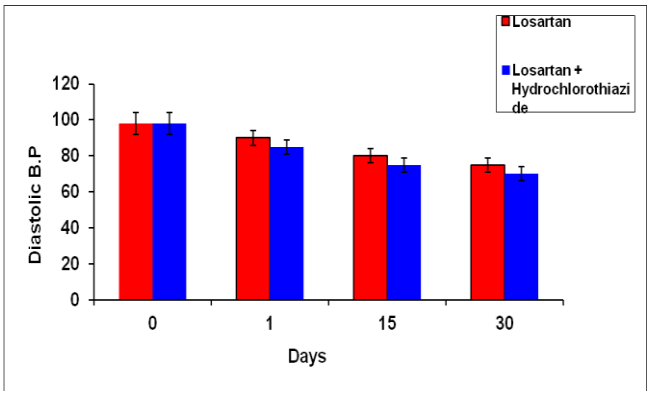
(All the values are expressed in mean ±SD)

Fig 1: Systolic Blood pressure of patients treating with Losartan alone and in combination with Hydrochlorothiazide

The decrease in systolic blood pressure levels were high in the patients treating with Losartan + Hydrochlorothiazide when compare to Losartan alone as shown in figure 1. Treatment with Losartan to hypertension patients resulted in significant reduction of Systolic Blood pressure (P<0.05) in patients in all the measured days. The % of B.P reduced with single dose of Losartan was 10% and were 13%, 16.7% at 15th and 30th day of treatment respectively. Whereas the treatment with Losartan and Hydrochlorothiazide to the hypertension patients results in the significant reduction of Systolic blood pressure (P<0.05) and the percentage of systolic blood pressure reduced with this combination was 16% and were, 19.2%, 26% at 15th and 30th day of treatment respectively.

Diastolic blood pressure

The diastolic blood pressure values of patients using Losartan alone, and combination of Losartan and Hydrochlorothiazide were shown in figure 2.



(All the values are expressed in mean±SD)

Fig 2: Diastolic Blood pressure of patients using Losartan alone and in Combination of Losartan and Hydrochlorothiazide.

The treatment with Losartan to hypertension patients results in the significant reduction of diastolic Blood pressure ($P<0.05$) in patients. The % of B.P reduced with single dose of Losartan was 8% and were 18%, 23% at 15th and 30th day of treatment respectively. Whereas the treatment with Losartan and Hydrochlorothiazide to the hypertension patients results in the significant reduction of diastolic blood pressure ($P<0.05$) and the percentage of diastolic blood pressure reduced with combination was 13% and were, 23%, 25.5 at 15th and 30th day of treatment respectively

Pharmacokinetic Parameters

The plasma concentrations of Losartan from the 30th day samples of patients receiving Losartan alone and in combination with Hydrochlorothiazide are given in Table 3.

Table 3: Plasma concentration of samples in Losartan alone and in combination with Hydrochlorothiazide on 30th day of study.

Time (hr)	Losartan alone (ng/ml)	Losartan and Hydrochlorothiazide (ng/ml)
0.5	90.5	95.7
1.0	125.0	139.8
1.5	101.5	112.6
2.0	80.6	93.6
2.5	81.7	94.0
4.0	42.5	53.2
8.0	26.5	34.9
12.0	22.5	30.4

The plasma Losartan concentrations in patients treating with Losartan alone were slightly morethan those of patients treating with Losartan and Hydrochlorothiazide. The maximum plasma Losartan concentration was found to be at 1st and was 135.2ng/ml, 141.6ng/ml in LOS alone and LOS +HCT groups respectively. From the estimated concentrations, the preliminary pharmacokinetic parameters were calculated using Ramkin Software, and the results were shown in Table no 4.

Preliminary data of Pharmacokinetic Parameters

Preliminary data of pharmacokinetic parameters after LOS alone, and in combination with hydrochlorothiazide treated patients was showed in table 4.

Table 4: Preliminary data of pharmacokinetic parameters after LOS, LOS + HCT

PK Parameter	Losartan	Losartan + Hydrochlorothiazide
AUC $_{0\text{-}t\ (\mu g\text{-}hr/ml)}$	392.5	420.4
AUC $_{0\text{-}\alpha(\mu g\text{-}hr/ml)}$	408.6	440.2
t $_{½(hr)}$	2.22	2.3
V d (ml/ug)	457.8	435.6
Cl/f (mg/kg)/(ug/ml)/hr	142.1	147.4
C max (µg/ml)	135.2	141.6
T max (hr)	1.06	1.0

In Losartan group, the AUC $_{0\text{-}\alpha}$ was 408.6 ng.hr/ml, where as it is in LOS+HCT group of patients, it was 440.2 ng.hr/ml indicates the elevation of AUC in (LOS +HCT) subjects. It indicates the bioavailability of Losartan was affected in presence of Hydrochlorothiazide. Hence it is confirmed that the bioavailability of Losartan was not significantly affected in presence of Hydrochlorothiazide.

4. Discussion

Oral administration of losartan and HCT was considered safe and also controlling the hypertension in all the treated subjects. After oral administration of losartan (50 mg) or losartan (50 mg) + hydrochlorothiazide (12.5 mg), the observed pharmacokinetic parameters of losartan of both oral administrations were similar to those reported in the literature for losartan (Mendes *et al*., 2015) [5]. As shown in Figure 1& 2, the systolic and diastolic blood pressures measurements revealed that there was a more significant reduction of BP in LOS+ HCT group than that of LOS alone, indicating the synergistic effect of HCT combined with LOS. Whereas for pharmacokinetic interaction between the drugs, there is statistically insignificant change in the pharmacokinetic parameters were found in group of LOS + HCT than those of LOS alone. However, the area under the curve (AUC), Cmax and other parameters were more in combination groups. The findings of present investigation have concluded that the efficiency of Losartan along with the Hydrochlorothiazide was high when compared to that of Losartan alone, and preliminary pharmacokinetic data also revealed that there were no interactions with the usage of combination of drugs. Further studies were needed with various pharmacokinetics, and Pharmacodynamics outcomes, with a higher number of patients, and regular inpatient monitoring for the conclusion of the safety profile of the combination of drugs.

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