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# Pharmacokinetic and Pharmacodynamic Interaction between Simvastatin and Diltiazem in Hypercholesterolaemia and Hypertension Induced Rats

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**Abstract:** 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors are often prescribed along with antihypertensive agents, including calcium antagonists. Simvastatin is an HMG-CoA reductase inhibitor and Diltiazem which calcium antagonist it is metabolized by the cytochrome P450 (CYP) 3A4. The purpose of this study is to investigate drug interactions between simvastatin and diltiazem. In the present investigation, we studied the pharmacokinetics and dynamics of simvastatin alone and in combination with diltiazem in hyperlipidemic and hypertensive rats. The standard cholesterol diet was used to induce hyperlipidemia and 10% fructose solution was used to induce hypertension in Wistar rats. The selected drugs were given for 2 weeks by oral route, simvastatin followed by 2 weeks co-administration of diltiazem and simvastatin. Combined treatment with simvastatin and diltiazem increased the peak concentration ( $C_{max}$ ) of HMG-CoA reductase inhibitors from  $15.64 \pm 0.29 \mu\text{g/mL}$  to  $19.45 \pm 0.07$  ( $P < 0.01$ ) and the area under the concentration-time curve (AUC) from  $60.19 \pm 2.24 \mu\text{g h/mL}$  to  $78.4 \pm 0.80 \mu\text{g h/mL}$  ( $P < 0.01$ ) without affecting the cholesterol-lowering effect of simvastatin. In hyperlipidemic rats, simvastatin caused a marked reduction in the lipid profiles but combination with diltiazem produced a significant change. In pharmacokinetics, witnessed insignificant change in the combination of simvastatin and diltiazem when compared with simvastatin alone. From the results synergistic activity of simvastatin with diltiazem was witnessed

**Keywords:** Pharmacokinetic; Pharmacodynamic; Hypertension; Hyperlipidemia; Simvastatin; Diltiazem

## 1. Introduction

In the broadest sense, a drug-drug interaction (DDI) may be defined as the pharmacological or clinical response to the administration of a drug combination that is different from that anticipated from the known effects of the two agents when given alone and that can result in reduced effectiveness or increased toxicity.(1) A DDI can be the consequence of various situations that reflect the growing number of drugs available on the market. The increasing complexity of poly therapy is a major source of DDIs. The very widespread practice of self-medication makes the situation more severe and difficult.

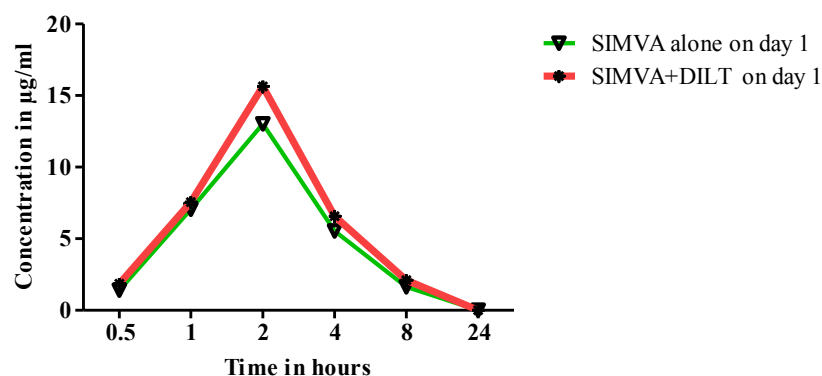
Simvastatin (SIMVA), a hydroxymethyl glutarate coenzyme A (HMG-CoA) reductase inhibitor, is a commonly used cholesterol lowering agent which is metabolized through the CYP3A4 pathway.(2) It is used in the treatment of hyperlipidemia and/or cardiovascular complications like coronary heart disease along with calcium channel blocker. Diltiazem (DILT) is a calcium channel blocker (CCB) widely used in the treatment of angina and hypertension, and may have potential is preventing stroke and ischemic heart disease.(3,4) DILT is extensively metabolized in the liver, primarily by deacetylation and demethylation by CYP3A4 into a host metabolite, N-desmethyl-DILT, which along with DILT, in turn selectively inhibits CYP3A4. Concomitant use of CYP3A4 inhibitors has the potential to increase exposure to SIMVA.(5) DILT is a substrates and inhibitors of CYP3A4 and therefore increase the plasma concentration ( $AUC_{0-24h}$ ) and maximum plasma concentration ( $C_{max}$ ) of SIMVA when co-administered.(6,7)

In this study we prospectively studied the pharmacokinetic and pharmacodynamic interactions between SIMVA and DILT in hypercholesterolemia and hypertension induced rats.

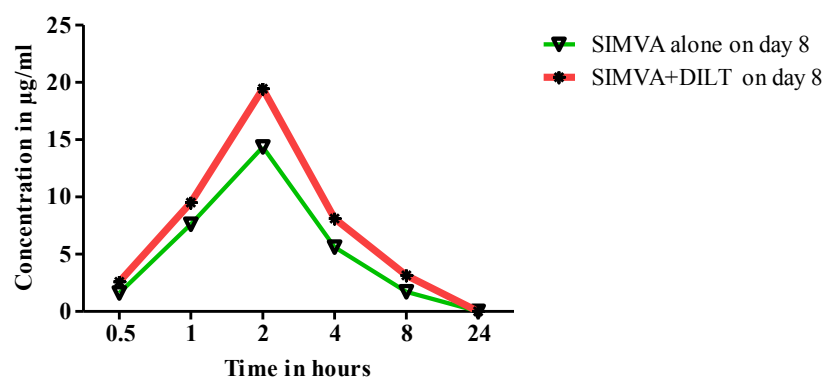
## 2. Results and Discussion

### 2.1. Pharmacokinetic Study

Pharmacokinetic parameters like  $C_{max}$ ,  $T_{max}$ , AUC,  $T_{1/2}$ , MRT, CL/f and  $V_d$  were calculated for each subject using a non-compartmental pharmacokinetic model. The plasma levels of Group-III and Group-IV at different time points, on day 1 and day 8 were shown in Fig 1 & 2 and respective pharmacokinetic parameters are shown in Table 1.



**Figure 1.** Plasma concentration-time profile of SIMVA alone and SIMVA + DILT on day 1



**Figure 2.** Plasma concentration-time profiles of SIMVA alone and SIMVA+DILT on day 8

**Table 1.** Pharmacokinetic parameters of SIMVA alone and combination with DILT on day1 and day 8

Parameter	SIMVA alone (day1)	SIMVA+DILT (day1) <sup>a</sup>	SIMVA alone (day8)	SIMVA+DILT (day8) <sup>b</sup>
$C_{max}$ (µg/ml)	13.02±0.46	15.64±0.29	14.36±0.32	19.45±0.07**
$T_{max}$ (hrs)	2±0	2±0	2±0	2±0
AUC <sub>tot</sub> (µg h/mL)	50.12±1.37	60.19±2.24	53.43±2.04	78.4±0.8**
$T_{1/2}$ (hrs)	2.04±0.17	2.12±0.12	2.00±0.11	2.35±0.03*
MRT(min)	3.77±0.25	3.88±0.17	3.68±0.12	4.2±0.04*
CL/f(mL/kg)	160.56±4.52	133.78±4.92	151.62±4.95	102.60±1.06**
$V_d$ (mL)	473.59±37.76	408.54±12.58	438.51±18.87	349.00±2.49**

All values were expressed as mean ± SEM, n= 6, \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 as compared to simvastatin alone on day 1. Results were done by one-way ANOVA followed by Dunnett's test.

The effect of DILT on the pharmacokinetics of SIMVA, increased  $C_{max}$  (13.02±0.46 to 19.45±0.07 µg/mL) and AUC (50.12±1.37 to 78.4±0.8 µg/mL) of SIMVA, after 8 days of treatment with DILT, which is accompanied by enhanced cholesterol-lowering effect of SIMVA in rats with hypercholesterolemia and hypertension. These results were consistent with a retrospective study demonstrating that SIMVA caused a 21.77 % cholesterol reduction in rats using DILT when compared with 15.88 % in those not using DILT.

This is compatible with our finding that a 1.5-fold increase in the  $C_{max}$  and AUC of HMG-CoA reductase inhibitor by co-administration of DILT with SIMVA was accompanied by further 5 % reduction in TC level. The results of our study suggest that rats which receive both SIMVA and DILT may need a lower dose of SIMVA rather than receiving SIMVA in prescribed to achieve the desired reduction in total and LDL cholesterol levels.

SIMVA monotherapy significantly reduces LDL-C from  $61.73 \pm 2.46$  to  $33.87 \pm 3.07$  and in combination therapy reduced  $63.01 \pm 5.33$  to  $25.60 \pm 3.53$  (1.32 % more reduction) because of DILT co-administration with SIMVA, probably by inhibiting CYP3A4-mediated metabolism.

Although the  $C_{max}$  and AUC of DILT were decreased by SIMVA, blood pressure-lowering effect of DILT was not influenced by SIMVA. Systolic BP significantly reduced from  $153 \pm 13$  to  $123 \pm 7$  (combined therapy  $150 \pm 11$  to  $127 \pm 6$ ), diastolic BP significantly reduced from  $121 \pm 4$  to  $99 \pm 5$  (combined therapy  $120 \pm 6$  to  $101 \pm 6$ ) and heart rate also significantly reduced from  $455 \pm 12$  to  $384 \pm 9$  (combined therapy  $455 \pm 13$  to  $390 \pm 5$ ) for DILT monotherapy. On the other hand combined treatment with SIMVA did not differ from that during the DILT monotherapy.

As there was no significant pharmacokinetic interaction between atorvastatin and DILT, the significant difference in lipid levels of SIMVA, SIMVA+DILT groups suggests the synergistic lipid lowering activity of DILT which may be suitable for the patients of hypertension associated with hyperlipidemia. In support of our investigations, the lipid lowering activity of calcium channel blockers was also reported. It can be suggested that the combination of these two drugs have potential benefits in safety, efficacy and tolerability than individual drugs.

## 2.2. Pharmacodynamic Study

The lipid profiles were estimated for all the groups on day1 & 8 at different time points. The average lipid profiles Group-III and IV shown in Table 2. Standard cholesterol diet effectively induced hyperlipidemia by increasing the plasma total cholesterol (TC), triglycerides (TG) and low density lipoprotein- cholesterol (LDL-C) levels and decreasing the plasma high density lipoprotein-cholesterol (HDL-C) levels. SIMVA alone and in combination with DILT statistically reduced hyperlipidemia. In Group-III and IV significantly ( $P < 0.01$ ), reduced the plasma TC and LDL-C levels in rats but the change in the lipid levels was more Group-IV.

**Table 2:** Lipid profiles of SIMVA alone, DILT alone and SIMVA+DILT on 1<sup>st</sup> & 8<sup>th</sup> day.

Lipid (mg/dl)	HL Control		SIMVA		DILT		SIMVA+DILT	
	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8
<b>TC</b>	172.92 ±3.23	169.37 ±4.74	170.83 ±3.23	143.14 ±3.04**	175.00 ±3.95	182.23 ±8.92**	169.79 ±4.7	133.33 ±3.34**
<b>TG</b>	245.62 ±2.72	238.60 ±2.72	246.49 ±3.96	237.72 ±2.15	245.62 ±2.72	242.99 ±2.15*	238.60 ±2.72**	237.72 ±2.15
<b>HDL-C</b>	54.90 ±0.76	54.93 ±0.95	59.81 ±0.76**	61.73 ±0.95**	59.31 ±0.76**	60.49 ±1.51**	59.065 ±0.60**	60.185 ±1.01**
<b>LDL-C</b>	68.89 ±3.22	66.72 ±4.61	61.73 ±2.46*	33.87 ±3.07**	66.57 ±4.04	73.14 ±9.48	63.01 ±5.33*	25.60 ±3.53**

TC: Total cholesterol; TG: Triglycerides. All values were expressed as mean ± SEM, n= 6, \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 as compared to the disease control group. Results were done by one-way ANOVA followed by Dunnett's test.

Blood Pressure and Heart rate were estimated on day1 & day 8. The average values of Group-II and IV were shown in Table 3. The 10% Fructose solution effectively induce hypertension by increasing systolic, diastolic BP and Heart rate in experimental animals. SIMVA lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic LDL receptors on the cell surface for enhanced uptake and catabolism of LDL-C which did not alter with TG levels and HDL-C. It was reported that the SIMVA therapy produced a statistically significant changes in TC, LDL-C within 8 days treatment. It has been shown in humans that plasma concentrations of DILT increased after multiple doses, which was accompanied by a greater increase of plasma concentrations of its active metabolites such as N-monodesmethyl diltiazem and deacetyl diltiazem.(8,9)

SIMVA+DILT are often prescribed together for the treatment of hypercholesterolemia in patients with hypertension and/or angina pectoris. In the Scandinavian SIMVA Survival Study (1994), which demonstrated a reduction in nonfatal myocardial infarction, cardiovascular death, and total mortality by simvastatin treatment in patients with angina pectoris or previous myocardial infarction, more than 30 %

of the study population were treated with calcium antagonists including diltiazem. The efficacy and safety profiles of simvastatin and diltiazem are widely accepted.(10,11)

**Table 3:** Mean Systolic Blood Pressure, Diastolic Blood Pressure and Heart Rates of SIMVA alone, DILT alone and SIMVA+DILT in rats on 1<sup>st</sup> and 8<sup>th</sup> day of the treatment.

Parameters		Hypertension control	DILT alone	DILT+SIMVA
Systolic BP (mm of Hg)	1 <sup>st</sup> day	153±12	153±13	150±11
	8 <sup>th</sup> day	144±12	123±7**	127±6**
Diastolic BP (mm of Hg)	1 <sup>st</sup> day	120±5	121±4	120±6
	8 <sup>th</sup> day	116±5	99±5**	101±6**
Heart rate (beats/min)	1 <sup>st</sup> day	457±8	455±12	455±13
	8 <sup>th</sup> day	440±11	384±9**	390±5**

All values were expressed as mean ± SEM, n= 6, \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 as compared to the disease control group. Results were done by one-way ANOVA followed by Dunnett's test

### 3. Materials and Methods

#### 3.1. Animals

In this study Male Wistar albino rats (150-180 gm) were selected and procured from Mahaveer enterprises, Hyderabad, India. All animals were maintained under controlled conditions of 25±2 °C, relative humidity of 45 to 55 % and 12 hr light-12 hr dark cycle. The animals were acclimatized to laboratory conditions for at least one week before starting the experiment and they had free access to food and water *ad libitum*. The study protocol was approved by Institutional Animal Ethics Committee (IAEC NO: 1047/ac/07/CPCSEA).

#### 3.2. Study design

Selected animals are divided into four groups and each group consists of 6 animals.

*Group I* - Served as hyperlipidemic and hypertensive control rats which receive only vehicle (0.5% Sodium CMC).

*Group II* - Received Diltiazem (15mg/kg) given alone in hyperlipidemic and hypertensive rats.

*Group III* - Received Simvastatin (2 mg/kg) given alone in hyperlipidemic rats.

*Group IV*- Received Simvastatin (2 mg/kg) + Diltiazem (15 mg/kg) in hyperlipidemic and hypertensive rats.

The weight of the individual animal and plasma cholesterol levels were estimated before the induction of hyperlipidemia, the BP and Heart rate of individual animals were also estimated. The standard cholesterol diet was administered for 30 days to induce hyperlipidemia and fructose 10% solution was administered for 30 days to induce hypertension

#### 3.3. Collection of Blood sample and Estimation of biochemical parameters

On 1<sup>st</sup> and 8<sup>th</sup> day, blood samples of 1 ml were withdrawn at 0.5, 1, 2, 4, 6, 8 and 24 hrs through retro-orbital sinus into heparinized eppendorff tubes and equal amount of saline were administered to replace blood volume at every blood withdrawal time. The plasma was obtained by immediate centrifugation at 5000-6000 rpm for 5 minutes at room temperature. All samples were stored at -4 °C until analysis (12,13) and TC, TG, HDL-C were estimated by using commercially available (14, 15) and LDL-C were calculated by using Friedewald's equation (16). The systolic blood pressure, diastolic blood pressure and heart rate were measured twice in each animal by using Noninvasive Tail Cuff Blood Pressure Amplifier (NIBP).

$$LDL = TC - [HDL + VLDL]$$

#### 3.4. Extraction procedure

Rat plasma samples were prepared for chromatography by precipitating proteins with 0.1 mL perchloric acid (60%) for each 0.5 ml of plasma. The resultant solution was mixed for 5 min on vortex shaker at room temperature and centrifuged at 3000-5000 rpm for 10 min. After that supernant was separated, from this supernant 300 µL was taken and 2 µL of internal standard was added to it followed by addition of 5 mL of diethyl ether and 1 mL of KOH (4 M) respectively. This solution was mixed for 5 min on vortex shaker, centrifuge at 3000-5000 rpm for 10 min. The supernant was collected and evaporated it to 4-6 hr under nitrogen stream. The residue was collected and 100 µL of mobile phase was added. For HPLC analysis 20 µL of the solution was used. The slope of the plots was determined by the method of least square regression analysis (r<sup>2</sup>=0.995) and was used to calculate the simvastatin and diltiazem concentration in the unknown sample.

### 3.5. Chromatographic Conditions

Shimadzu high performance liquid chromatography unit equipped with the LC-8A Solvent delivery module, SPD-10AVP UV-Visible spectrophotometer detector, Class CR-10 Data Processor, Rheodyne (with 20  $\mu$ l capacity loop) Injection Port and Wakosil II C-18 Column (stainless steel column of 25 cm length and 4.6 mm internal diameter packed with porous silica spheres of 5 $\mu$  diameter, 100 Å pore diameter) were used for analysis of samples. The mobile phase consists of acetonitrile, water and orthophosphoric acid in a combination of (65:35:0.1% v/v). Before using the mobile phase, it was degassed by passing it through a 0.22  $\mu$ m membrane filter. The mobile phase was pumped at an isocratic flow rate of 1 mL/min. 20  $\mu$ L quantity of the sample was injected and the retention time obtained for simvastatin is 3.88 min and atorvastatin (Internal Standard; 5 mg/kg) is 5.91 min. The UV-detection wavelength was set at 237 nm and sensitivity of 0.001 AUFS was used for the analysis. Typical HPLC chromatogram for the estimation of Simvastatin in rat plasma was given in Fig 3.

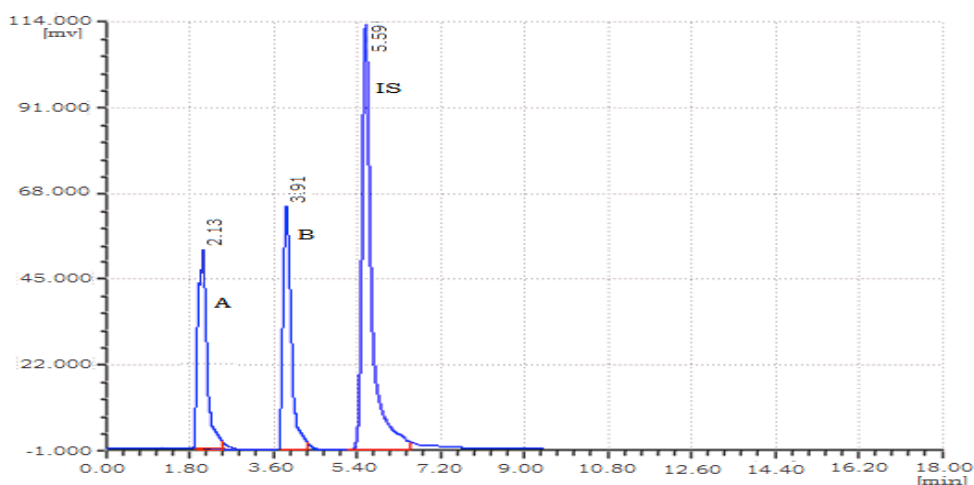


Figure 3. Typical HPLC chromatogram for the estimation of Simvastatin in rat plasma. A= Plasma peak, B= Simvastatin, IS= Atorvastatin.

### 3.6. Statistical Analysis:

Time to reach peak concentration ( $T_{max}$ ) was directly taken from observed data. Area under the curve [AUC], elimination half-life [ $T_{1/2}$ ], volume of distribution [ $V/f$ ], total clearance [ $CL/f$ ], mean retention time [MRT] and peak plasma concentrations ( $C_{max}$ ) were calculated for each subject using a noncompartmental pharmacokinetic model "WIN NONLIN". All the pharmacodynamic data were statistically evaluated with ANOVA and the differences among groups were determined by Dunnett's multiple comparison tests using Graph pad prism 5.0. Values were considered to be significant when  $P < 0.05$ . All the results were presented as mean  $\pm$  SEM for six rats in each group.

### 4. Conclusion

Combined treatment with diltiazem and simvastatin increases the  $C_{max}$  and AUC of HMG-CoA reductase inhibitor and further reduces to TC and LDL-CI levels. These interactions should therefore be taken into consideration, and pharmacokinetic and pharmacodynamic monitoring may be necessary when these drugs are used concomitantly.

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