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The Effect of Cytochrome P450 and Organic Anion Transporter Polypeptide on Macrolides–Atorvastatin Drug Interaction

[1]Zainab Farooq Abdalhameed*, [2]Orooba Mohammed Saeed Ibrahim

[1]Veterinary Drug Research and Production Center, Corporation of Research and Industrial Development, Iraq

[2]Physiology & Biochemistry & Pharmacology Department, College of Veterinary Medicine, University of Baghdad, Iraq

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Corresponding Author:
Zainab Farooq Abdalhameed
zainab.abd2206m@covm.uobaghdad.edu.iq

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Abstract

Cytochrome P450 (CYP3A4) enzymes and organic anion-transporting polypeptides (OATPs) are critical in drug pharmacokinetics. Fifteen rabbits were divided into three groups: the atorvastatin group received a single oral dose of atorvastatin (0.86 mg/kg B.W.); the clarithromycin-atorvastatin group received a single oral dose of clarithromycin (43.7 mg/kg B.W.), followed by atorvastatin (0.86 mg/kg B.W.) 30 minutes later; and the azithromycin-atorvastatin group received a similar dosing protocol with azithromycin instead of clarithromycin. High-performance liquid chromatography (HPLC) measured atorvastatin concentrations in hyperlipidemic rabbit serum, while ELISA assessed macrolides' CYP3A4 and OATP inhibition potential. Coadministration of clarithromycin significantly increased atorvastatin's area under the curve (AUC) by 2.74-fold, compared to a slight increase of 1.11-fold with azithromycin. CYP3A4 inhibition was higher in the clarithromycin group (1.67-fold in the liver, 1.50-fold in the intestine) than the azithromycin group (1.64-fold in the liver, 1.17-fold in the intestine). Similarly, OATP inhibition in serum, liver, and intestine was greater in the clarithromycin group (1.4-fold, 1.5-fold, and 1.9-fold, respectively) compared to the azithromycin group (1.1-fold, 1.2-fold, and 1.1-fold, respectively). The results suggest that atorvastatin does not interact with azithromycin, while clarithromycin strongly interacts with it, indicating potential pharmacokinetic concerns in coadministration.

1. Introduction

Drug-drug interactions (DDIs) happen when two or more medications that are given together interact, causing one drug to change the impact of the other [1]. These interactions have the potential to impact therapy targets, clinical efficacy, and treatment results [2, 3]. Common risk variables involve advanced age, the use of many medications, an elevated number of prescribing healthcare professionals, and the presence of concurrent medical conditions. The process of aging, together with the associated decrease in drug metabolism, damage to the liver and kidneys, and changes in drug levels in the blood, make medicine use more complicated and increase

susceptibility to drug-related interactions [3]. Pharmacokinetic interactions are primarily influenced by the induction or inhibition of hepatic enzymes and the organic anion transporting polypeptides (OATPs). Cytochrome P450 enzymes (CYP) which are the most well-known drug-metabolizing enzymes primarily expressed in the liver & intestine [4], and have an impact on how drugs are processed in the body, as well as their safety, bioavailability, and toxicity Among the several isoforms of CYP450, CYP3A4 is considered the most significant [5]. The organic anion transporting polypeptides (OATPs) are a family of transporters comprised of eleven proteins. Among them, OATP1B1, OATP1A2, OATP1B3, and OATP2B1 have been extensively studied for their impact on drug absorption. OATP1B1 and OATP1B3 are primarily expressed in the hepatocyte membranes, while OATP 2B1 found in the hepatocytes and enterocyte where they determine the distribution of different drugs including statins and other cholesterol-lowering agents [6].The Food and Drug Administration have provided instructions for conducting drug-drug interaction studies for substances that function as substrates or inhibitors of this transportation pathway. Different statins have varying degrees of substrate activity for OATP1B1, OATP1B3, and OATP2B1. When an OATP1B1 inhibitor is used, the statins that are most affected are pitavastatin, lovastatin, atorvastatin, and simvastatin [7]. Statins are a category of medications that decrease cholesterol levels and are now the most commonly recommended treatment for hyperlipidemia [8]. The impact of statins on lipid profile is a result of their ability to competitively inhibit hydroxymethylglutaryl-CoA (HMG-CoA) reductase [9]. It plays a crucial role for regulating cholesterol synthesis in hepatocytes, as it is heavily metabolized by the CYP3A4 enzyme, the main metabolizing enzyme in regard to simvastatin, lovastatin, and atorvastatin is CYP3A4, the most readily available CYP450 isoenzyme [10]. Increased atorvastatin concentrations when combined with OATP inhibitors like clarithromycin can lead to serious drug interactions since they slow down the metabolism of statins. Clarithromycin and azithromycin is member of macrolides which are antibacterial medications with a macro cyclic lactone ring in their chemical structures, they are absorbed by the gastrointestinal system and undergo hydrolysis to produce an active open ring hydroxyl acid in the liver and gut [11]. These drugs undergo significant metabolism by the CYP3A4 isoenzyme, although azithromycin is not metabolized and is primarily eliminated from the body without undergoing any changes [12]. CYP3A4 inhibitors encompass a wide range of drugs that vary in their ability to inhibit this enzyme and also have diverse effects on various OATP transporters [13]. Understanding affects these interactions is vital for optimizing therapeutic strategies and ensuring the safe and effective use of macrolide antibiotics in patients with elevated lipid levels [14]. The aim of our study was to evaluate the results of the interaction between macrolide drugs (clarithromycin and azithromycin) with atorvastatin through the role of macrolide drugs in inhibiting cytochrome P450 (CYP) isoenzyme systems and membrane transporters organic anion transporting polypeptide.

2. Material and Methods

2.1. Ethics

The study was conducted in accordance with ethical guidelines for animal experiments. Ethical approval for the care and treatment of animals was granted by the Local Committee for the Care and Use of Animals at the University of Baghdad, College of Veterinary Medicine (Approval No. P.G. 123, dated 15-01-2024).

2.2. Experimental Animals

Fifteen New Zealand White male rabbits, ranging between 3–4 months with body weights of 1.7 to 2.0 kg were used. At least two weeks for adaptation the animals were kept in the house collage of Veterinary Medicine / University of Baghdad.

2.3. Induced Hyperlipidemia

All groups fed with 0.4% cholesterol and 4% hard fat ad libitum. The feeding was done for 8 consecutive weeks. During this period, the weight of each of the rabbits was measured and lipid profile [15].

2.4. Experimental Design

Animals were randomized into the three groups, 5 rabbits for each group. Atorvastatin group was received orally at one dose of atorvastatin (0.86 mg/kg B.W.) given by stomach tube once time [16]. The clarithromycin-atorvastatin group received one oral dose of clarithromycin (43.7 mg/kg. B.W.) After 30 minutes the animals received one dose of atorvastatin (0.86 mg/kg B.W.) [17], and azithromycin-atorvastatin group received orally

one dose of azithromycin (43.7 mg/kg. B.W.) After 30 minutes the animals received a one dose of atorvastatin (0.86 mg/kg B.W).

2.5. Blood Samples

Blood samples (3 mL) were obtained from jugular vein from each animals using (plastic syringe 3mL) at time intervals of 0, 0.15, 0.30, 1, 2, 3, 4, 6, 8, 12, and 24 hours after administration of atorvastatin and the drug concentration was estimated using HPLC. Blood was collected with a jell test tube and centrifuged for ten minutes at 4000 rpm, and the serum was collected in 2 mL eppendorf tubes for pharmacokinetic study. All tubes were labeled with time; date of blood drawing then stored in freezer at -20 °C till use, before vein puncture, the skin above the vessel was wiped with a wet cotton ball with (70%) isopropyl alcohol [18, 19].

2.6. Samples Preparation

Each serum sample (0.5 mL) was mixed with 2.5 mL of cooled pure ethanol and then centrifuged at 3,000 rpm for 10 minutes to extract it. After transferring the liquid portion to separate tubes, the solid material was mixed with 1 mL of acetonitrile by vigorously shaking for 1 minute. The mixture was then spun at a speed of 6,000 rpm for 10 minutes. The acetonitrile was combined with the ethanol, and the resulting mixture was evaporated at room temperature using nitrogen gas until it was completely dry. The samples were dissolved again in 200 µl of the mobile phase, and then 20 µl of the solution was injected onto the HPLC column [20].

2.7. Statistical Analysis

The data was subjected to Two-Way Analysis of Variance (ANOVA) with a significance level of ($P \leq 0.05$). The method of least significant differences (LSD) was employed to determine the specific differences between groups.

2.8. Pharmacokinetics Assessment

The drugs were administered via injection into a High Performance Liquid Chromatography (HPLC) system manufactured by Shimadzu in Tokyo, Japan, with the serial number L20105027316. The separation was conducted using a C18 column. The detection was performed at a wavelength of 244 nm. Chromatographic curves were generated by creating 6-point calibration curves using standard solutions of different concentrations. The temperature of the column was maintained at 25 °C [18].

2.9. Tissue Sampling

Animals were anesthetized by intramuscular injection of 40 mg/kg B.W xylazine and 90 mg/kg B.W of ketamine. After sacrificing, midline incision in the abdominal wall was performed to expose the liver and intestine. The liver and intestine was cleaned and rinsed in ice-cold Phosphate buffer saline to remove excess blood and store in -4°C.

2.10. Tissue Homogenate

Liver and intestine, weighed, minced into small pieces, subsequently, the tissue fragments were thoroughly mixed in phosphate buffer slain using a glass homogenizer while maintaining a low temperature on ice. The ratio between the weight of the tissue (in grams) and the volume of PBS solution (in milliliters) was 1:9. An ultrasonic cell disruptor is used to sonicate the resultant suspension until the solution turns clear. The homogenates are then centrifuged at a speed of 10000×g for a period of 5 minutes. The supernatants should be promptly collected for assay testing and stored at a temperature of -80℃ for later use.

3. Results

The linear equation, $y = 894896x + 494119$, was used to analyze samples for atorvastatin, with a correlation coefficient of 0.998 in a limit of detection of 1.35ng/mL and a limit of quantification of 2.76ng/mL (Figure 1).

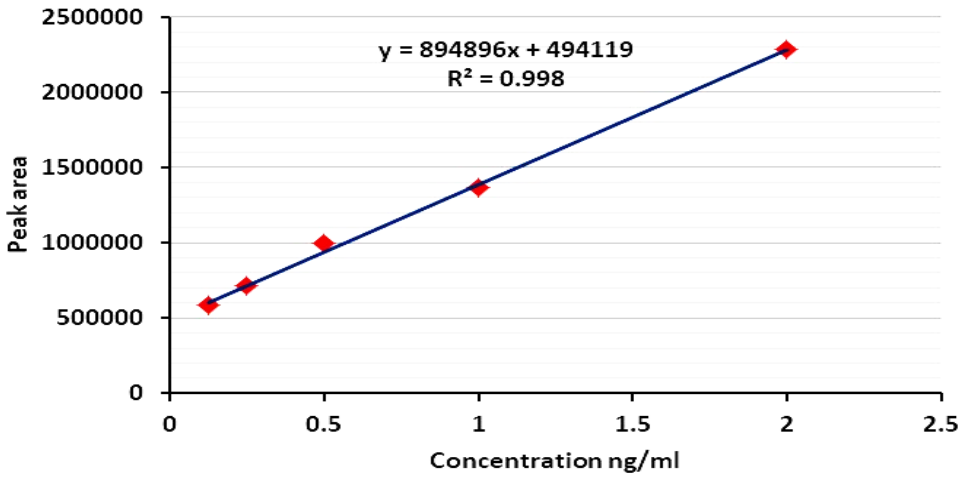

Figure (1): Calibration curve of atorvastatin in serum as a standard

3.1. Atorvastatin Concentration ng/mL in Blood Serum

At atorvastatin group peak concentration of atorvastatin was registered (2.0 h) after oral administration (PO) of 0.86 mg/kg and it was (9.8 ± 0.11) ng/mL. Then the concentration after (24h) declined to (1.05 ± 0.02) ng/mL as shown in Figure (2). While atorvastatin + clarithromycin group peak concentration of atorvastatin co-administration with clarithromycin was registered statistically significant increase (P≤0.05) (2.0 h) after oral administration (PO) and it was (13.5 ± 0.0.6) ng/mL as shown in Figure (3). Then the concentration after (24 h) declined to (5.7 ± 0.13) ng/mL. On the other hand at atorvastatin + azithromycin group peak concentration of atorvastatin co-administration with azithromycin was registered a slight increase compared with ATV group (2.0 h) after oral administration (PO) and it was (10.6 ± 0.10) ng/mL. Then the concentration after (24 h) declined to (1.5 ± 0.08) ng/mL as shown in Figure (4).

3.2. Biological Half-Life (t 1/2. h)

Biological half-life (t 1/2 β) for the atorvastatin to disappear for the rabbit's body after oral administration was (14.43±0.43) h, as demonstrated in Table (1). While registered statistically significant a longer time of biological half-life of elimination of atorvastatin from the rabbit body when co-administration with clarithromycin and was (20.38±0.07) h, and disappeared the atorvastatin from the rabbit body after (15.06±0.02) h in a slightly longer time when co-administration with azithromycin compared with the atorvastatin group.

3.3. Apparent Volume of Distribution Vd (L/kg)

After oral administration of atorvastatin, we noticed the value of volume of distribution was (206.75±0.67) L/kg, as demonstrated in Table (1). While the value of volume of distribution decreased after co-administration with clarithromycin to (106.71 ±0.02) L/kg, a little decrease in the value of volume of distribution was registered when co-administration with azithromycin (202.03 ±0.02) L/kg compared with the atorvastatin group.

3.4. Area Under the Curve AUC (ng/mL.h)

After oral administration of rabbit in atorvastatin group, the value of area under curve was (68.52±0.42) ng/mL.h. While in atorvastatin + clarithromycin group after oral administration it was (187.43±0.04) ng/mL.h, an increase statistically significant (P≤0.05) compared with atorvastatin group Table (1), and in atorvastatin + azithromycin group the value of area under curve was (73.17±0.05) ng/mL.h, a slight increase compared with atorvastatin group.

3.5. Clearance CL (L/kg/h)

After oral administration of atorvastatin, the clearance from the body was (12.55±0.12) L/kg/h While significantly decrease (P≤0.05) after co administration of clarithromycin with atorvastatin it was (4.59±0.03) L/kg/h, but in atorvastatin plus azithromycin group the value of atorvastatin clearance from the body was slight decrease no significant (11.75±0.01) L/kg/h as demonstrate in Table (1).

Table (1): Pharmacokinetic parameters for atorvastatin after oral administration atorvastatin only and co-administration with clarithromycin or azithromycin

Pharmacokinetics parameters	ATV Mean ± SE	ATV + ClA Mean ± SE	ATV + AZI Mean ± SE	LSD
Absorption rate constant (Ka).h^{-1}	0.34 ±0.92	0.31 ±0.01	0.32 ±04	0.041 NS
Elimination rate constant (Ke) h^{-1}	0.048 ±0.10 A	0.034 ±0.06 b	0.046 ±002 ab	0.013*
Conc. at absorption phase (CPa) ng/mL	1.14± 1.05	1.12± 1.28	1.28 ± 1.29	0.371 NS
Conc. at elimination phase(CPe) ng/mL	1.56± 1.24 B	2.48 ± 1.37 a	2.01 ± 1.38 ab	0.84*
Half-life at absorption phase (t 1/2a) h	2.03 ±0.21	2.23 ±0.31	2.16 ±03	0.451 NS
Half-life at elimination phase (t 1/2e) h	14.43 ±0.43 B	20.38 ±0.07 a	15.06 ±0.02 b	1.727*
Volume of distribution Vd (L/kg)	206.75 ±0.67 A	106.71 ±0.02 b	202.03 ±0.02 a	6.934*
Area under curve AUC (ng/mL.h)	68.52 ±0.42 C	187.43 ±0.04 a	73.17 ±0.05 b	5.094*
Clearance CL (L/kg/h)	12.55 ±0.12 B	4.59 ±0.03 a	11.75 ±0.01 b	0.961*

ATV= atorvastatin, ATV + ClA = atorvastatin with clarithromycin, ATV + AZI = atorvastatin with azithromycin

- Values represent mean ± S.E.
- Significant differences are signed as the asterisk *.
- Different small letters mean significant (P≤0.05) between groups.

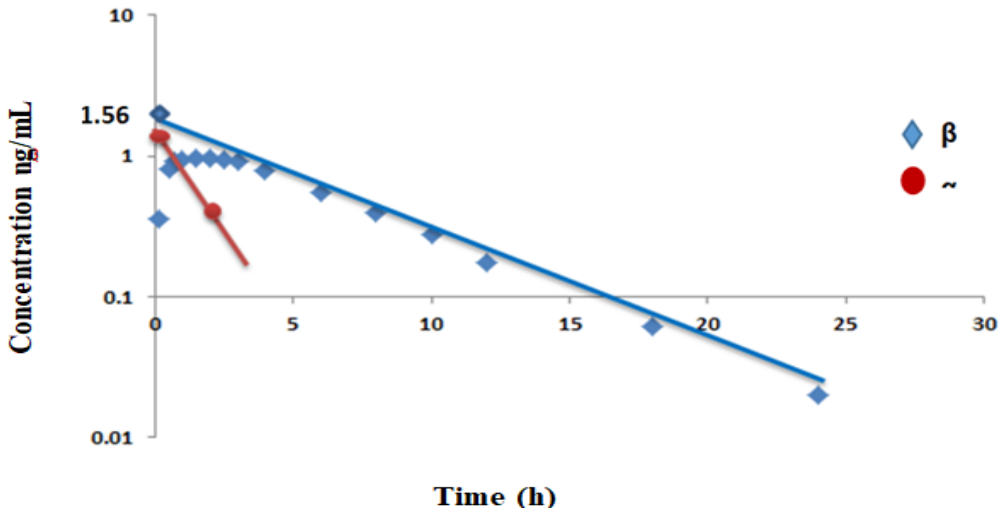

Figure (2): Semi-log plot of serum concentration of atorvastatin (ng/mL) versus time (h) after a single oral dose (0.86 mg/kg)

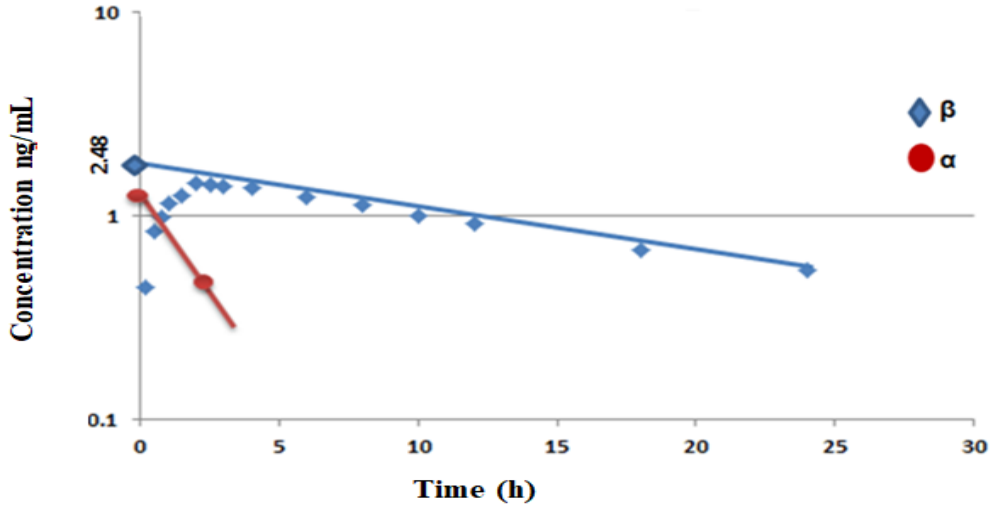

Figure (3): Semi-log plot of plasma concentration of atorvastatin versus time curve (mean ±SE) after single oral dose (0.86mg/kg) when co-administration with clarithromycin (43.7 mg/kg)

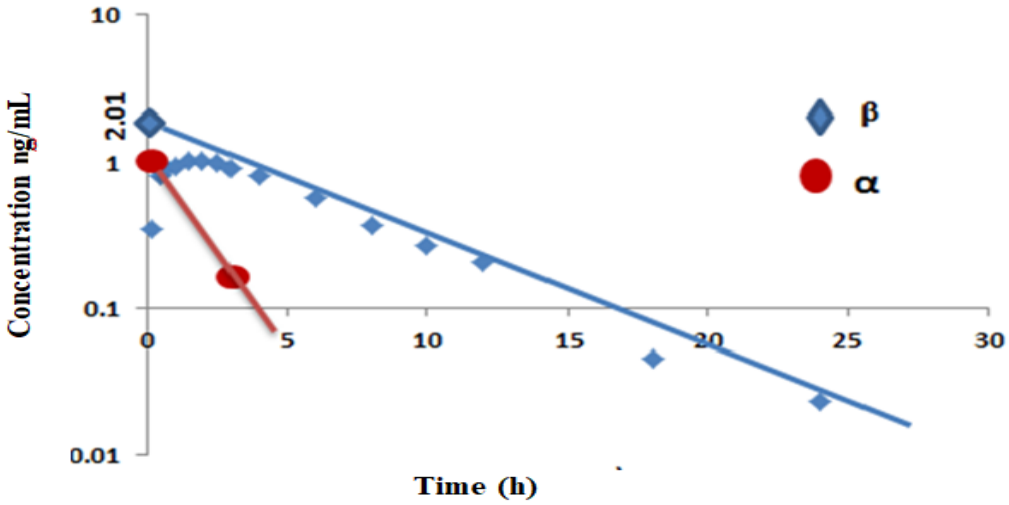

Figure (4): Semi-log plot of serum concentration of atorvastatin versus time curve (mean ±SE) after single oral dose (0.86mg/kg) when co administration with azithromycin (43.7 mg/kg)

3.6. Measurement of Tissue CYP3A4 Isozymes

Using the ELISA assay, we investigated the potential CYP inhibition ability of macrolide. Figure (5) shows the effect of clarithromycin and azithromycin on CYP3A4 inhibition. We observed significant differences in CYP3A4 inhibition in both the liver and intestine tissue compared to the control group that only received atorvastatin. After oral administration of atorvastatin, we noticed the active concentration of cytochrome P450 of hyperlipidemic rabbit was (1.369±0.21) and (1.088±0.04) ng/g in liver and intestine, respectively, while the significant diminish ($P \leq 0.05$) concentration of cytochrome P450 after co-administration with clarithromycin was (0.815 ±0.04) and (0.724±0.10)ng/g in the atorvastatin +clarithromycin group, and (1.029±0.16) and (0.926±0.07) ng/g with azithromycin compared with the control atorvastatin group. The administration of atorvastatin plus clarithromycin resulted in a CYP3A4 fold inhibition of 1.67 and 1.5 fold in the liver and intestine, respectively. Conversely, the administration of atorvastatin plus azithromycin reduced the fold inhibition of CYP3A4 to 1.64 and 1.17 fold in the liver and intestine, respectively, compared to the administration of clarithromycin.

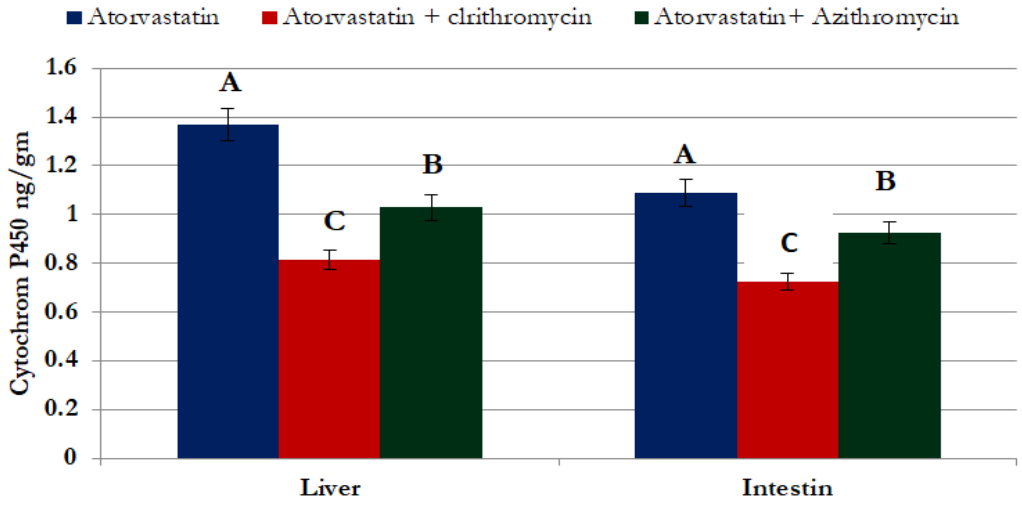

Figure (5): Active concentration of cytochrome P450 of hyperlipidemic rabbit after treatment with single dose of atorvastatin and atorvastatin with clarithromycin or azithromycin. Values represent mean ± S.E different capital letters mean significant (P≤0.05) between groups

3.7. Measurement of OATP

The impact of clarithromycin and azithromycin on OATP inhibition is presented in Figure (6). We found significant variations in OATP inhibition between atorvastatin group and atorvastatin +clarithromycin, and atorvastatin + azithromycin group in serum, liver, and intestinal tissue. Following atorvastatin oral administration, we observed that the hyperlipidemic rabbit's active OATP concentration in serum, liver, and intestine was (83.41±0.06), (82.90±0.14) and (95.32±0.13) pg/mL respectively. While Significant diminish (P≤0.05) concentration of OATP after co-administration with clarithromycin group to 57.15 ±0.20, 54.35±0.06, and 48.81±0.17 pg/mL, respectively. In other hand, the third group which co-administration with azithromycin the concentration were (71.23±0.08), (68.99±0.12) and (79.82±0.04) pg/mL, respectively, compared with control group. The fold of OATP inhibition in serum, liver and intestine at atorvastatin +clarithromycin group compare with atorvastatin group was 1.4, 1.5 and 1.9, respectively. Greater fold inhibition in atorvastatin + azithromycin group was 1.1, 1.2 and 1.1 folds, respectively.

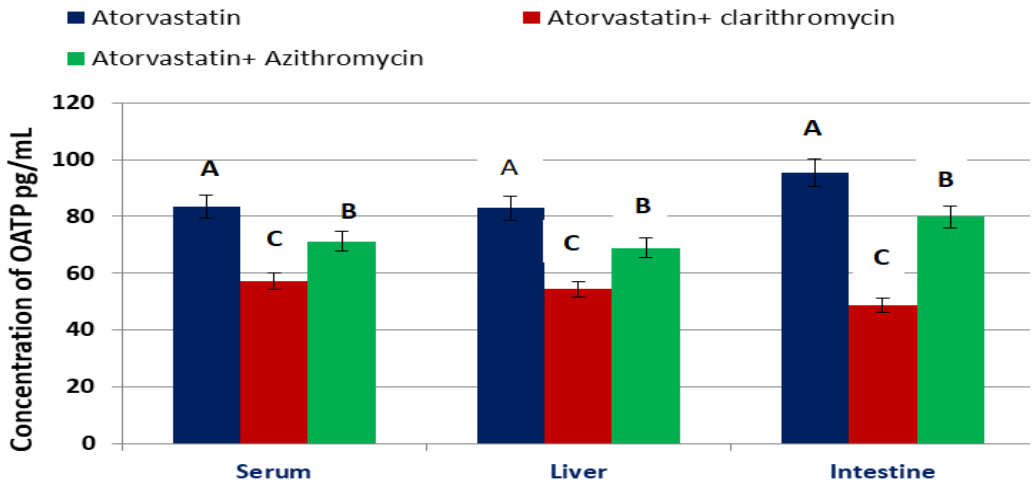

Figure (6): Concentration of OATP pg/mL of hyperlipidemic rabbit after treatment with single dose of atorvastatin and atorvastatin with clarithromycin or azithromycin. Values present mean ± S.E Different capital letters mean significant (P≤0.05) between groups.

4. Discussion

All statins, including atorvastatin, are metabolized by the liver, resulting in low systemic bioavailability. The cytochrome P450 (CYP) isoenzyme systems extensively metabolized most statins in microsomes. The role of particular CYP enzymes or transporters in the disposition of statins differs depending on the specific statin being considered. Contribution of specific "CYP enzymes or transporters to the metabolism and elimination of statin varies between different statins. The CYP3A4 isoenzyme is in charge of atorvastatin, lovastatin, and simvastatin metabolism [20]. Major variations in atorvastatin treatment results were observed in the clarithromycin group, which might be related to the suppression of cytochrome P450 (CYP) isoenzyme systems and membrane transporters [21]. Drug-drug interactions refer to measurable alterations in the duration or strength of a drug's effects caused by the prior or concurrent use of other medications [22]. Pharmacokinetic interactions primarily arise from the interactions between drugs during four distinct phases: absorption, distribution, metabolism, and excretion. Clinically common drug-drug interactions (DDIs) primarily occur in the field of pharmacokinetics [23]. Through the results of our study above, we note; Clarithromycin considerably increased serum concentrations of atorvastatin in each animal group. A comparison of pharmacokinetic parameters confirmed a drug-drug interaction between atorvastatin and clarithromycin [24]. Concomitant administration of clarithromycin increased the mean area under the curve of atorvastatin concentration versus time [25]. The mean area under the curve of atorvastatin concentration versus time AUC (0–24) was increased 2.73 folds from 68.52±0.42ng/mL.h to 187.43±0.04 ng/mL.h and the mean peak serum concentration C max was increased 2.1 folds from 6.2± 0.27 ng/mL to13.5±1.24 ng/mL due to clarithromycin concomitant administration. There were no statistically significant changes in the Tmax and t1/2α of atorvastatin, but there were statistically significant changes in the t1/2β of atorvastatin from 14.43 ±0.43 h to 20.38 ±0.07 h, and statistically significant decrease in the volume distribution and clearance from 206.75 ±0.67 to 106.71 ±0.02L/kg and from 12.55 ±0.12 to 4.59 ±0.03 L/kg/h, respectively.

On the other hand when comparing pharmacokinetic parameters for atorvastatin with azithromycin its drug interaction existed with a low percentage about clarithromycin. The AUC and C max recorded an increase of 1.06 and 1.5 fold respectively. But there were no statistically significant changes in the t1/2β, the volume distribution and clearance. Macrolide antibiotics are a type of antibacterial medications made of large aliphatic lactone rings, which are mostly effective against gram-positive bacteria and typical pathogens. Clarithromycin, erythromycin, and troleandomycin are characteristic medications of the fourteen-membered macrolide ring, whereas azithromycin is indicative of the fifteen-membered ring. The fourteen-membered ring inhibited CYP450 activity more than the fifteen-membered ring [26].

Clarithromycin has been proposed as a possible statin interacting agent [26, 27], because they are eliminated via the same metabolic route regulated by CYP3A4. Clarithromycin inhibits CYP3A4 by a mechanism: when the medication is metabolized at the isozyme's active site, a reactive metabolite is produced, which binds permanently to the isozyme, disabling it and lowering the quantity of accessible CYP3A4. The inhibition of CYP450 isoenzymes is a significant source of medication interactions [28]. Competitive inhibition between medicines at the enzymatic level is widespread and may contribute to change the disposition of atorvastatin, resulting in higher blood levels [29]. Clarithromycin inhibits CYP3A4-mediated first-pass metabolism of the HMG.-CoA reductase inhibitor [30]. Clarithromycin, a powerful CYP3A4 and OATP inhibitor, had a significant influence on atorvastatin pharmacokinetics whereas azithromycin had a little effect, suggesting that atorvastatin hepatic clearance is mostly regulated by hepatic uptake [31, 32]. We observed that co-administration of clarithromycin significantly raised the atorvastatin AUC 2.74 fold and a small fold increase in the AUC of atorvastatin 1.11 when co-administered with azithromycin. The rise in AUC might be attributed to the enhanced intestinal availability of atorvastatin caused by the inhibition of intestinal CYP3A4 and efflux transporters. These findings show that changes in atorvastatin's hepatic metabolic clearance had an effect on its pharmacokinetics. The main cause of drug interactions with macrolides is believed to be the inhibition of the key drug metabolizing enzyme CYP3A4 in the small intestine and liver. However, macrolides can also cause drug interactions by inhibiting the uptake of concurrently administered drugs through OATPs [33, 34].

5. Conclusion

The study found that coadministration of clarithromycin with atorvastatin significantly alters its pharmacokinetics, increasing plasma concentrations, prolonging elimination half-life, and reducing

clearance by inhibiting CYP3A4 and OATP transporters. This interaction led to a 2.74-fold increase in AUC and reduced hepatic metabolism and clearance. However, azithromycin showed minimal effects, with only a slight increase in atorvastatin levels. The study recommends caution when prescribing clarithromycin with atorvastatin and suggests stopping statin medication during antibiotic treatment, while considering azithromycin with caution.

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Conflict of Interest: The authors declare that there are no conflicts of interest associated with this research project. We have no financial or personal relationships that could potentially bias our work or influence the interpretation of the results.

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