



Evaluation of Clarithromycin Pharmacokinetics after Single and Repeated oral Administration of Atorvastatin in Hyperlipidemic Wistar Rats

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Received: 4 March 2013; | Revised: 5 April 2014; | Accepted: 28 May 2014

Abstract

To evaluate the clarithromycin pharmacokinetics after atorvastatin single and repeated dose administration in induced hyperlipidemic Wistar rats. A standard cholesterol diet (2% cholesterol) was used to induce hyperlipidemia and feed was given to rats for 6-8 weeks. Group's allocation as follows: G1-G3: Induced hyperlipidemic rats pretreatment of atorvastatin as a single dose followed by clarithromycin single doses of 10, 20 and 100 mg/kg; G4-G6: Induced hyperlipidemic rats pretreatment of atorvastatin for 7 consecutive days followed by clarithromycin single doses of 10, 20 and 100 mg/kg; G7-G8: Atorvastatin treated hyperlipidemia rat livers harvested on day 1 and 7 post dose; G9-G10: Concomitant administration of atorvastatin and clarithromycin in presence and absence of hyperlipidemia for biliary and urinary excretion studies. Effect of atorvastatin on hyperlipidemia and alterations of clarithromycin kinetics due to various states of hyperlipidemia like induced, reduced and absence of hyperlipidemia was evaluated. The C_{max} and AUC_{0-t} of clarithromycin on day 1 was found to be $0.56 \pm 0.061 \mu\text{g/mL}$ and $2.4 \pm 0.59 \mu\text{g. h/mL}$ for 10 mg/kg; $1.60 \pm 0.24 \mu\text{g/mL}$ and $9.48 \pm 3.05 \mu\text{g.h/mL}$ for 20 mg/kg; $14.9 \pm 3.87 \mu\text{g/mL}$ and $113 \pm 41.4 \mu\text{g.h/mL}$ for 100 mg/mL, respectively and similarly on day 7 clarithromycin C_{max} and AUC_{0-t} was $0.47 \pm 0.08 \mu\text{g/mL}$ and $1.81 \pm 0.44 \mu\text{g.h/mL}$ for 10 mg/kg; $1.34 \pm 0.121 \mu\text{g/mL}$ and $5.61 \pm 0.66 \mu\text{g.h/mL}$ for 20 mg/kg; $12.1 \pm 3.3 \mu\text{g/mL}$ and $67.4 \pm 16.9 \mu\text{g. h/mL}$ for 100 mg/kg, respectively. The in-vitro clarithromycin results suggesting down / up regulation of CYP mediated metabolism in induced and reduced hyperlipidemia rats. The % dose of clarithromycin in biliary excretion was significantly increased ($P < 0.05$) after atorvastatin pretreatment in induced hyperlipidemic rats as compared non-hyperlipidemic rats. The % dose excretion of clarithromycin in urine showed almost similar results across the groups. Clarithromycin pharmacokinetics was considerably affected by hyperlipidemia and also reported that recovered kinetics and metabolism in presence of atorvastatin and compared on day 1 and 7. In-vitro up regulation of clarithromycin metabolism on day 7 was observed in induced hyperlipidemic rat liver homogenates. The decreased area under the curve of clarithromycin in hyperlipidemic rats after co-administration of

atorvastatin might be mainly due to up regulation of the CYP mediated metabolism by reducing the hyperlipidemia in the liver.

Keywords: Clarithromycin, Atorvastatin, Hyperlipidemia, Area under the curve, Peak plasma, Rats.

1. Introduction

Hyperlipidemia (HL) is defined as an elevation of one or more of the plasma lipids, including cholesterol, cholesterol esters, triglycerides, or phospholipids [1]. An elevation in plasma lipids may be caused by a primary genetic defect or secondary to diet, drugs, or diseases [1]. The pharmacokinetics (PK), particularly lipoprotein binding of drugs, may be altered in hyperlipidemic conditions, resulting in a decrease [2] or increase in pharmacological response [3]. Despite the differences in lipoprotein distribution and metabolism between humans and rats, hyperlipidemic (HLM) rat models are used extensively in lipid research. Many hyperlipidemic rat models are existed [4]. Antimicrobial drugs manifest a wide variety of drug interactions, which can differ greatly in their extent of severity and clinical relevance. Not only co-medication, but also food and herbal medicine can interact with antimicrobial drugs and vice versa. The nature of these interactions can be of pharmacodynamic (PD) and/or pharmacokinetic (PK) origin. Historically, the relevance of drug distribution, particularly of protein binding, has been over-emphasized in the assessment of drug interactions, and nowadays the main cause of drug-drug interactions has been recognized to be modulation of the activity, i.e., inhibition or induction, of cytochrome P450 (CYP) enzymes and transporters [5].

HL was recently proven to decrease the liver uptake of the more potent (-)-ketoconazole (KTZ) enantiomer 1 which raises a question about effect of HL on the CYP inhibitory potency of KTZ [6]. Similarly, impact of HL on pharmacokinetics of many drugs evaluated was including nifedipine [7], docetaxel [8], amiodarone [9], and cyclosporine A [10]. Reported higher exposure and lower clearance of nifedipine in the HLM rats due to the decrease in fraction unbound in plasma [6]. Docetaxel

showed significantly low unbound fraction and intrinsic hepatic metabolism probably due to the lower expression of cytochrome P 450 (CYP) 3A [8]. Henceforth, Clarithromycin (CLR) is a lipophilic and extensively distributed in the body and metabolized by CYP 3A [11], might also be changed in the HLM state. CLR showed significantly higher exposure in HL (Data was not shown). To further evaluation of impact of HL we have used atorvastatin (AT) is a 3-hydroxy-3-methylglutaryl (HMG) coenzyme A (CoA) reductase inhibitor that efficiently and dose-dependently lowers both cholesterol [12] and triglyceride [13] levels. AT was administered orally as a single and repeated dose (consecutive 7 days) to HL rats after successful induction. The reduced hyperlipidemia (RHL) term was used since after repeated dose of AT, HL has been reduced but not cured. This group also named as Atorvastatin-treated hyperlipidemic group (AHL).

In our previous study, HL was induced successfully using standard cholesterol diet (2% cholesterol) to rats which significantly increases high plasma total cholesterol (TC) and triglycerides (TG) levels [14]. Rats which will have elevated TC and TG levels are called Induced Hyperlipidemic rats (IHL).

CLR has been previously demonstrated that the total cytochrome P 450 (CYP) content was significantly lower in the microsomal protein of the liver of HLM rats than of control rats [15] showed significant decreases in the protein expressions of CYP2C11, CYP3A1, and CYP3A2 in the hepatic microsomes of HLM rats compared to control rats [16]. Henceforth, no studies have been conducted to evaluate CLR metabolism in presence of IHL and AT post dose (single and repeated). Therefore in view of the foregoing, we isolated induced and reduced HL rat livers and homogenized with suitable buffers to establish CLR metabolism in various HLM conditions following AT pre-treatment.

Fat-enriched diets have been used for decades to model obesity, HLM, dyslipidemia and insulin intolerance in rodents. It has been observed that the disorders achieved by high-fat feeding resemble the human metabolic syndrome closely, and this also may extend to the cardiovascular diseases. High fat diet fed groups, showed significant increase in cholesterol level after 7th day with respect to control attaining the maximum level (17) which supports present study day 7 regimen treatment. HLM is associated with heart diseases which is the leading cause of death in the world. The lowering of the levels of harmful lipid to satisfactory values have been confirmed by several experimental animals and interventional studies indicating lowered morbidity and mortality in coronary heart diseases (18) which evidenced current selection of animal model as rat.

Our main aim is to study the CLR pharmacokinetics and excretion at different phases of HL like IHL, RHL (AT treatment for consecutive 7 days) and absence of HL in Wistar rats. The scope is to present an overview of pharmacokinetic interactions studies on drug-hyperlipidemia of commonly prescribed antimicrobial drug like CLR in daily clinical practice. However, no studies have been conducted regarding the possible effects of HL and RHL on CLR pharmacokinetics and excretion in Wistar rats. Therefore we focused to investigate CLR pharmacokinetics and excretion following single and repeated dose of AT 7 consecutive days in HLM rat models to evaluate any potential interactions of CLR with HL and in RHL rat models.

2. Materials and Methods

2.1 Chemicals and apparatus

The active pharmaceutical ingredients like CLR, Erythromycin (internal standard) and AT were purchased from Sigma–Aldrich Co. (India). High performance liquid chromatography (HPLC) grade methanol and acetonitrile were obtained from Merck Co. (Darmstadt, Germany). All other chemicals in this study were of analytical grade and used without further purification. The apparatus used in this study

included a MS/MS equipped with a Agilent isocratic HPLC Pump including auto sampler (Agilent, USA), an HPLC column with temperature controller (Discovery C18, Waters), a Branson ultrasonic cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex mixer (Scientific Industries Inc., Bohemia, NY, USA), and a high-speed micro centrifuge (Hitachi Co., Tokyo, Japan).

2.2 Induction of hyperlipidemia and Animal experiments

The required animals Male Wistar rats (weighing 250–300 g) were procured from the In-house, animal resource, Wockhardt Research Centre (MS, India) and were given access to a commercial rat chow diet (Nutrilab, provimi pvt, Ltd, India) for control group and for induction of hyperlipidemia, in-house prepared standard cholesterol diet [14] was fed for 6-8 weeks separately, selected rats were fed twice daily with butter (0.5 mL) to hasten the HLM after 6-8 weeks, lipid profiles were evaluated as per the procedure published [14] and animals were selected based on TC (>150 mg/dL) and TG (> 100 mg/dL) levels (Table 1) which named as an IHL rats. Diet and water was provided *ad libitum* and The selected rats were housed individually for pharmacokinetic and biliary excretion study and maintained temperature at 22 ± 3 °C and 42–65% relative humidity under a 12:12 h light/dark cycle. The animals were allowed 6-8 days for acclimation. The Institute of Animal Care and Ethics Committee of Drug metabolism pharmacokinetics (DMPK) department (Regd No.: 13/1999/CPCSEA, Wockhardt research Centre, India) approved the design and conduct of this study. Experimental animals were fasted for at least 12 h before the experiments and each animal was anesthetized with isoflurane during the catheterization. The right Jugular vein, bile duct and duodenum were cannulated using polyethylene tubing are PE-50 (i.d. 0.58 mm, o.d. 0.98 mm; Portex, Smiths medicals, USA) and PE-10 (i.d. 0.28 mm, o.d. 0.61 mm; Portex, Smiths medicals, USA) to allow for blood sampling (Jugular vein, for pharmacokinetics) and bile sampling (Bile duct, for excretion) following oral administration.

2.3 Pharmacokinetic study

2.3.1 Effect of AT on CLR pharmacokinetics in presence of IHL following single and repeated administration

The rats were divided into the following groups (n = 6, each group): Doses were selected are 10, 20 and 100 mg/kg; (suspended in 0.5% carboxymethyl cellulose (CMC) containing 2% Tween 80, 5 ml/kg), in this study, CLR doses we have been selected at three dose levels to investigate CLR dose proportionality in hyperlipidemic conditions after single AT treatment. AT dose is 20 mg/kg suspended in 0.5% CMC solution was administered intragastrically using an oral feeding tube, AT was administered as a single and repeated dose up to day 1 and day 7, respectively. Groups allocation was as scheduled, G1: IHL rats treated with CLR (10 mg/kg) and single dose of AT (20 mg/kg); G2: IHL rats treated with CLR (20 mg/kg; 11 and 19) and single dose of AT (20 mg/kg); G3: IHL rats treated with CLR (100 mg/kg; 20) and single dose of AT (20 mg/kg); G4: IHL rats treated with repeated dose of AT (20 mg/kg) up to day 7 consecutive days followed by single dose of CLR (10 mg/kg); G5: IHL rats treated with repeated dose of AT (20 mg/kg; 21) up to 7 consecutive days followed by single dose of CLR (20 mg/kg); G6: IHL rats treated with repeated dose of AT (20 mg/kg) up to day 7 consecutive days followed by single dose of CLR (100 mg/kg). After oral gavage co-administration of CLR and AT in IHL rats, blood samples were collected at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h post-dose and 0.2-ml aliquot of blood was collected (n=6) into heparinized tubes from the jugular vein catheter and equal volume was replaced. Plasma samples were centrifuged at 6,000 g for 6 min at +2 to +8 °C and the separated plasma samples were stored at -70 °C until analyzed by LC-MS/MS.

2.3.2 In-vitro CLR metabolism in induced hyperlipidemic rat liver homogenates following AT single and repeated dose administration

Rat liver homogenates (n=4) were prepared in-house as IHL rat liver homogenates were not

commercially available. Liver tissues were harvested under isoflurane anesthesia after perfusion. Perfusion was performed using Dulbecco's modified eagle's medium (DMEM) at 37 °C by portal vein. AHL rat livers were harvested at 2 h post dose of AT treatment of single (day 1) and repeated (consecutive 7 days) from G7 and G8. G7: AHL group (n=4) on day 1 livers were harvested at 2 h AT post dose; G8: AHL group (n=4) on day 7 livers were harvested at 2 h AT post dose. The homogenation procedure was as detailed, isolated rat liver tissues were diluted with DMEM buffer ; 1 part of liver with 4 parts of DMEM buffer and homogenized at 10,000 rpm for 5 minutes using ultra-Turrax® homogenizer (IKA) at 37 °C. Neat CLR (10 µM) was incubated with AT treated induced hyperlipidemic rat liver homogenate (IHLMRL) at 37 °C. A 100 µL of IHLMRL homogenate was quenched with 200µL of acetonitrile at 0 h, and 0.083, 0.25, 0.5, 0.75 and 1 h. All samples were centrifuged and stored at -70 °C until analyzed by LC-MS/MS.

2.4 Excretion study

2.4.1 Effect of AT on CLR excretion following co-administration of AT and CLR single dose

The % dose excretion of CLR following co-administration of single dose of AT and CLR formulation was investigated. Study group details as detailed: Group 9: Absence of hyperlipidemia or non-hyperlipidemic (NHL) rats were co-administered with AT and CLR at doses of 20 mg/kg; Group 10: IHL rats were co-administered with AT and CLR at doses of 20 mg/kg. For both groups the common bile duct and duodenum were cannulated using PE-10 and 50 catheters under light isoflurane anesthesia. A surgical probe was used to tunnel subcutaneously to the dorsal scapular region where each catheter then exited from the body. The exit incision and abdominal cavity were sutured as soon as the bile could be seen flowing freely through the catheter. A U-shaped coupler (stainless steel) then connected both catheters to maintain normal flow of bile to the intestines during the recovery period. Injectable analgesics were given as needed and

provided 72 h recovery period for cannulated animals. The cannulated rats were installed into metabolic cages, with utilities to separate feces and retain urine at a temperature of 4 ± 2 °C. Bile and urine samples were collected at pre-determined time points are 0-2 h, 2-4 h, 4-6 h and 6-8 h after oral gavage dosing and simultaneously blank bile was infused at a rate of 1.3 mL/hr [22, 23] through duodenum (non -vascular) catheter by using infusion pump (Model 11, Harvard apparatus). Cumulative bile and urine samples were stored at -70 °C until bioanalysis.

2.4.2 Calculation of % dose excreted from bile and urine

After oral administration of CLR, the % CLR recovered (IHL Vs NHL; CLR alone and co-administration of AT) in bile and urine was calculated by cumulative dose excreted at respective time points / the actual dose administered to rat *100 for bile and urine.

2.5 LC-MS/MS analysis

The plasma concentrations of CLR were determined using a LC-MS/MS method. Briefly, 10 µL of erythromycin, as an internal standard and a 0.2-ml aliquot of acetonitrile were mixed with a 50 µL of plasma sample and same method was employed for bile and urine. The resulting mixture was then vortex mixed for 2 min and centrifuged at 10,000 rpm for 10 min. A 100 µL aliquot of the supernatant was injected (Injection volume, 10 µL) into the LC-MS/MS system. The chromatographic separations were achieved using waters, discovery C18 column (4.6 x 50 mm, 5 µm). The mobile phase consisted of 30% of 5 mM ammonium formate and 70% of acetonitrile containing 0.1% formic acid. The flow rate of the mobile phase was maintained at 0.5 mL/min. Chromatography was performed at 40 °C, which was set by an HPLC column temperature controller. CLR and internal standard were eluted with retention times of 3.47 and 1.87 min, respectively. A linear correlation coefficient of ≥ 0.99 was obtained with calibration range of 20 ng/mL to 3000 ng/mL. A 10-fold dilution was applied for the calibration range. The operational parameters for the tandem mass spectrum of analyte were obtained after running in

quantitative optimization mode. The turbo ion spray setting and collision gas pressure were obtained (IS voltage: 5500 V, temperature: 550 °C, nebulizer gas: 37 psi, heater gas: 45 psi, curtain gas: 20 psi). The clean chromatogram of matrix blank obtained from the injection of a matrix blank extract immediately after an ULOQ sample demonstrated that this method had neither injector carryover nor analytical column carryover [24].

2.6 Pharmacokinetic analysis

The plasma concentration data were analyzed using a non-compartmental method on WinNonlin software version 2.1 (Pharsight Co., Mountain View, CA, USA). The elimination rate constant (K_{el}) was calculated by the log linear regression of CLR concentration data during the elimination phase, and the terminal half-life ($T_{1/2}$) was calculated by $0.693/K_{el}$. The peak plasma concentration (C_{max}) and time to reach the peak concentration (T_{max}) of CLR in the plasma were obtained by visual inspection of the data from the concentration–time curve. The area under the plasma concentration–time curve (AUC_{0-t}) from time zero to the time of the last measured concentration (C_{last}) was calculated using the linear trapezoidal rule. The AUC zero to infinite ($AUC_{0-\infty}$) was obtained by adding AUC_{0-t} and the extrapolated area as determined by C_{last}/K_{el} .

2.7 Statistical analysis

Statistical analysis was carried out using one way ANOVA followed by a student's paired t-Test with a two tailed distribution (performed by Graph pad prism 6.0). The differences were considered significant at a level of $P < 0.05$ for 10, 20, and 100 mg/kg. All mean values are presented with their standard deviation (Mean \pm SD).

3. Results

The main objective of this work was to investigate the severity of IHL on CLR pharmacokinetics and the severity of IHL was evaluated by AT following single (day-1) dose and repeated (up to day-7) dose for 7 consecutive days by oral gavage administration followed by

single oral CLR administration to evaluate CLR pharmacokinetics in IHL rats. Hyperlipidemia was successfully induced by standard cholesterol diet along with butter twice a day administration. Considered and confirmed successful induction

of hyperlipidemia by examination of TC levels (TC > 150 mg/dL) and TG levels (TG > 100 mg/dL) levels and results are presented in Table 1.

Table 1 Total cholesterol and Triglyceride levels (mg/dl) of selected rats on day 1 and day 7 after CLR alone and co-administration of Atorvastatin for pharmacodynamic assessment

Treatment Groups (n=6 per group)	Units of (mg/dl)			
	Total Cholesterol		Triglyceride	
	Mean	SD	Mean	SD
IHL_CL_AT_Baseline	186	15.7	121	5.79
IHL_CL_AT_Day 1	179	2.07	123	2.04
IHL+AT_Day 7	130	1.08	106	1.53

NHL: Non-hyperlipidemia; IHL: presence of induced hyperlipidemia

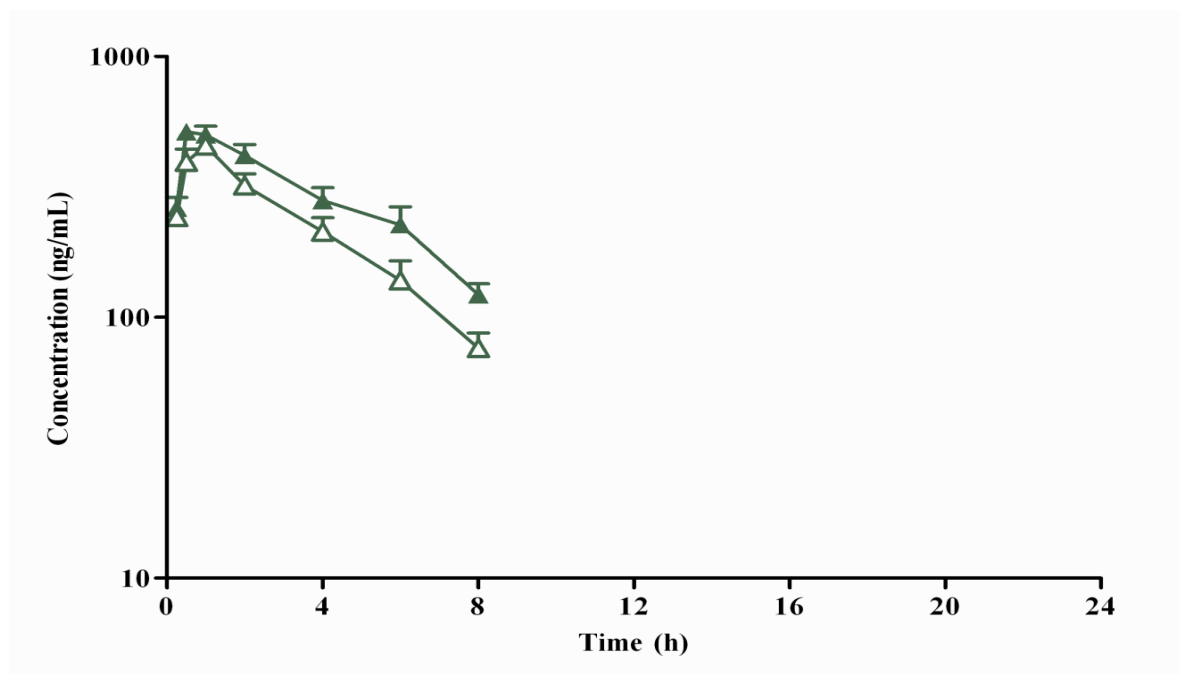


Figure 1 Mean plasma concentration-time profiles of clarithromycin (10 mg/kg) following co-administration of atorvastatin (20 mg/kg) in hyperlipidemic rats. Concomitant administration of clarithromycin and atorvastatin on day 1 (▲), repeated administration of atorvastatin up to 7 days followed by clarithromycin single dose (Δ)

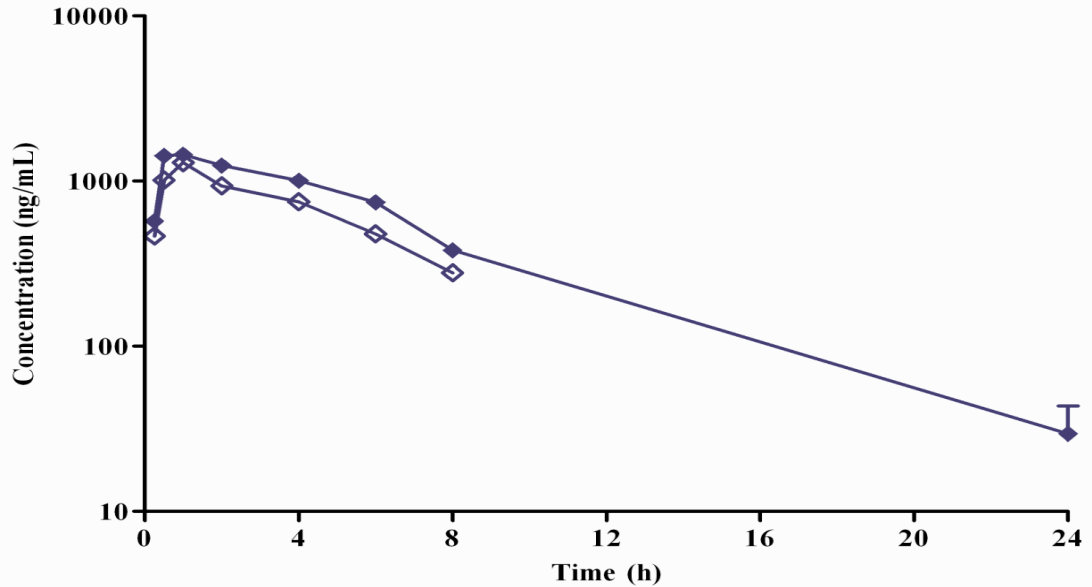


Figure 2 Mean plasma concentration-time profiles of clarithromycin (20 mg/kg) following co-administration of atorvastatin (20 mg/kg) in hyperlipidemic rats. Concomitant administration of clarithromycin and atorvastatin on day 1 (◆), repeated administration of atorvastatin up to 7 days followed by clarithromycin single dose (◇).

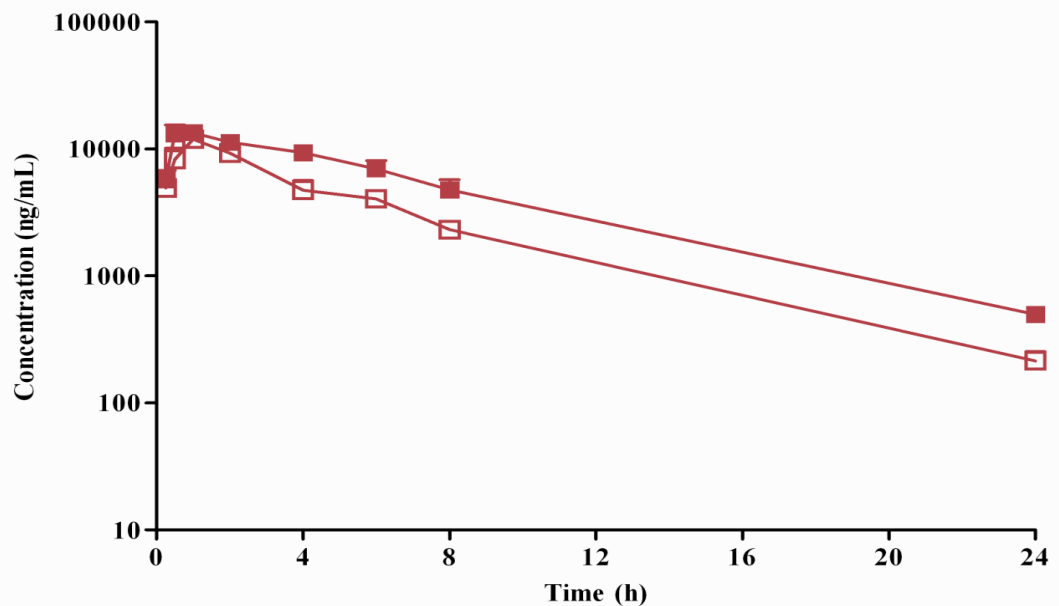


Figure 3 Mean plasma concentration-time profiles of clarithromycin (100 mg/kg) following co-administration of atorvastatin (20 mg/kg) in hyperlipidemic rats. Concomitant administration of clarithromycin and atorvastatin on day 1 (■), repeated administration of atorvastatin up to 7 days followed by clarithromycin single dose (□).

Table 2 Pharmacokinetic parameters of Clarithromycin after the oral concomitant Administration of Atorvastatin (20 mg/kg, single and repeated up to day 7) and co-administration of Clarithromycin as a single dose (10, 20, and 100 mg/kg; n=6, mean \pm SD) to rats in the presence of induced hyperlipidemia

PK parameters	Induced Hyperlipidemic rats + CLR + AT day 1			Induced Hyperlipidemic rats + CLR + AT day 7		
	10 mg/kg	20 mg/kg	100 mg/kg	10 mg/kg	20 mg/kg	100 mg/kg
C_{\max} ($\mu\text{g}/\text{mL}$)	0.560 \pm 0.061	1.60 \pm 0.201	14.9 \pm 3.87	0.47 \pm 0.080	1.34 \pm 0.121	12.1 \pm 3.30
T_{\max} (h)	0.67 \pm 0.261	0.67 \pm 0.261	0.67 \pm 0.261	0.83 \pm 0.261	0.92 \pm 0.201	0.92 \pm 0.201
AUC_{0-t} ($\mu\text{g}\cdot\text{h}/\text{mL}$)	2.40 \pm 0.590	9.48 \pm 3.05	113 \pm 41.4	1.81 \pm 0.44	5.61 \pm 0.66*	67.4 \pm 16.9*
$AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{h}/\text{mL}$)	3.13 \pm 0.82	10.6 \pm 2.35	116 \pm 42.4	2.16 \pm 0.57	6.91 \pm 0.66*	68.7 \pm 17.5*
AUC % Extrapolated	23	11	3	16	19	2
MRT_{last} (h)	3.16 \pm 0.250	4.33 \pm 1.10	5.61 \pm 0.361	2.92 \pm 0.301	3.20 \pm 0.132	4.90 \pm 0.421
$T_{1/2}$ (h)	3.74 \pm 0.402	4.10 \pm 0.911	4.74 \pm 0.241	2.93 \pm 0.692	3.08 \pm 0.650	4.23 \pm 0.331

$AUC_{0-\infty}$ Area under the plasma concentration–time curve from 0 h to infinity; AUC_{0-t} Area under the plasma concentration–time curve from 0 h to last quantifiable concentrations; C_{\max} maximum plasma concentration, T_{\max} time to reach maximum plasma concentration, $T_{1/2}$ terminal half-life, MRT_{last} last Mean resident time. * $P < 0.05$ significant difference compared with day 1 of respective groups.

3.1 Effect of AT on CLR pharmacokinetics in IHL following single AT (day 1) and repeated AT (up to day 7) dose administration followed by single dose of CLR

The mean plasma concentration–time profiles of CLR after concomitant oral administration of AT (20 mg/kg; dosed for single as day 1 and repeated up to day 7) following CLR (10, 20, and 100 mg/kg; only single dose) to IHL rats are shown in Fig. 1, 2 and 3. The corresponding pharmacokinetic parameters are shown in Table 2. The effect of AT on IHL found that CLR pharmacokinetic parameters were significantly recovered and the same was re-evaluated by administration of AT on day 1 and up to day 7 (negative control for hyperlipidemia). The C_{\max} and AUC_{0-t} of clarithromycin on day 1

were found to be 0.56 \pm 0.06 $\mu\text{g}/\text{mL}$ and 2.4 \pm 0.59 $\mu\text{g}\cdot\text{h}/\text{mL}$ for 10 mg/kg; 1.6 \pm 0.201 $\mu\text{g}/\text{mL}$ and 9.48 \pm 3.05 $\mu\text{g}\cdot\text{h}/\text{mL}$ for 20 mg/kg; 14.9 \pm 3.87 $\mu\text{g}/\text{mL}$ and 113 \pm 41.4 $\mu\text{g}\cdot\text{h}/\text{mL}$ for 100 mg/kg, respectively and similarly on day 7 clarithromycin C_{\max} and AUC_{0-t} was 0.47 \pm 0.08 $\mu\text{g}/\text{mL}$ and 1.81 \pm 0.44 $\mu\text{g}\cdot\text{h}/\text{mL}$ for 10 mg/kg; 1.34 \pm 0.121 $\mu\text{g}/\text{mL}$ and 5.61 \pm 0.66 $\mu\text{g}\cdot\text{h}/\text{mL}$ for 20 mg/kg; 12.1 \pm 3.30 $\mu\text{g}/\text{mL}$ and 67.4 \pm 16.9 $\mu\text{g}\cdot\text{h}/\text{mL}$ for 100 mg/kg, respectively. The AUC_{0-t} and the $AUC_{0-\infty}$ of CLR were significantly ($P < 0.05$, 20 and 100 mg/kg) decreased. The percentage differences of C_{\max} and AUC_{0-t} are 84–69%, 84–65% and 81–59%, respectively, in IHL AT day 7 group (given oral AT repeated dose up to 7 days followed by CLR single dose) as compared with the IHL AT day 1 group (given

oral co-administration of CLR and AT on day 1). IHL rats treated with AT up to day 7 followed by single dose of CLR, exposures were similar to NHL group (data was not shown). There was no significant difference in the T_{max} , MRT and the $T_{1/2}$ across the groups. CLR kinetic exposure was high in IHL rats and after single AT treatment slightly recovered to normal and completely recovered on day 7 AT post dose.

3.2 In-vitro CLR metabolism in induced hyperlipidemic rat liver homogenates following AT single and repeated dose administration

AT effect on CLR metabolism was evaluated in IHLMRL homogenates and livers were harvested at 2 h post dose of AT as a single (day 1) and repeated (up to day 7) oral administration. Thereafter livers were homogenized and incubated at 37 °C with CLR at 10 µM. Results were summarized in Table 3. The CLR metabolism at 5 and 15 min was 6% and 18% for IHLMRL homogenates on day 1, similarly 17% and 33% for IHLMRL homogenates on day 7, respectively. At 0.5 and 0.75 h was reported 40% and 66% for day 1 and 58% and 81% for day 7, respectively and finally

at 1 h post dose the % metabolism was noted as 80% for day 1 and 87% for day 7.

The outcome of *in-vitro* study on CLR metabolism in IHLMRL homogenates supports that hyperlipidemia was a rate limiting step for CLR metabolism and also evaluated the CLR metabolism with AT post dose treatment on day 1 and day 7. IHLMRLs were harvested at respective days of AT post dose treatment at 2 h and homogenized. The same was incubated with CLR to know the % remaining and % metabolism at respective intervals results states that at 0.083 h the % metabolism was very rapid in all treated groups i.e. almost 50% was higher in IHL group on day 7 than day 1 later on significantly decreased to 25-30% of CLR metabolism in day 7 as compared to day 1 group. Thereafter the difference in metabolism was low among the all groups. So based on results we can also conclude that hyperlipidemia have an impact on CLR pharmacokinetics and same was confirmed with AT single dose (day 1) and repeated dose administration (day 7), AT concludes that CLR metabolism was recovered and up-regulated on day 7 as compared with day 1 in induced HLM rat liver homogenates.

Table 3 Clarithromycin % remaining and metabolism in induced hyperlipidemic rat liver (IHLMRL) tissue homogenates after oral co-administration of Atorvastatin (dose: 20 mg/kg) on day 1 and day 7

Time (h)	IHL_RLH treated AT day 1		IHL_RLH treated AT up to day 7	
	% remaining	% metabolism	% remaining	% metabolism
0	100	0	100	0
0.083	94	6	83	17*
0.25	82	18	67	33*
0.5	60	40	42	58*
0.75	34	66	19	81*
1	20	80	13	87

* P<0.05, significant difference compared with day 1; IHLMRL homogenates treated AT day 1: hyperlipidemic rat liver homogenates (n=4) were harvested 2h post dose of atorvastatin treatment as a single dose on day 1; IHLMRL homogenates treated AT up to day 7: induced hyperlipidemic rat liver homogenates (n=4) were harvested 2 h post dose of atorvastatin treatment for consecutive 7 days. CLR incubated at 10 µM.

Table 4 Biliary excretion of Clarithromycin after the oral co-administration of Atorvastatin (20 mg/kg) to rats in the presence or absence of hyperlipidemia

Mean Parameters	NHL+CLR+AT	IHL+CLR+AT
Cumulative Concentration ($\mu\text{g/mL}$)	123 \pm 16.8	172 \pm 14.2*
Total Bile (mL)	12.0 \pm 0.131	16.3 \pm 0.382*
Total Amount (mg)	1.47 \pm 0.210	2.80 \pm 0.191
Rat body weight (kg)	0.290 \pm 0.01	0.272 \pm 0.01
Dose per rat (mg)	5.80 \pm 0.182	5.44 \pm 0.251
% Dose Excreted	25.1 \pm 3.80	51.1 \pm 1.62*

NHL Non-hyperlipidemia, CLR Clarithromycin, IHL Induced Hyperlipidemia, AT Atorvastatin; * $P < 0.05$, significant difference compared with controls (NHL + CLR+AT)

Table 5 Urinary excretion of Clarithromycin after the oral administration of Clarithromycin alone (20 mg/kg; $n=4$, mean \pm SD) and concomitant administration of Atorvastatin (20 mg/kg) and Clarithromycin (20 mg/kg) to rats in the presence or absence of hyperlipidemia

Mean Parameters	NHL+CLR+AT	IHL+CLR+AT
Cumulative Concentration ($\mu\text{g/mL}$)	189 \pm 16.2	206 \pm 30.6
Total Urine (mL)	13.6 \pm 0.64	12.0 \pm 0.29
Total Amount (mg)	2.56 \pm 0.15	2.47 \pm 0.36
Rat body weight (kg)	0.290 \pm 0.01	0.272 \pm 0.01
Dose per rat (mg)	5.80 \pm 0.18	5.44 \pm 0.25
% Dose Excreted	44.0 \pm 3.32	46.0 \pm 6.15

NHL Non-hyperlipidemia, CLR Clarithromycin, IHL Induced Hyperlipidemia, AT Atorvastatin;

3.3 Effect of IHL and AT on the CLR biliary excretion

As *in-vivo* is a metabolically intact system than *in-vitro* so we have performed a study to support the severity of hyperlipidemia on the CLR pharmacokinetics in awakening rats cannulated with bile duct to study the biliary excretion of CLR. The study was performed in

presence and absence of HL and AT (single administration) was used as negative control for hyperlipidemia. Reported the CLR percentage dose recovered in rat bile after co-administration of single AT with CLR in presence and absence of hyperlipidemia.

Table 4 represents the mean cumulative biliary concentration ($\mu\text{g/mL}$) for 0-6 h, mean % biliary

excretion of CLR (20 mg/kg) after oral co-administration of AT (20 mg/kg, only single dose) and CLR in presence and absence of hyperlipidemia. The cumulative biliary excretion of CLR in NHL (non-hyperlipidemic) rats treated with CLR was $123 \pm 16.8 \mu\text{g/mL}$ and similarly in IHL rats was $172 \pm 14.2 \mu\text{g/mL}$ demonstrated higher significant ($P > 0.05$, 20 mg/kg) differences. The mean percentage biliary excretion was found to be 25.1 ± 3.80 in NHL rats and 51.1 ± 1.62 in IHL rats. To summarize, IHL rats treated with CLR was significantly ($P < 0.05$, 20 mg/kg) elevated almost 100% increase in mean percentage biliary excretion as compared with NHL rats treated with concomitant administration of CLR and AT (51% and 25%, respectively).

3.4 Effect of IHL and AT on the CLR urinary excretion

Table 5 represents the mean cumulative urinary concentration ($\mu\text{g/mL}$) for 0-6 h, mean % urinary excretion of CLR (20 mg/kg) followed by co-administration of AT single dose (20 mg/kg) in presence and absence of IHL. The cumulative urine concentrations were found in NHL and IHL groups were $189 \pm 16.2 \mu\text{g/mL}$ and $206 \pm 30.6 \mu\text{g/mL}$, respectively. NHL rats treated with CLR and AT exhibited no significant difference in mean percentage urinary excretion as compared with IHL rats treated with CLR and AT (44% and 46%, respectively).

4. Discussion

We Aimed to explore CLR pharmacokinetics in presence of IHL and compared with RHL which was achieved by AT repeated dose. IHL was successfully achieved by standard cholesterol diet (14). Hyperlipidemia is state of elevated levels of lipoproteins like Total cholesterol (TC), triglycerides (TG), Low density lipoproteins (LDL), high density lipoproteins (HDL), very low density lipoproteins (VLDL) etc. Hyperlipidemia proved alteration of unbound fraction clearance and volume of distribution of ketoconazole being a high potent CYP inhibitor [25]. Hypercholesterolemia was induced in rats by feeding them with a

cholesterol-enriched diet consisting of 3% cholesterol. Parallel studies have shown that diets supplemented with 1% cholesterol and 0.5% cholic acid for 5 days is sufficient in elevating the serum total cholesterol level to 238 mg/dl (17) strongly supports our current methodology used for induction of hyperlipidemia and lipid levels.

In disease state, physiological changes can influence drug pharmacokinetics, although the mechanism remains to be elucidated. In many critically ill patients extracellular fluids have increased, possibly resulting in a higher volume of distribution that might affect pharmacokinetics [26]. One should bear in mind that findings in pharmacokinetics interaction studies performed in healthy volunteers might not be observed in clinical practice in specific patient populations. Furthermore, pharmacokinetics interaction studies administering both single doses and multiple doses to study subjects were used in this overview. It need hardly be mentioned that multiple-dose studies will reflect best clinical practice. This is particularly true for pharmacokinetics interaction studies with biotransformation as possible underlying mechanism since induction of enzyme systems might require days to weeks to develop fully [27]. The interaction may also persist at a similar length of time when the inducing agent is stopped. Unlike induction, inhibition of enzyme systems can occur within 2–3 days (5). In current study AT was used as repeated dosing for consecutive 7 days to treat the IHL. Clarithromycin pre-treatment increased the AUC of atorvastatin lactone in all of the subjects, and the magnitude of the increase was greater (37%) in those who carried a CYP3A5*1 allele (28). No studies were performed as AT pre-treatment in IHL rat models to prove CLR kinetics with IHL and atorvastatin treated rat models on day 1 and 7.

The current study was planned, as they have been reported hyperlipidemia shown a significant impact on PK including of potent CYP inhibitors henceforth, we have selected CLR which is a potent CYP inhibitor and its kinetics has been evaluated in IHL rat models following single and repeated dose for consecutive 7 days of AT. Our previous study demonstrated hyperlipidemia has

an impact on CLR kinetics as compared with presence and absence of HL (data was not shown). This study is concentrated only to monitor the reversal effect of hyperlipidemia following AT treatment as a single and repeated dose for 7 consecutive days. AT was anti-hyperlipidemic drug used to reduce hyperlipidemia and compared clarithromycin pharmacokinetics with hyperlipidemic (single dose pre-treatment) and reduced hyperlipidemic (consecutive 7 days pre-treatment) rat models. Since no kinetic parameters data was reported on CLR pharmacokinetics in presence of IHL and RHL rat models to overcome, we performed the experiment in rat as a model to study the effect of AT on CLR pharmacokinetics in IHL following single AT (dose: 20 mg/kg; day 1) and repeated dose of AT (dose: 20 mg/kg; up to day 7) administration followed by single dose of CLR at three dose levels (Dose: 10, 20 and 100 mg/kg) to evaluate dose linearity. The current study revealed that CLR AUC_{0-t} at the doses of 20 and 100 mg/kg was significantly lower ($P < 0.05$) on day 7 as compared with day 1 but there were not significantly different at lower dose (10 mg/kg). The MRT and $T_{1/2}$ was not altered in induced and reduced hyperlipidemic rat models. Hyperlipidemia potentiates the anti-microbial effect by increasing the plasma concentration of CLR and same hyperlipidemic effect was supported by publication [7, 9, and 29].

Our current study clearly shows that in-vitro metabolism of CLR in IHL MRL homogenates significantly lowered ($P < 0.05$) at 10 μ M on day 1 as compared with day 7. Current data supporting to CLR metabolism was altered in presence of IHL which might be down regulation of CYP mediated metabolism and changes of unbound drug concentrations, however previous publications [7,16,24] substantially emphasized hyperlipidemia was altered the liver and intestine metabolism and unbound drug concentrations which supports our observations. In addition to this we reported CLR biliary and urinary cumulative excretion and percentage of dose excreted in bile and urine after co-administration of AT and CLR in presence and absence of induced HL. The groups without AT treatment exhibited higher levels of CLR excretion through

bile (data was not shown). The data has been suggested that the % dose excretion of CLR in IHL rat significantly higher ($P < 0.05$) as compared with NHL rats. Increased levels of CLR cumulative urine concentrations were observed in IHL rats but not significantly different from NHL rats.

Overall results demonstrated that CLR pharmacokinetics was significantly altered in plasma and bile by IHL and the same was concluded from in-vitro rat induced liver homogenates. So further we need to evaluate or focus on particular transporters involved or responsible for the uptake or efflux in the presence of hyperlipidemia. Results obtained from in-vitro and in-vivo studies were co-related and concludes that CLR pharmacokinetics in induced hyperlipidemic rats models after AT post dose (Day 1 and Day 7) are significantly altered might be the reason of change of fraction unbound drug concentration, increased volume of distribution by lowering the clearance and alteration of transport mechanism's.

5. Conclusion

It can be concluded that the hyperlipidemic stages like induced hyperlipidemia, reduced hyperlipidemia and absence of hyperlipidemia causes down / up regulations of CYP mediated metabolism in the liver (AT post treatment) which leads in term of high / low systemic availability of CLR. Hyperlipidemia might have an impact on CLR free drug concentrations. Therefore, a more detailed investigation is required to get clear understand of transporters involved and affected in presence of induced hyperlipidemia in rat and other species as well for safety perspective.

Acknowledgments

The author was thankful to Wockhardt Research Centre for providing necessary laboratory facilities to carry out present research work.

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