Journal of Experimental Pharmacology

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Simultaneous administration of fluoxetine and simvastatin ameliorates lipid profile, improves brain level of neurotransmitters, and increases bioavailability of simvastatin

This article was published in the following Dove Press journal:
Journal of Experimental Pharmacology
11 April 2017
Number of times this article has been viewed

Abdulrahman K Al-Asmari[1]
Zabih Ullah[1]
Aqeel Salman Al Masoudi[2]
Ishtiaque Ahmad[1]

[1]Department of Research, Prince Sultan Military Medical City, Riyadh, [2]Department of Research and Education, King Abdulaziz Airbase Armed Forces Hospital, Dhahran, Saudi Arabia

Abstract: Simvastatin (STT), a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, is widely prescribed for dyslipidemia, whereas fluoxetine (FLX) is the first-choice drug for the treatment of depression and anxiety. A recent report suggests that selective serotonin reuptake inhibitors can interact with the cytochrome P450 3A4 substrate, and another one suggests that STT enhances the antidepressant activity of FLX. However, the data are inconclusive. The present study was designed to explore the pharmacokinetic and pharmacodynamic consequences of coadministration of STT and FLX in experimental animals. For this, Wistar rats weighing 250±10 g were divided into four groups, including control, STT (40 mg/kg/day), FLX (20 mg/kg/day), and STT+FLX group, respectively. After the dosing period of 4 weeks, the animals were sacrificed, and the blood and brain samples were collected for the analysis of STT, simvastatin acid (STA), FLX, total cholesterol, triglyceride, high-density lipoprotein (HDL), 5-hydroxytryptamine, dopamine, and hydroxy indole acetic acid. It was found that the coadministration resulted in a significant increase in the bioavailability of STT in the plasma (41.8%) and brain (68.7%) compared to administration of STT alone ($p<0.05$). The maximum drug concentration (C_{max}) of STT was also found to be increased significantly in the plasma and brain compared to that achieved after monotherapy ($p<0.05$). However, STT failed to improve the pharmacokinetics of FLX up to a significant level. The results of this study showed that the combined regimen significantly reduced the level of cholesterol and triglyceride and increased the level of HDL when compared to STT monotherapy. Furthermore, the coadministration of STT with FLX led to an elevated level of neurotransmitters in the brain ($p<0.05$). FLX increased the concentration of STT in the plasma and brain. The coadministration of these drugs also led to an improved lipid profile. However, in the long-term, this interaction may have a vital clinical importance because the increase in STT level may lead to life-threatening side effects associated with statins.

Keywords: fluoxetine, simvastatin, lipid levels, neurotransmitters, bioavailability, drug interaction

Introduction

The 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors (statins) are a well-established class of drugs in the treatment of hypercholesterolemia. Drugs of this class have also been shown to reduce the risk of ischemic heart disease and cerebrovascular stroke and have potential use in multiple sclerosis and traumatic brain injury.[1] The use of statins in cardiac patients as well as in healthy elderly has increased tremendously. In fact, statins have been termed as "drug of 21st century"[2] with more

Correspondence: Abdulrahman K Al-Asmari
Research Center, Prince Sultan Military Medical City, PO Box 7897 (S-775), Riyadh 11159, Saudi Arabia
Tel +966 1 479 1000 (Extension 25100)
Fax +966 1 477 7714 (Extension 23060)
Email abdulrahman.alasmari@gmail.com

submit your manuscript | www.dovepress.com
Dovepress
http://dx.doi.org/10.2147/JEP.S128696

than 13 million annual prescription in the USA[3] and a massive increase in its consumption in Europe and Asia.[4,5] Some investigators have strongly suggested statin for all by the age of 50 years.[6] On the other hand, the use of statins has been associated with a spectrum of skeletal muscle complaints, ranging from myalgia to skeletal muscle destruction.[7] Chronic use of statins has been associated with severe myopathy and rhabdomyolysis in patients with hypothyroidism, renal diseases, and hepatobiliary disorders.[8]

In spite of numerous criticisms, the newly emerging evidence indicates that the beneficial effects of simvastatin (STT) extend to the central nervous system (CNS).[9] Elderly patients commonly receive statin medications for the primary or secondary prevention or cure of cardiovascular and cerebrovascular diseases.[10] Most of the time, these patients also receive antidepressant medications, generally selective serotonin reuptake inhibitors (SSRIs), for the management of depression, anxiety, or other CNS conditions. Recent reports suggest that SSRIs are associated with inhibition of the cytochrome P450 (CYP) metabolic pathways and are responsible for many pharmacokinetic drug interactions.[11] It is a matter of concern that drugs that interfere with statin metabolism can trigger adverse effects associated with it, especially potentially severe myopathy (rhabdomyolysis).[12] Therefore, the potential for drug–drug interactions emerges as a relevant factor in shaping the safety and efficacy of statins. Pharmacological dissimilarities are evident among the statins, and these may affect their safety and potential for drug interactions. The prolonged administration of these two classes of drugs may also produce convergent effects on different neurotransmitter systems or signaling targets in the brain.[13] There are reports that STT combined with fluoxetine (FLX) provides a potential mechanism for the anxiolytic and antidepressant properties of drugs in animal models.[14] Despite increasing indication for the role of STT in CNS diseases, there is relatively little knowledge of its interaction with CNS drugs, especially with SSRIs.[15] So, the current study was warranted to assess the pharmacokinetic and pharmacodynamic consequences that follow the combined administration of STT and FLX by oral route in experimental animals.

Materials and methods

Materials

STT and FLX were obtained from Sigma-Aldrich (St Louis, MO, USA). Reference standards were purchased from Clearsynth Ltd. (Mumbai, India). Kits for the determination of biochemical parameters (cholesterol, triglycerides) were obtained from Human Diagnostics (Wiesbaden, Germany). Acetonitrile of liquid chromatography/mass spectrometry (LCMS) grade and methanol (purity 99.9%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Liquid ammonia (about 25% NH_3, analytical grade), orthophosphoric acid (analytical grade), and ammonium formate (99.0%, LCMS grade) were purchased from Sigma-Aldrich. Milli-Q water used for the analysis was prepared in house by using Milli-Q water purification unit procured from EMD Millipore (Billerica, MA, USA). The blank control plasma containing K3 EDTA was collected from animal house of Prince Sultan Military Medical City (Riyadh, Saudi Arabia) and stored below −70°C in the deep freezer. All other reagents used for liquid chromatography/tandem mass spectrometry (LCMSMS) analysis were obtained from Merck Scientific (Kenilworth, NJ, USA).

Study design and sample collection

Adult male hyperlipidemia Wistar rats raised in our animal breeding facility were used in this study. The protocol of the study was approved by Research and Ethical Committee (REC) of Prince Sultan Military Medical City (Riyadh, Saudi Arabia). The experiments were performed based on the guidelines set forth for the use of animals by the REC. Hyperglycemia was induced by feeding a high-fat diet to the rats for up to 4 weeks.[16] The animals were weighing approximately 200–225 g, and housed in stainless steel wire cages, with not more than six animals per cage, in a controlled environment (temperature 25°C±2°C and relative humidity 50%±15%). The animals were allowed to have access to tap water and feed ad libitum. The rats were randomly divided into four groups (G1, G2, G3, and G4). Group G1 was treated as control, whereas G2 and G3 were administered with FLX (20 mg/kg body weight [b.w.]) and STT (40 mg/kg b.w.), respectively. The animals of the G4 group were administered with FLX (20 mg/kg b.w.) and STT (40 mg/kg b.w.) simultaneously. The dosing of FLX and STT to the respective groups was done orally for up to 4 weeks. At the last day after dosing, the animals of the treatments groups were further divided into subgroups (n=3 each) as per the sample collection time points (0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 8.0, 15.0, and 18.0 h). On the last day, after dosing as per schedule, the blood specimens were collected at above-stated time points by cardiac puncture under ether anesthesia. Immediately after bleeding, animals were euthanized by cervical dislocation. Each blood specimen was centrifuged (4000 rpm for 10 min at 4°C), and plasma was separated. The plasma samples were stored at below −70°C until analysis.

Sample processing protocol

Plasma samples were taken out of the freezer and allowed to thaw in an ice-cold water bath and then vortexed for proper mixing. Two hundred microliters of plasma sample was pipetted and transferred to a microcentrifuge tube containing an internal standard (ISTD) mevastatin. Samples were vortexed again for complete mixing. Further, these sample ISTDs were diluted with 500 µL of 5% orthophosphoric acid (OPA) solution and vortexed. The samples were then centrifuged at 14,000 rpm for 10 min (at 4°C). The supernatant thus obtained was loaded onto the preconditioned extraction cartridge (HLB, 30 mg/cc; Waters, Milford, MA, USA) and centrifuged at 2000 rpm for up to 1 min at 4°C. The samples were washed with 1 mL of 5% ammonia solution followed by 1 mL of Milli-Q water. The analytes of interest were eluted with 1 mL of methanol twice. The extracted samples were dried using nitrogen stream (Turbo Vap; Biotage, Uppsala, Sweden) at a pressure and temperature of 20 psig and 45°C, respectively. The dried samples were reconstituted with 300 µL of mobile phase and transferred to an autosampler glass vial, and 30 µL of extracted sample was injected into LCMSMS system for analysis. Furthermore, for the extraction of drugs from the brain tissue, the sample was accurately weighed and homogenized with the deionized water. This tissue homogenate was mixed with ISTD dilution followed by 1 mL of acidified acetonitrile (0.1% HCl) and 500 µL of working solution (5% OPA). This mixture was then spun at 14,000 rpm for 10 min. The supernatant obtained was loaded onto preconditioned HLB cartridges and processed as per plasma samples.

Conditions for chromatography and mass spectrometry

The liquid chromatography separation was performed by using ultrahigh-performance liquid chromatography (UHPLC) system of Dionex Ultimate 3000 (serial no. 7248679, part no. 5035.9200; Thermo Fisher Scientific, Waltham, MA, USA). The UHPLC system was equipped with a quaternary pump, an autosampler, and a solvent manager (serial no. 8074857, part no. 5082.0010). Chromatographic separation was achieved on an Aquasil C_{18} column (dimension 100×2.1, particle size 5 µm, lot no. 10268848; Thermo Fisher Scientific) using 5 mm ammonium formate buffer having pH 5±0.1 and acetonitrile (70:30, v/v) as a mobile phase at an isocratic flow rate of 0.4 mL/min. A mixture of methanol and water (50:50, v/v) was used as a rinsing solution. The column oven temperature was fixed at 35±5°C and autosampler temperature at 10°C.

The quantitation was performed by LCQ Fleet Ion Trap Mass Spectrometer (serial no. LCF 10356; Thermo Fisher Scientific) in positive electrospray ionization mode. Quantitation was carried in multiple-reaction monitoring mode of the transitions *m*/z 314/154, 415/285.2, 395/185.1, and 265.1/201.2 for FLX, STT, simvastatin acid (STA), and ISTD mevastatin, respectively. The optimized parameters for FLX were as follows: STT – spray voltage 6.0 kV, sheath gas 30, auxiliary gas 8 (highly pure nitrogen); STA – spray voltage 6.0 kV, sheath gas 30, auxiliary gas 8; and ISTD – spray voltage 5.0 kV, sheath gas 45, auxiliary gas 5, and sweep gas 1.0. Capillary temperature was set at 325°C for all analytes and ISTD.

Neurotransmitter analysis

Concentrations of dopamine and serotonin and hydroxy indole acetic acid (HIAA) were determined by the slightly modified method described by Eghwrudjakpor et al.[17] The brain samples were weighed (100 mg) and were homogenized in 1 mL of 0.1 M perchloric acid containing 0.05% EDTA using a Teflon homogenizer and centrifuged at 18,000 rpm for 10 min (at 4°C). Supernatants were filtered and injected to high-performance liquid chromatography (HPLC) system (Waters). The HPLC system consisted of an isocratic pump (1525), an autosampler (2707), and an electrochemical detector (2465) with a potential maintained at 0.8 V at 5 nA sensitivity. The separation was achieved using µBondapak C_{18} column (3.9×10 µm×150 mm; Waters), and a mobile phase of 0.1 M citric acid monohydrate, 0.1 M sodium acetate, 0.01% octane sulfonic acid, 100 µM EDTA, and 7% methanol (pH 2.8). The flow rate was 1.2 mL/min, and retention time was 6.2, 8.7, and 15.2 min for dopamine, 5 HIAA, and serotonin, respectively.

Pharmacokinetic analysis

Using the plasma levels at various time intervals, the following pharmacokinetic parameters were calculated by using the least-squares program of SummitPK® (Summit Research Services, Montrose, CO, USA): AUC_{0-t} (area under the concentration–time curve from time zero to t), $AUC_{0-\infty}$ (area under the concentration–time curve from time zero to infinity), C_{max} (maximum plasma drug concentration), T_{max} (time to reach maximum concentration following drug administration), $t_{1/2}$ (elimination half-life associated with terminal slope of a semilogarithmic concentration–time curve), and K_{el} (the rate at which a drug is removed from the body).

Biochemical analysis

Levels of cholesterol, triglyceride, and HDL were measured spectrophotometrically (Evolution 300; Thermo Fisher

Scientific) using the commercially available kits from Human Diagnostics. The levels of cholesterol, triglycerides, and HDL are expressed as mg/dL.

Statistical analyses

Differences between treatment groups were analyzed for statistical significance by the multiple-comparison tests (Dennett's test). Differences between pre- and post-administration values were analyzed using the Student's *t*-test. A *p* value of less than 0.05 was defined as an indicator of a significant difference.

Results

Validation of bioanalysis method

The developed method was found to be sensitive enough to quantify the analytes in plasma as well as in the brain. No interference was observed from the endogenous matrix at the retention time of analytes and ISTD, which proposes that the method was selective for the analytes of interest. The calibration range was established between 5 and 500 ng/mL for each analyte. Mean extraction recoveries of STT, STA, and FLX from plasma were found to be 84.58%±4.2%, 75.8%±5.4%, and 81.6%±4.2%, respectively, and 74.5%±3.5%, 71.4%±5.3%, and 85.4%±4.8% for STT, STA, and FLX, respectively, from the brain. The intra- and interday precision for all the analytes ranged between 4.5% and 8.9% for plasma and 5.6% and 9.2% for the brain, and intra- and interday accuracies of the developed method for each analyte ranged between 96.4% and 106.7% for plasma and 93.6% and 103.4% for the brain.

Pharmacokinetic parameters in plasma

The potentiation effect of STT with FLX in improving the lipid profile and neurotransmitters level could involve higher bioavailability and/or changes in drug metabolism exerted by each other. To test this hypothesis, we examined the plasma concentrations of STT and its metabolite STA, and FLX after a 4-week treatment with STT (40 mg/kg) and FLX (20 mg/kg) separately or in combination. The mean plasma concentration–time curves of STT, STA, and FLX are presented in Figure 1. All the analytes demonstrated the similar pattern of plasma levels; however, in the case of STT+FLX

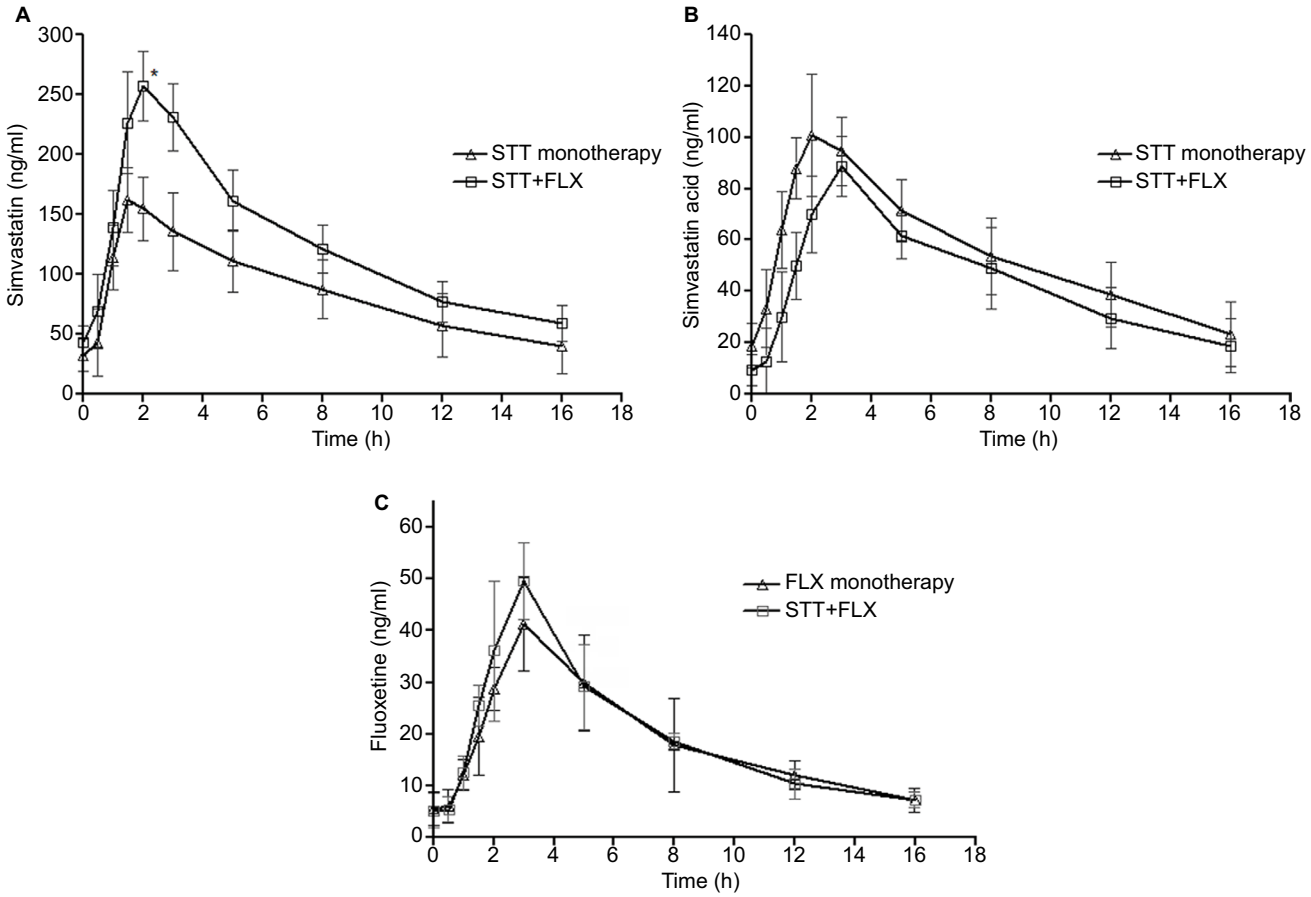

Figure 1 Mean plasma levels of STT, STA, and FLX after single and combined administration of STT and FLX. Time course for (**A**) STT plasma levels, (**B**) STA plasma levels, and (**C**) FLX plasma levels after single administration of STT at a dose of 40 mg/kg b.w. and FLX at a dose of 20 mg/kg b.w. and combined administration of STT with FLX for a period of 4 weeks. Results are expressed as mean±SD; n=3 (three animals per time points). FLX administered simultaneously with STT was able to increase the plasma concentration of STT. Significant difference at the C_{max} level was observed with combined treatment. *$p<0.05$, compared to STT alone.
Abbreviations: STT, simvastatin; STA, simvastatin acid; FLX, fluoxetine; b.w., body weight; SD, standard deviation; C_{max}, maximum plasma drug level.

combination, the plasma levels of STT were found to be significantly higher as compared to STT-alone treatment. The plasma levels of STA and FLX were not affected up to a significant level in the combination regimen.

Pharmacokinetic parameters of STT, STA, and FLX following the administration of the multiple doses as monotherapy and as combination are shown in Table 1. Coadministration of STT orally with FLX for 4 weeks resulted in a significant increase in the AUC and C_{max} of STT ($p<0.05$). The AUC and C_{max} of STT increased up to 41.6% and 57.9%, respectively, as compared to STT-alone treatment. The other parameters like $t_{1/2}$ and K_{el} were not altered up to a significant level ($p>0.05$). On the other hand, no significant change was observed in the value of AUC and C_{max} for the STA and FLX administered in combination as compared to the monotherapy, except the $AUC_{0-\infty}$ value of the STA, which was found to be significantly decreased ($p<0.05$) when compared to combined treatment. The reduction in the bioavailability of STA was found to be 36% as compared to monotherapy as it decreased from 1108.59±341.74 to 703.87±333.84 ng·h/mL. Similarly, as the STT, no significant change was observed in the other pharmacokinetic parameters of STA and FLX.

Pharmacokinetic parameters in the brain

The comparative mean brain concentrations and time profile of STT, STA, and FLX after separate and combined administrations of STT and FLX are illustrated in Figure 2, and pharmacokinetic data are shown in Table 2. As evident from the figure, the time to reach maximum concentration in the brain was delayed in comparison to plasma for all three analytes. Similar to plasma level, the brain level of STT after combined administration with FLX was found to be significantly higher when compared to administration of STT alone ($p<0.05$). The brain C_{max} level was increased up to 196.65±10.01 ng/g with combined therapy as compared to STT monotherapy which was 103.80±19.87 ng/g, whereas the percentage increase in AUC_{0-t} and $AUC_{0-\infty}$ was found to be 79% and 68.7%, which was significantly higher as compared to STT monotherapy. The reduction in bioavailability of STA in the brain was not as much as plasma, where it was found to be significantly lower when compared to STT monotherapy. On the other hand, brain level of FLX was not affected up to a significant level with coadministration with STT. Moreover, after multiple-dose administration, the accumulation of FLX was multifold higher in the brain than plasma. The combined administration of STT and FLX was found to achieve a significantly higher level of STT in plasma and the brain after multiple-dose administration. A significant increase in C_{max} of STT in plasma and the brain was observed when the STT was given along with FLX.

Lipid levels

The bar graph of different plasma lipid levels in lipid control animals and other treatment groups is illustrated in Figure 3. After 4 weeks of treatment, a significant reduction ($p<0.05$) in total cholesterol and triglyceride levels was observed in the hyperlipidemic Wistar rats treated with STT alone or in combination with FLX. STT administration resulted in an increase in plasma HDL level as well as a documented effect. However, FLX alone was also able to reduce the total cholesterol up to a significant level ($p<0.05$) after a treatment period of 4 weeks. FLX reduced the total cholesterol level up to 16.3% in comparison to control. However, FLX alone failed to produce any significant effect on plasma triglyceride or HDL level. The animals treated with combined regimen

Table 1 Pharmacokinetic profile of STT, STA, and FLX in plasma of Wistar rat after treatment with different regimens (data are represented as mean±SD)

Parameters	STT		STA		FLX	
	STT monotherapy	STT+FLX	STT monotherapy	STT+FLX	FLX monotherapy	STT+FLX
AUC_{0-t} (ng·h/mL)	1389.26±163.63	1966.54±108.18*	907.00±335.62	647.95±217.37	301.75±102.32	254.81±83.03
$AUC_{0-\infty}$ (ng·h/mL)	1579.33±391.64	2240.25±226.13*	1108.59±341.74	703.87±333.84*	367.83±133.26	321.65±53.28
$t_{1/2}$ (h)	4.86±0.51	4.88±0.31	5.95±0.65	5.80±0.60	6.59±1.27	6.12±0.56
K_{el} (h^{-1})	0.15±.03	0.14±0.03	0.12±0.02	0.12±0.03	0.13±.02	0.12±0.03
C_{max} (ng/mL)	162.79±20.38	257.06±19.13*	103.39±24.56	88.59±11.89	43.84±16.85	55.26±11.76
T_{max} (h)	1.67±0.29	2.00±0.00	2.33±0.58	3.00±0.00	3.67±1.15	3.33±0.67

Note: *$p<0.05$, compared with monotherapy.

Abbreviations: STT, simvastatin; STA, simvastatin acid; FLX, fluoxetine; SD, standard deviation; AUC_{0-t}, area under concentration–time curve from time zero to time t; $AUC_{0-\infty}$, area under concentration–time curve from time zero to infinity; $t_{1/2}$, terminal elimination half-life; K_{el}, rate of drug removal from the body; C_{max}, maximum plasma drug level; T_{max}, time to maximum concentration.

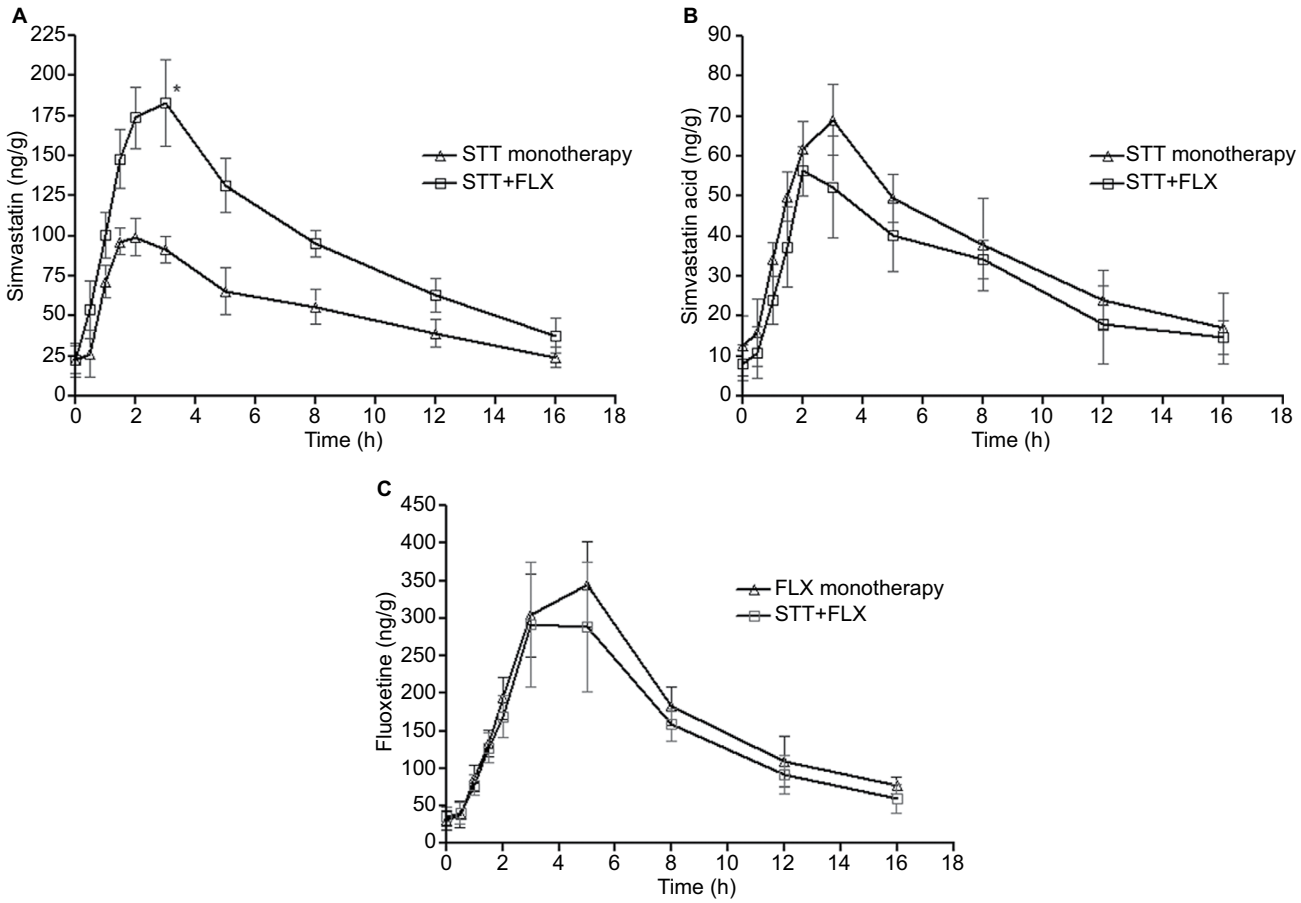

Figure 2 Brain concentration–time profile of (**A**) STT, (**B**) STA, and (**C**) FLX obtained after oral administration of FLX and STT, alone and in combination, to Wistar rats for a period of 4 weeks. The data are represented as mean±SD; n=3. Combined administration of STT with FLX led to a significant increase in the level of STT in brain in comparison to STT monotherapy ($p<0.05$). However, STT did not alter the brain concentration of FLX up to a significant level. *$p<0.05$, compared to STT alone.
Abbreviations: STT, simvastatin; STA, simvastatin acid; FLX, fluoxetine; SD, standard deviation.

Table 2 Pharmacokinetic profile of STT, STA, and FLX in the brain of Wistar rats after treatment with different regimens (data are represented as mean±SD)

Parameters	STT		STA		FLX	
	STT monotherapy	STT+FLX	STT monotherapy	STT+FLX	FLX monotherapy	STT+FLX
AUC_{0-t} (ng·h/g)	663.68±163.85	1188.39±314.11*	453.32±157.20	361.79±162.48	2823.78±382.73	2380.06±426.63
$AUC_{0-\infty}$ (ng·h/g)	898.58±161.36	1515.91±355.16*	625.23±190.44	499.83±160.91	3556.09±492.20	2913.11±482.40
$t_{1/2}$ (h)	6.67±0.34	7.52±0.59	6.92±0.20	6.58±1.01	6.48±00.90	6.24±0.86
K_{el} (h^{-1})	0.11±0.01	0.12±0.01	0.1±0.01	0.11±0.01	0.11±0.01	0.11±0.02
C_{max} (ng/g)	103.80±19.87	196.65±10.01*	70.32±18.08	58.69±5.84	374.45±28.91	348.00±35.93
T_{max} (h)	2.33±0.29	2.67±0.0.58	2.67±0.58	2.67±0.58	4.33±0.00	3.67±1.02

Note: *$p<0.05$, compared with monotherapy.
Abbreviations: STT, simvastatin; STA, simvastatin acid; FLX, fluoxetine; SD, standard deviation; AUC_{0-t}, area under concentration–time curve from time zero to time t; $AUC_{0-\infty}$, area under concentration–time curve from time zero to infinity; $t_{1/2}$, terminal elimination half-life; K_{el}, rate of drug removal from the body; C_{max}, maximum plasma drug level; T_{max}, time to maximum concentration.

demonstrated a significant reduction in cholesterol and triglyceride level when compared to lipid control group as well as animals treated with STT alone ($p<0.05$). The combination (STT+FLX) resulted in the highest reduction in total cholesterol (57.1%) and triglyceride level. STT alone was able to reduce the cholesterol level to 41.6% in comparison to control. However, an additional increment of 15.5% in cholesterol-lowering capacity was observed when STT was combined with FLX. The combined regimen failed to produce any significant effect on plasma HDL level, although its level was found to be reduced in this group of animals.

Neurotransmitter levels

The neurotransmitter levels in the control and experimental animal groups are illustrated in Figure 4. As expected, during the study period, FLX alone increased the brain concentration

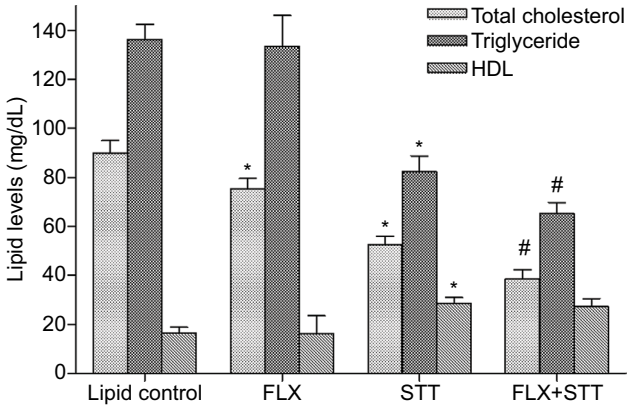

Figure 3 Bar graph of cholesterol, triglyceride, and HDL plasma concentrations in Wistar rats after the treatment with FLX and STT alone and their combination for a period of 4 weeks. The data are presented as mean±SD; n=6. Significant differences were observed between the lipid control and the other experimental groups. There was a significant decrease in cholesterol levels in the animals treated with FLX alone. Significant improvement in lipid profile was observed when STT was administered along with FLX. FLX alone did not affect the level of triglyceride or HDL, but in combination with STT, exerted a synergistic effect in reducing cholesterol and triglyceride level. *p<0.05, compared to lipid control; #p<0.05, compared to STT alone.

Abbreviations: HDL, high-density lipoprotein; FLX, fluoxetine; STT, simvastatin; SD, standard deviation.

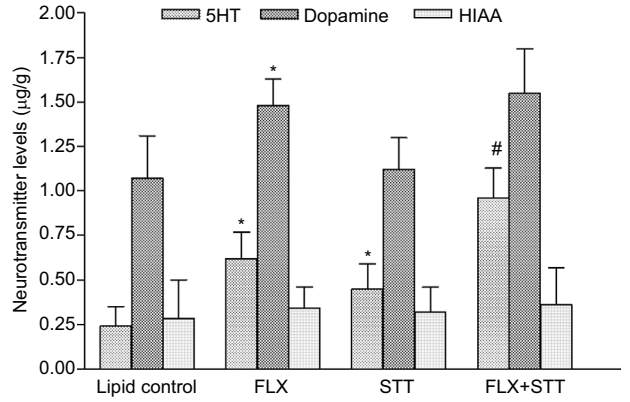

Figure 4 Levels of neurotransmitters in Wistar rat brain after the combined and separate treatment with FLX and STT for 4 weeks. STT significantly increased 5HT level in the brain when administered alone and produced a synergistic effect in combination with FLX, whereas it did not alter the level of dopamine up to a significant level. Furthermore, FLX produced significant increases in the brain level of dopamine and 5HT after 4-week systemic administrations. In addition, both of these drugs failed to alter the concentration of HIAA up to a significant level. The data are presented as mean±SD; n=6. *p<0.05, compared to lipid control; #p<0.05, compared to FLX alone.

Abbreviations: FLX, fluoxetine; STT, simvastatin; 5HT, 5-hydroxytryptamine; HIAA, hydroxy indole acetic acid; SD, standard deviation.

of 5-hydroxytryptamine (5HT) (0.62±0.15 µg/g) and dopamine (1.48±0.14 µg/g) up to a significant level (p<0.05) in comparison to control. However, FLX alone failed to alter the brain level of HIAA. STT was also found to increase the level of 5HT (0.45±0.14 µg/g) in comparison to control. However, the increase was insignificant when compared to animals treated with FLX alone. The dopamine and HIAA levels were unaffected by the STT-alone treatment. The combined regimen (FLX+STT) was found to exert a synergistic effect on the elevation of 5HT concentration in the brain (0.96±0.17 µg/g), as the increment was significant in comparison to control group or group treated with FLX alone; however, the combination did not affect the concentration of dopamine and HIAA when compared to animals treated with FLX alone.

Discussion

Potential statin–drug interactions are common in patients receiving treatment for cardiovascular ailments. Around 20% of patients taking statins are exposed to at least one or more coprescriptions that can be linked to adverse drug reactions.[18] Even though statins are well tolerated with an acceptable safety profile, adverse effects may occur in some patients at prescribed doses. Myopathy and rhabdomyolysis are occasional with statin monotherapy at the permitted dose ranges, but even at the approved doses, the risk increases with use of interacting drugs including SSRIs.[19] SSRIs are the drug of choice for anxiety and depression, which may be ideally suited to hypercholesterolemic patients. These medicines may also decrease cardiovascular risks and improve clinical cardiovascular end points through several mechanisms.[20] However, SSRIs are commonly associated with pharmacokinetic drug interactions, and many of these inhibit the metabolism of other drugs, thereby raising the blood levels of many coadministered drugs. Hence, the risk of adverse effects is increased. In the case of statins, elevated plasma concentration by SSRIs can result in an increased level of hepatic enzyme and myopathies that can range from troublesome myalgia, cramps, weakness, and life-threatening disorder (rhabdomyolysis).[21] Therefore, it is important to know how SSRIs affect the pharmacokinetics and pharmacodynamics of statins and the effect of coadministration on neurotransmitter level.

The results of this study revealed that concomitant administration of STT with FLX up to 4 weeks led to a significant increase (p<0.05) in the bioavailability of STT in plasma as well as in the brain (Figures 1 and 2). The AUC of STT was increased by 41.6% in plasma and 79% in the brain when STT was given in combination with FLX (Tables 1 and 2). These findings focus on the possible interaction between these two most widely used drugs. After ingestion, STT is converted to metabolite STA by esterases and several other metabolites by CYP3A4.[22] FLX may alter the metabolism of STT by various possible mechanisms including CYP3A4,[23] glucuronidation,[24] and meddling with transport systems across membrane including organic anion transport peptide (OATP) 1B1[25] and P-glycoprotein (Pgp).[26] The drug-metabolizing enzyme

CYP3A4 plays a major role in the first-pass metabolism of STT and is abundantly expressed in the intestinal wall mucosa and liver.[27] Inhibition of this enzyme in the intestinal wall as well as in the liver tends to increase the plasma concentration of CYP3A4 substrate including STT.[28,29] In this study, the C_{max} and AUCs of STT were significantly increased by FLX with no significant change in the elimination half-life of STT, clearly suggesting that inhibition of the CYP3A4 by FLX may be responsible for inhibiting the metabolism of STT. FLX and its metabolite norfluoxetine comprise a multiple-inhibitor system that causes reversible and time-dependent inhibition of the CYP family members including CYP2D6, CYP2C19, and CYP3A4, which increases the plasma concentration of many drugs.[30–32]

Our findings of increased bioavailability of STT by CYP3A4 inhibitor FLX are in agreement with the several earli`er studies showing that CYP3A4 inhibitors such as itraconazole,[33] erythromycins, verapamil,[34] diltiazem,[35] ketoconazole,[36] and grapefruit juice[37] can increase the bioavailability of STT significantly. Furthermore, a recent report found that after 2-week dosing of FLX, the bioavailabilities of omeprazole and dextromethorphan were increased by 7.1- and 27-fold, respectively.[32,38] CYP3A4 is the most copious enzyme of CYPs in the liver and gut that metabolizes nearly about 50% of currently available drugs. Some essential medicines have been identified as substrates, inducers, and/or inhibitors of CYP3A4. Inhibition of CYP3A4 is mediated through NADPH-, time-, and concentration-dependent enzyme inactivation, which takes place when CYPs transform drugs to active metabolites. At this stage, chemical modification of CYP3A4 occurs as a consequence of covalent binding of modified heme to the protein, finally inhibiting the activity of the enzyme.[39]

On the other hand, other possible mechanism for interaction would be the Pgp. Pgp is an ATP-powered drug efflux pump present in the intestine, kidney, liver, brain, and placenta, and plays a critical role in absorption, distribution, and elimination of a variety of drugs, including STT.[26,40,41] Recently, it has been reported that FLX downregulates Pgp.[42] Hence, the hepatocellular transport of STT may be affected due to downregulation of Pgp. Besides drug-metabolizing enzymes, active transport system is an emerging concept in drug pharmacokinetics.[43] It has become evident that significant drug–drug interaction may result from inhibition and induction of transporter function.[25,44] The role of OATP in drug–drug interaction may be challenging, since many OATP substrates are also substrates for other drugs. Recently, it has been shown that OATP1B1 mediates hepatocellular influx of STT in the liver.[45] Since both are substrates of the OATP transport system, OATP1B1 may interfere with the pharmacokinetics of STT.

In addition to the well-known P450-mediated oxidation processes, and transport systems, glucuronidation is also a common metabolic pathway for the statins.[24] Glucuronidation may play a major role in facilitating the lactonization process of statin drugs in vivo. This complex metabolic pathway of statins involves hydroxy acid/lactone interconversion through UDP-glucuronosyl transferase (UGT1A1and UGT1A3) enzyme system, and concomitant administration of FLX can alter this process. The FLX and its active metabolite norfluoxetine are substrates for UDP-glucuronosyl transferase enzymes in the liver and can affect the disposition and biological effects of STT by interfering with its glucuronidation.[46] Hence, it may be concluded that FLX-induced alteration of STT pharmacokinetics involves several complex mechanisms.

Furthermore, we observed another important and innovative finding in the study that the STT increased 5HT level in the brain when administered alone or in combination with FLX. STT produced a synergistic effect on level of 5HT when given along with FLX (Figure 4). Recent reports also revealed the elevated level of 5HT and synergistic antidepressant activity after treatment with the STT.[47,48] It can be explained that statins dose-dependently attenuate the activity of indoleamine 2,3-dioxygenase (IDO), which is an interferon γ-inducible enzyme that degrades tryptophan in the kynurenine pathway. Tryptophan is a known precursor of the neurotransmitter serotonin. So, it can be suggested that STT increases 5HT concentration by increasing tryptophan levels as a result of IDO enzyme blockade.[14] However, STT fails to alter the level of dopamine up to a significant level.

On the other hand, FLX was found to increase brain dopamine level. FLX produced robust increases in concentrations of dopamine after 4-weak systemic administrations (Figure 4). Our results of elevated dopamine level are in agreement with the earlier reports which suggest that FLX blocks binding to the serotonin transporter, but it does not affect the norepinephrine transporter. The increase in dopamine level is not due to blockade of norepinephrine uptake, but FLX at higher concentration blocks 5-HT_{2C} receptors, which can be said as a potential mechanism for the FLX-induced increase in brain dopamine level.[49] So, it can be concluded that FLX increases concentrations of serotonin as well as dopamine by different mechanisms.

Moreover, another interesting result was observed that there was a significant decrease in serum cholesterol

levels in animals treated with FLX alone ($p<0.05$), whereas fall in serum triglyceride level and HDL was found to be insignificant. Treatment of rats with STT alone as expected produced a significant decrease in plasma cholesterol and triglyceride levels (Figure 3). Concomitant administration of FLX significantly improved STT-induced reduction in cholesterol and triglyceride level ($p<0.05$) when compared to STT monotherapy. The results of our study showing a reduction in cholesterol in animals treated with FLX alone are in agreement with a clinical study by Dryden et al[50] in which they reported a decrease in cholesterol level upon FLX-alone treatment. However, in other studies in human, a decrease in triglyceride level was reported rather than a reduction in cholesterol level.[51,52] These contradictory findings may be attributed to differences in species and races.

Additionally, the lipid-lowering effect of STT (a prodrug) depends on the plasma concentration of STT and its metabolite STA, which is produced by esterases or/and CYP3A4 enzymes and other metabolic pathways in the liver and intestinal wall during the first-pass metabolism.[22] However, this mechanism cannot be justified here as the FLX is a known CYP3A4 inhibitor, which leads to decrease in STA level. The absence of expected reversal of STA-induced cholesterol-lowering effect, due to a significant decline in STA bioavailability in STT- and FLX-treated animals, may be attributed to potentiation of lipid-lowering effect of statins by FLX. FLX inhibits serotonin reuptake, and this neurotransmitter is known to decrease food intake by inhibiting neuropeptide Y neurones, and leptin levels, possibly by an effect on the white adipose tissue resulting in reduced cholesterol level.[50] Overall, the study provides useful information about the possible outcomes of this most widely used combination as follows: FLX has the potential to elevate the bioavailability of STT, and combined administration of these drugs demonstrates an increase in the brain level of neurotransmitters and improved lipid profile. At first, this combination seems to be beneficial with respect to pharmacodynamic responses of each other; however, there are chances that elevated level of STT due to FLX may predispose to myopathy or rhabdomyolysis. Due to the short duration of treatment and differences in species and races, further clinical studies of longer duration are warranted to establish the outcomes of interactions between STT and FLX.

Conclusion

The coadministration of STT and FLX resulted in a significant increase in the AUC and C_{max} of STT in plasma as well as in the brain, when compared to STT-alone treatment. Conversely, the $AUC_{0-\infty}$ of STA was reduced significantly in the combined treatment arm, whereas STT failed to alter the plasma or brain concentration of FLX up to a significant level. The mechanism of the interaction is most probably the inhibition of the CYP3A4 enzyme by the FLX, which is responsible for the metabolism of STT. However, another possible mechanism would be an alteration in the functioning of OATP, Pgp, or various transporters in the intestinal wall. Combined treatment was also proved beneficial as it improved lipid profile and increased the neurotransmitters level in the brain. From a pharmacodynamic viewpoint, this interaction seems to be beneficial, but it may have a vital clinical importance because the increase in STT level may cause serious side effects associated with STT.

Acknowledgments

The authors are grateful to Dyanidhi Yesunayagam at the animal house of Prince Sultan Military Medical City and Manea A Al-Ahmari for the care of the animals during the study period and providing rat plasma for the development and validation of method investigated in this study. The authors are also thankful to Biju Prasad for the assistance in neurotransmitters analysis and to Tariq Al Zamil and Ruba Al Mohini for the help in the measurement of lipid levels.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Wang Q, Wang PH, McLachlan C, Wong PT. Simvastatin reverses the downregulation of dopamine D1 and D2 receptor expression in the prefrontal cortex of 6-hydroxydopamine-induced Parkinsonian rats. *Brain Res*. 2005;1045(1–2):229–233.
2. Ginter E, Simko V. Statins: the drugs for the 21st century? *Bratisl Lek Listy*. 2009;110(10):664–668.
3. Al-Asmari AK, Ullah Z, Al-Sabaan F, Tariq M, Al-Eid A, Al-Omani SF. Effect of vitamin D on bioavailability and lipid lowering efficacy of simvastatin. *Eur J Drug Metab Pharmacokinet*. 2015;40(1):87–94.
4. Hankey GJ, Wong KS, Chankrachang S, et al; Working Group on Stroke and Lipids Management in Asia Consensus Panel. Management of cholesterol to reduce the burden of stroke in Asia: consensus statement. *Int J Stroke*. 2010;5(3):209–216.
5. Stroes ES, Thompson PD, Corsini A, et al; European Atherosclerosis Society Consensus Panel. Statin-associated muscle symptoms: impact on statin therapy—European Atherosclerosis Society Consensus Panel statement on assessment, aetiology and management. *Eur Heart J*. 2015;36(17):1012–1022.
6. Ebrahim S, Casas JP. Statins for all by the age of 50 years? *Lancet*. 2012;380(9841):545–547.
7. Parker BA, Capizzi JA, Grimaldi AS, et al. Effect of statins on skeletal muscle function. *Circulation*. 2013;127(1):96–103.
8. Mancini GB, Baker S, Bergeron J, et al. Diagnosis, prevention, and management of statin adverse effects and intolerance: proceedings of a Canadian Working Group Consensus Conference. *Can J Cardiol*. 2011;27(5):635–662.

9. McFarland AJ, Anoopkumar-Dukie S, Arora DS, et al. Molecular mechanisms underlying the effects of statins in the central nervous system. *Int J Mol Sci*. 2014;15(11):20607–20637.
10. Thavendiranathan P, Bagai A, Brookhart MA, Choudhry NK. Primary prevention of cardiovascular diseases with statin therapy: a meta-analysis of randomized controlled trials. *Arch Intern Med*. 2006;166(21):2307–2313.
11. Spina E, Santoro V, D'Arrigo C. Clinically relevant pharmacokinetic drug interactions with second-generation antidepressants: an update. *Clin Ther*. 2008;30(7):1206–1227.
12. Bottorff MB. Statin safety and drug interactions: clinical implications. *Am J Cardiol*. 2006;97(8A):27C–31C.
13. Santos T, Baungratz MM, Haskel SP, et al. Behavioral interactions of simvastatin and fluoxetine in tests of anxiety and depression. *Neuropsychiatr Dis Treat*. 2012;8:413–422.
14. Kilic FS, Ozatik Y, Kaygisiz B, Baydemir C, Erol K. Acute antidepressant and anxiolytic effects of simvastatin and its mechanisms in rats. *Neurosciences (Riyadh)*. 2012;17(1):39–43.
15. Wang Q, Zengin A, Deng C, et al. High dose of simvastatin induces hyperlocomotive and anxiolytic-like activities: the association with the up-regulation of NMDA receptor binding in the rat brain. *Exp Neurol*. 2009;216(1):132–138.
16. Vijaya C, Ramanathan M, Suresh B. Lipid lowering activity of ethanolic extract of leaves of Aegle marmelos (Linn.) in hyperlipidaemic models of Wistar albino rats. *Indian J Exp Biol*. 2009;47(3):182–185.
17. Eghwrudjakpor PO, Miyake H, Kurisaka M, Mori K. Central nervous system bioaminergic responses to mechanical trauma. An experimental study. *Surg Neurol*. 1991;35(4):273–279.
18. Ansari J. Drug interaction and pharmacist. *J Young Pharm*. 2010;2(3):326–331.
19. Hu M, Cheung BM, Tomlinson B. Safety of statins: an update. *Ther Adv Drug Saf*. 2012;3(3):133–144.
20. Andrade C, Kumar CB, Surya S. Cardiovascular mechanisms of SSRI drugs and their benefits and risks in ischemic heart disease and heart failure. *Int Clin Psychopharmacol*. 2013;28(3):145–155.
21. Bellosta S, Corsini A. Statin drug interactions and related adverse reactions. *Expert Opin Drug Saf*. 2012;11(6):933–946.
22. García MJ, Reinoso RF, Sánchez Navarro A, Prous JR. Clinical pharmacokinetics of statins. *Methods Find Exp Clin Pharmacol*. 2003;25(6):457–481.
23. Schwartz JB. Effects of vitamin D supplementation in atorvastatin-treated patients: a new drug interaction with an unexpected consequence. *Clin Pharmacol Ther*. 2009;85(2):198–203.
24. Prueksaritanont T, Subramanian R, Fang X, et al. Glucuronidation of statins in animals and humans: a novel mechanism of statin lactonization. *Drug Metab Dispos*. 2002;30(5):505–512.
25. Ho RH, Kim RB. Transporters and drug therapy: implications for drug disposition and disease. *Clin Pharmacol Ther*. 2005;78(3):260–277.
26. Holtzman CW, Wiggins BS, Spinler SA. Role of P-glycoprotein in statin drug interactions. *Pharmacotherapy*. 2006;26(11):1601–1607.
27. Kivistö KT, Niemi M, Schaeffeler E, et al. Lipid-lowering response to statins is affected by CYP3A5 polymorphism. *Pharmacogenetics*. 2004;14(8):523–525.
28. Kolars JC, Schmiedlin-Ren P, Schuetz JD, Fang C, Watkins PB. Identification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes. *J Clin Invest*. 1992;90(5):1871–1878.
29. Fromm MF, Busse D, Kroemer HK, Eichelbaum M. Differential induction of prehepatic and hepatic metabolism of verapamil by rifampin. *Hepatology*. 1996;24(4):796–801.
30. Italiano D, Spina E, de Leon J. Pharmacokinetic and pharmacodynamic interactions between antiepileptics and antidepressants. *Expert Opin Drug Metab Toxicol*. 2014;10(11):1457–1489.
31. Vlase L, Neag M, Popa A, Muntean D, Leucuta SE. Pharmacokinetic interaction between fluoxetine and omeprazole in healthy male volunteers: a prospective pilot study. *Curr Ther Res Clin Exp*. 2010;71(6):360–368.
32. Sager JE, Lutz JD, Foti RS, Davis C, Kunze KL, Isoherranen N. Fluoxetine- and norfluoxetine-mediated complex drug-drug interactions: in vitro to in vivo correlation of effects on CYP2D6, CYP2C19, and CYP3A4. *Clin Pharmacol Ther*. 2014;95(6):653–662.
33. Maxa JL, Melton LB, Ogu CC, Sills MN, Limanni A. Rhabdomyolysis after concomitant use of cyclosporine, simvastatin, gemfibrozil, and itraconazole. *Ann Pharmacother*. 2002;36(5):820–823.
34. Kantola T, Kivistö KT, Neuvonen PJ. Erythromycin and verapamil considerably increase serum simvastatin and simvastatin acid concentrations. *Clin Pharmacol Ther*. 1998;64(2):177–182.
35. Kanathur N, Mathai MG, Byrd RP Jr, Fields CL, Roy TM. Simvastatin-diltiazem drug interaction resulting in rhabdomyolysis and hepatitis. *Tenn Med*. 2001;94(9):339–341.
36. Jerling M, Huan BL, Leung K, Chu N, Abdallah H, Hussein Z. Studies to investigate the pharmacokinetic interactions between ranolazine and ketoconazole, diltiazem, or simvastatin during combined administration in healthy subjects. *J Clin Pharmacol*. 2005;45(4):422–433.
37. Lilja JJ, Kivistö KT, Neuvonen PJ. Grapefruit juice-simvastatin interaction: effect on serum concentrations of simvastatin, simvastatin acid, and HMG-CoA reductase inhibitors. *Clin Pharmacol Ther*. 1998;64(5):477–483.
38. Amchin J, Ereshefsky L, Zarycranski W, Taylor K, Albano D, Klockowski PM. Effect of venlafaxine versus fluoxetine on metabolism of dextromethorphan, a CYP2D6 probe. *J Clin Pharmacol*. 2001;41(4):443–451.
39. Zhou SF, Xue CC, Yu XQ, Li C, Wang G. Clinically important drug interactions potentially involving mechanism-based inhibition of cytochrome P450 3A4 and the role of therapeutic drug monitoring. *Ther Drug Monit*. 2007;29(6):687–710.
40. Barthe L, Woodley J, Houin G. Gastrointestinal absorption of drugs: methods and studies. *Fundam Clin Pharmacol*. 1999;13(2):154–168.
41. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266–281.
42. Weiss J, Dormann SM, Martin-Facklam M, Kerpen CJ, Ketabi-Kiyanvash N, Haefeli WE. Inhibition of P-glycoprotein by newer antidepressants. *J Pharmacol Exp Ther*. 2003;305(1):197–204.
43. Christians U, Strom T, Zhang YL, et al. Active drug transport of immunosuppressants: new insights for pharmacokinetics and pharmacodynamics. *Ther Drug Monit*. 2006;28(1):39–44.
44. Chan LM, Lowes S, Hirst BH. The ABCs of drug transport in intestine and liver: efflux proteins limiting drug absorption and bioavailability. *Eur J Pharm Sci*. 2004;21(1):25–51.
45. Karlgren M, Vildhede A, Norinder U, et al. Classification of inhibitors of hepatic organic anion transporting polypeptides (OATPs): influence of protein expression on drug-drug interactions. *J Med Chem*. 2012;55(10):4740–4763.
46. Nałęcz-Jawecki G. Evaluation of the in vitro biotransformation of fluoxetine with HPLC, mass spectrometry and ecotoxicological tests. *Chemosphere*. 2007;70(1):29–35.
47. Vevera J, Valeš K, Fišar Z, et al. The effect of prolonged simvastatin application on serotonin uptake, membrane microviscosity and behavioral changes in the animal model. *Physiol Behav*. 2016;158:112–120.
48. ElBatsh MM. Antidepressant-like effect of simvastatin in diabetic rats. *Can J Physiol Pharmacol*. 2015;93(8):649–656.
49. Bymaster FP, Zhang W, Carter PA, et al. Fluoxetine, but not other selective serotonin uptake inhibitors, increases norepinephrine and dopamine extracellular levels in prefrontal cortex. *Psychopharmacology (Berl)*. 2002;160(4):353–361.
50. Dryden S, Brown M, King P, Williams G. Decreased plasma leptin levels in lean and obese Zucker rats after treatment with the serotonin reuptake inhibitor fluoxetine. *Horm Metab Res*. 1999;31(6):363–366.
51. O'Kane M, Wiles PG, Wales JK. Fluoxetine in the treatment of obese type 2 diabetic patients. *Diabet Med*. 1994;11(1):105–110.
52. Daubresse JC, Kolanowski J, Krzentowski G, Kutnowski M, Scheen A, Van Gaal L. Usefulness of fluoxetine in obese non-insulin-dependent diabetics: a multicenter study. *Obes Res*. 1996;4(4):391–396.