ORIGINAL ARTICLE

THE IMPACT OF COMBINED ADMINISTRATION OF SEROTONIN-SPECIFIC REUPTAKE INHIBITORS WITH ATORVASTATIN ON THE LIVER AND KIDNEYS. *IN VITRO* AND *IN VIVO* STUDIES

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Abstract

In the treatment of depression coexisting with cardiovascular disease, serotonin-specific reuptake inhibitors (SSRIs) are the most frequently prescribed in combination with statins used for treating hypercholesterolemia in the primary and secondary prevention of cardiovascular and cerebrovascular incidents. Because of a risk of adverse interactions between above-mentioned drugs, our research was aimed to assess the impact of combined administration of SSRIs (fluoxetine or paroxetine) and atorvastatin on the physiological kidney cell culture and the functions of liver and kidneys in rats. The results of *in vitro* assays suggest an adverse effect of combination of fluoxetine and atorvastatin on cell viability. Conversely, the results of *in vivo* experiments indicate this combination superior safety profile in comparison to atorvastatin coupled with paroxetine. The adverse effect observed may point out the possibility of minor liver and kidney disorders during the long-term treatment with paroxetine and atorvastatin.

Rezumat

În comorbiditatea depresie – boală cardiovasculară, inhibitorii specifici de recaptare ai serotoninei (ISRS) sunt cei mai frecvent prescrişi în combinaţie cu statinele, acestea având rol în prevenţia primară şi secundară a incidenţei accidentelor cardiovasculare. Din cauza riscului interacţiunilor nedorite dintre medicamentele menţionate anterior, cercetarea noastră a urmărit să evalueze impactul administrării combinate a ISRS (fluoxetină sau paroxetină) şi atorvastatină asupra unor linii celulare renale, precum şi asupra funcţiilor hepatice şi renale la şobolani. Rezultatele testelor *in vitro* sugerează un efect negativ asupra viabilităţii celulare a combinaţiei fluoxetină – atorvastatină. Pe de altă parte, rezultatele experimentelor *in vivo* indică un profil de siguranţă superior al asocierii fluoxetină – atorvastatină, în comparaţie cu atorvastatina asociată cu paroxetină.

Keywords: SSRIs, atorvastatin, hepatotoxicity, nephrotoxicity

Introduction

Depression and cardiovascular disease (CVD) are currently two of the most common causes of disability in high-income economies. In addition, depression and the related symptoms are strong predictors of further cardiac adverse events and mortality in patients with ischemic heart disease [18, 20]. Unfortunately, depression in patients with CVD is extremely widespread with a prevalence of 17 - 47%. In these patients, selective serotonin reuptake inhibitors (SSRIs) are given precedence over other drugs, in particular tricyclic antidepressants, which are associated with significantly more frequent and serious cardiac events [1, 13]. Fluoxetine is a first-line SSRI in the treatment of depression with moderate and mild depressive states comorbid with CVD, however paroxetine, as one of the strongest and the most selective serotonin reuptake inhibitor, has also a very high efficiency [19, 22]. In the aetiology of cardio-vascular diseases, lipid disorders have a critical importance. Clinical trials provide the evidence of a relationship between cardiovascular improvement and the degree of reduction in low-density lipoprotein cholesterol (LDL-C) level. Reducing high blood cholesterol is a crucial purpose of pharmacotherapy. Statins are the most efficient lipid-lowering drugs. It is particularly atorvastatin that is usually prescribed for the treatment of hypercholesterolemia and lately confirmed as a neuroprotective agent [13]. Recent research has demonstrated that this statin is able to exert antidepressant-like effect through the serotonergic system modulation in the animal model. In addition, a combinatory antidepressant-like effect was observed when mice were co-administered atorvastatin and fluoxetine or paroxetine [12]. Likewise, in certain groups of patients with moderate to severe depression, statins decreased some depressive symptoms, which may be attributed to their anti-inflammatory and neurotransmitter modulatory potentials. These studies

pointed to simvastatin as an effective adjuvant therapy to fluoxetine for patients suffering from depression [8].
The benefits of statin and SSRIs use for individuals with co-existing depression, anxiety and coronary disease are well established. Alongside the increasingly broader use of these groups of drugs, close attention is being paid to their adverse effects. Both statins and antidepressants pose a risk of numerous drug interactions. SSRIs are associated with many pharmacokinetic drug interactions related to the inhibition of the cytochrome P450 metabolic pathways. The combined fluoxetine and its circulating metabolite norfluoxetine are predicted to reduce *in vivo* CYP3A4 activity by about 60%, and atorvastatin is metabolized by CYP3A4/3A5 [4, 21]. The inhibition of metabolism by SSRIs results in the increased of blood levels of co-administered drugs. In the case of statins, raised blood levels pose the risk of the hepatic enzyme elevation and dose-dependent myopathies ranging from common and troublesome myalgia, cramps and weakness to life-threatening but very rare rhabdomyolysis. A detailed review of the literature suggests that paroxetine is generally safe with all statins [4]. However, co-medication of paroxetine and pravastatin might have a significant and unfavourable impact on kidney function as well as blood anti-coagulation and glucose homeostasis [3]. Furthermore, although fluoxetine is considered generally safe and well-tolerated drug, adverse events are observed at high doses, including organ failure (liver failure, renal failure) in rare cases [23].
Accordingly, our studies have involved *in vitro* and *in vivo* experiments to discover possible interactions of the aforementioned drugs with biological systems. The *in vitro* models used extensively in research and drug development give the opportunity of analysing the cellular response in a closed system with constant experimental conditions. The procedures are easy to follow, less time consuming and less expensive [6, 17]. Our studies included the measurement of the cytotoxic effects of combined application of SSRIs and atorvastatin on green monkey kidney cells (GMK). In addition, the *in vitro* models provide preliminary information on the outcome of an *in vivo* experiment, because still the studies using live organisms (e.g. rodents) are necessary to evaluate drug safety better [6, 17]. Therefore, our research also covered the assessment of the chosen hepatic and renal serum markers in rats after a 28-day combined treatment with SSRIs and atorvastatin. The elevated enzyme activities (e.g. aspartate and alanine aminotransferases as well as γ-glutamyltransferase - GGT) are the common markers of liver disease in experimental animals [7, 9, 14]. The activity levels of the aforementioned enzymes were determined. The concentrations of β_2-microglobulin, creatinine and urea were also assessed as routine kidney function biomarkers.

Materials and Methods

Chemicals and diagnostic kits

The following substances were used in the study: fluoxetine (fluoxetine hydrochloride, Fluoxetine®, EGIS Pharmaceuticals PLC, Budapest, Hungary), paroxetine (paroxetine hydrochloride semihydrate, Xetanor®, Glenmark Pharmaceutical, Prague, Czech Republik), atorvastatin (atorvastatin calcium salt trihydrate, Atorvastatin®, Ranbaxy, Warsaw, Poland), MTT (thiazolyl blue tetrazolium bromide, Sigma-Aldrich, Steinheim, Germany), DMSO (dimethyl sulfoxide, POCH S.A. Gliwice, Poland) and also *aqua pro injectione* (Polfa Lublin, Poland). The cell culture medium RPMI-1640 (with L-glutamine and phenol red), Foetal Bovine Serum (FBS) and antibiotics solutions: penicillin, streptomycin and amphotericin B were obtained from the PAA - The Cell Culture Company GmbH, Pasching, Austria. The ready-made diagnostic kits were used to determine: aspartate (AST) and alanine (ALT) aminotransferases activities, total protein level, creatinine and urea concentrations, being purchased from Cormay Diagnostic S.A. (Lublin, Poland). β_2-microglobulin levels were estimated using an assay kit from IBL (Hamburg, Germany). The Cytotoxicity Detection Kit LDH from Roche Diagnostic GmbH (Mannheim, Germany) was also used. For the determination of aminotransferases activity levels the kinetic optimized, modified method was used, without pyridoxal phosphate according to International Federation of Clinical Chemistry (IFCC). This method is based on two enzymatic reactions catalysed by AST or ALT and MDH (malate dehydrogenase) or LDH (lactate dehydrogenase), respectively. Both dehydrogenases catalyse the oxidative reactions using the reduction of NAD^+ to NADH. The principle of measurement is based on the rate of change of absorbance at λ = 340 nm. GGT activity was also evaluated by kinetic, enzymatic method based on reaction with L-γ-glutamyl-3-carboxy-4-nitroanilide. In the case of GGT rate of change in absorbance was measured at λ = 405 nm. The rate of change in absorbance is directly proportional to the activity levels of the enzymes. The absorbance was measured using an automated absorbance microplate reader EL_x808_{IU} (Bio-Tek Instruments Inc.).

Cell Culture

The research was performed on green monkey kidney cells (GMK) obtained from the "Biomed" Serum and Vaccine Production Plant Ltd in Lublin, Poland. GMK cell line was grown in RPMI-1640 medium (with L-glutamine and phenol red) supplemented with 10% foetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin and 2.5 μg/mL amphotericin

B. GMK cells were cultured as monolayer in CO_2 cell incubator at 37ºC in an atmosphere of 5% CO_2. After multiplication, the cells were peeled away from the bottom of the culture flask with a cell scraper. The resulting cell suspension was added to the RPMI-1640 medium and used for further investigations. Afterwards, GMK cells were counted in Neubauer hemocytometer (BlauBrand, BRAND GmbH, Wertheim, Germany) by means of the compact inverted microscope Olympus CKX41. The suspension of cells was prepared at a density of $1x10^6$ cells/mL and then transferred to 96-well cell culture plates (SPL Lifescience, Gyeonggi-do, Korea) to assay the cytotoxicity.

MTT and LDH Assay

For assaying the of cell viability, MTT test based on DB-ALM Protocol n°17 (ECVAM - European Centre for the Validation of Alternative Methods) was used. The cells viability was determined in a mitochondrial-dependent reaction (reduction in mitochondrial dehydrogenase activity), measured formazan production from MTT salt and is expressed as % of control cells. The experiments began by designation of IC_{10} and IC_{50} values for fluoxetine, and paroxetine. The IC_{10} and IC_{50} values for atorvastatin were determined in a previous research [11]. To determine the effects on cells viability drugs and their combinations were added to GMK cells in the same volume (100 µL/well) and incubated for 24 h. Atorvastatin first was dissolved in DMSO and diluted to the required concentration with RPMI-1640 medium. The solutions of fluoxetine and paroxetine were *ex tempore* prepared in culture medium. In our research fluoxetine at the concentration of 86 µM, paroxetine 2 µM and atorvastatin 126 µM, as the initial concentrations were used for the evaluation of drug-drug interactions. These initial concentrations are about IC_{10} - the previously determined inhibitory concentration of 10% the GMK cells viability. The combinations of fluoxetine or paroxetine with atorvastatin were used in the following proportions of concentrations: IC_{10} - IC_{10} i.e. fluoxetine (86 µM) or paroxetine (2 µM) with atorvastatin (126 µM); IC_{50} - IC_{50} i.e. fluoxetine (122 µM) or paroxetine (15 µM) with atorvastatin (266 µM); IC_{10} - IC_{50} i.e. fluoxetine (86 µM) or paroxetine (2 µM) with atorvastatin (266 µM); IC_{50} - IC_{10} i.e. fluoxetine (122 µM) or paroxetine (15 µM) with atorvastatin (126 µM). After the incubation 10 µL MTT solution (5 mg/mL) was added to each well microplate and was incubated for 3 h at 37°C. At the end of the incubation the culture medium was removed carefully from each well and 100 µL DMSO was added. The absorbance of each well was measured at 550 nm using an automated absorbance microplate reader EL_x808_{IU}.

LDH activity was determined by the enzymatic test. The cytotoxicity detection kit (LDH) is a colorimetric assay for the quantification of cytotoxicity/cytolysis based on the measurement of LDH activity released from the damaged cells. The drugs were added to GMK cells and incubated for 24 h at the same concentrations as in the MTT assay. Cytotoxicity of single drugs and their combinations was calculated with a formula provided in the manufacturer's instructions and was expressed in %.

All the experiments were performed at least five times.

Experimental animals

The study was conducted on male Wistar rats (weighing initially 200 - 275 g) obtained from the licensed breeder (Breeding of Laboratory Animals, Zbigniew Lipiec, Brwinów, Poland). The animals were kept at room temperature (20 ± 1ºC) under 12 h light/dark cycles in constant environmental conditions (relative humidity between 40 and 70%). They had access to food and water *ad libitum*. The experiment was approved by the Local Ethics Committee on Animal Experimentation of the Medical University of Lublin, Poland.

Study design

The experimental groups consisted of eight animals each. The animals received intraperitoneally fluoxetine (10 mg/kg bw), atorvastatin (10 mg/kg bw) and paroxetine (10 mg/kg bw), separately or in combination, once a day for 28 days. The doses were selected based on those reported in the literature which shows that they are efficient [2, 4, 5, 9, 10, 16, 23]. Aqueous solutions of drugs were prepared *ex tempore* and administered intraperitoneally (*ip*) once daily for 28 days separated or combined in constant volumes 0.5 mL/100 g of body weight (bw). The control group received the same amounts of *aqua pro injectione*. The rodents were decapitated 24 hours after the last injection.

Serum tests

The blood from each animal was collected into a clean centrifuge tube and allowed to clot at room temperature. The serum was separated from the clot by centrifugation for 10 minutes at 3000 rpm. In fresh serum the activities of the following enzymes: aspartate aminotransferase (AST), alanine amino-transferase (ALT) and γ-glutamyl transferase (GGT) were determined. The other part of serum was transferred into a clean tube and stored at -20ºC until performing the other biochemical analyses. The concentrations of urea, creatinine and β_2-microglobulin were assayed using this serum.

Data analysis

Results were expressed as mean ± SEM. Groups of single drugs were compared to the control group and one-way analysis of variance (ANOVA) was used (followed by Dunnett test). Double-drug groups were compared with control and groups of single drug and two-way ANOVA with Tukey's *post hoc* test was used to determine statistical significance.

The *p*-value less than 0.05 were chosen as the criterion of statistical significance.

Results and Discussion

In vitro study

The research began with *in vitro* experiment because of methodology which can be routinely used for a preliminary screening of drugs' potential toxicity [6]. The cell viability was estimated by the MTT and LDH assays. The MTT assay allows the measurement of the activity of mitochondria energy conversion and can be used to determine the viability of metabolically active cells. The LDH assay is used to assess the degree of toxicity of a substance on cells in culture.

The IC_{10} and IC_{50} values for fluoxetine, paroxetine and atorvastatin were determined based on dose-response curves (Table I). All the mentioned parameters were evaluated in the presence of atorvastatin solvent (control GMK cells + solvent), and there were no significant differences between the control and the solvent-treated cells. After 24 hours incubation of GMK cells with the combination of fluoxetine (86 µM) and atorvastatin (126 µM), a significant increase of cytotoxic effect by 56.65% was found. Increasing the dose of atorvastatin to 266 µM also increased the cytotoxicity of the combination by 95%.

Table I

IC_{10} and IC_{50} values for fluoxetine, paroxetine and atorvastatin (MTT assay)

Drug	IC_{10} (µM)	IC_{50} (µM)
Fluoxetine	86.03	122.15
Paroxetine	2.07	15.76
Atorvastatin	126.69	266.58

The highest increase of cytotoxicity was noted after the simultaneous incubation of GMK cells with fluoxetine (122 µM) and atorvastatin (126 or 266 µM) (Figure 1). Similar results were obtained with the LDH assay (Figure 2). In turn, the cytotoxicity of the combination of paroxetine (2 µM) and atorvastatin (126 µM) was increased to 25.17% as compared with the results of the groups of GMK cells incubated with each drug separately in the MTT assay (Figure 1).

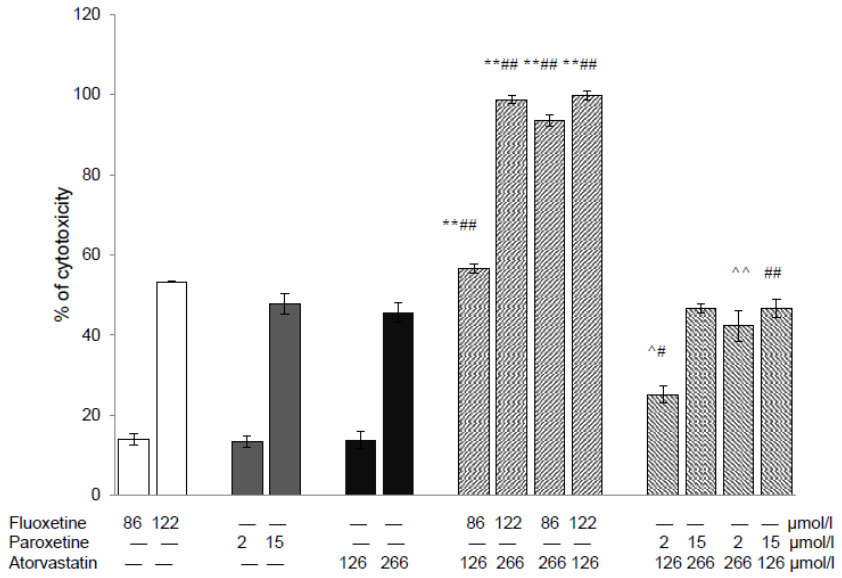

Figure 1.

Cytotoxicity of fluoxetine, paroxetine, atorvastatin and their combination after 24 h incubation with GMK cells in the MTT test

Data are presented as percentage of cytotoxicity ± SEM. Significance: $^{*}p < 0.05$; $^{**}p < 0.001$. Data was analysed by one-way ANOVA followed by Dunnett test (groups 1 - 6 *vs*. control) and two-way ANOVA with Tukey's *post hoc* test (group 7 *vs.* 1, 5; group 8 *vs.* 2, 6; group 9 *vs.* 1, 6; group 10 *vs.* 2, 5; group 11 *vs.* 3, 5; group 12 *vs.* 4, 6; group 13 *vs.* 3, 6; group 14 *vs.* 4, 5); ^ # p < 0.05, ** ^^ ## p < 0.001; * comp. with fluoxetine ^, comp. with paroxetine, # comp. with atorvastatin.

After incubation of GMK cells with paroxetine (2 or 15 µM) and atorvastatin (126 µM), no significant changes indicative of their increased cytotoxic action were reported in the LDH assay (Figure 2). However, the cytotoxicity of the combination of paroxetine (2 or 15 µM) with atorvastatin (266 µM) was enhanced by about 40%.

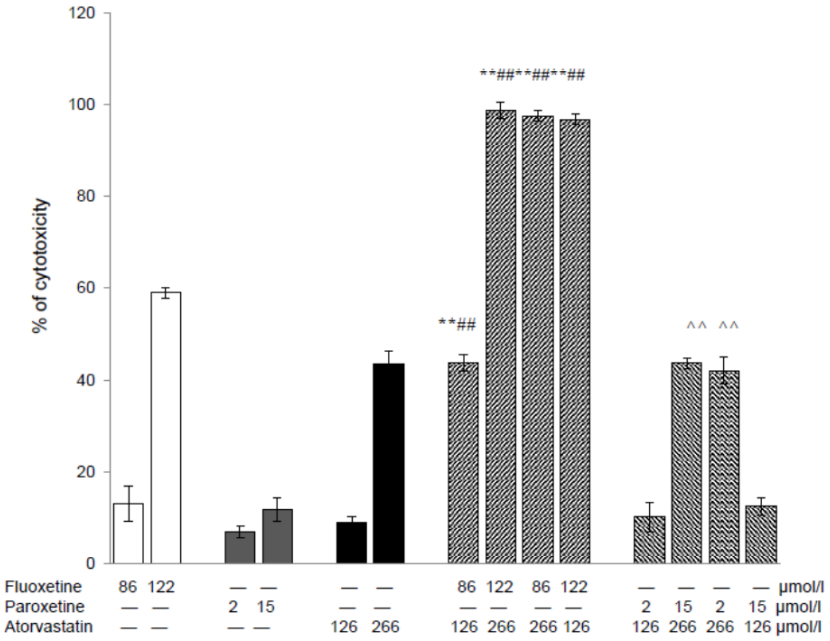

Figure 2.

Cytotoxicity of fluoxetine, paroxetine, atorvastatin and their combination after 24 h incubation with GMK cells in the LDH test

Data are presented as percentage of cytotoxicity ± SEM. Significance: $^{*}p < 0.05$; $^{**}p < 0.001$. Data was analysed by one-way ANOVA followed by Dunnett test (groups 1-6 *vs*. control) and two-way ANOVA with Tukey's *post hoc* test (group 7 *vs.* 1, 5; group 8 *vs.* 2, 6; group 9 *vs.* 1, 6; group 10 *vs.* 2, 5; group 11 *vs.* 3, 5; group 12 *vs.* 4, 6; group 13 *vs.* 3, 6; group 14 *vs.* 4, 5); ** ^^ ## p < 0.001; * comp. with fluoxetine, ^ comp. with paroxetine, # comp. with atorvastatin.

The results suggest a negative influence of the combined application of fluoxetine and atorvastatin on GMK cell viability, and these changes cannot be ignored. Additionally, the results of both the MTT and LDH assays are very similar. Particularly noteworthy is the significant (approximately 55%) increase of the cytotoxic effect of combination of both drugs used in the ratio of IC_{10} - IC_{10}. This may suggest an adverse drug-drug interaction (hyper-additive synergy). The influence of the couple paroxetine - atorvastatin on the GMK cell viability is not so clear. The MTT assay indicated that the combination of both drugs used in the ratio of IC_{10} - IC_{10} led to the enhancement of cytotoxicity, which may suggest an adverse interaction between both drugs. However, this hypothesis was not confirmed by the LDH assay results. The increase of cytotoxicity in the groups of GMK cells subjected to the combined action of paroxetine with atorvastatin in other used concentrations does not result from the combination of drugs. This significant increase of cytotoxicity was caused by the action of a higher concentration (about IC_{50}) of paroxetine or atorvastatin. Based on the obtained results, it should be noted that the MTT test was more sensitive for the examination of the cytotoxic effect of paroxetine (15 µM) and the combinations of paroxetine with atorvastatin. The absence of clear results in the LDH assay may be due to the differences in the evaluation of cytotoxicity in both assays. In the LDH assay, the activity of lactate dehydrogenase, an enzyme released from injured cells, was measured. It may be assumed that the combination of paroxetine with atorvastatin did not cause a damage to the cell membrane, so the increased release of the enzyme was not registered.

In vivo study

The *in vivo* experiment focused on the analysis of selected indicators of the liver and kidney function, these organs being particularly vulnerable to the adverse effect of drug interactions. The results showed that the administration of either SSRIs or atorvastatin alone for 28 days did not significantly influence the activity levels of GGT, ALT and AST (Figure 3 and Table II). However, the combination of atorvastatin and paroxetine administered to rats caused the increase of the GGT activity in comparison with the group of animals receiving atorvastatin alone. The enhancement of this parameter was also significant when compared to the control group (Figure 3).

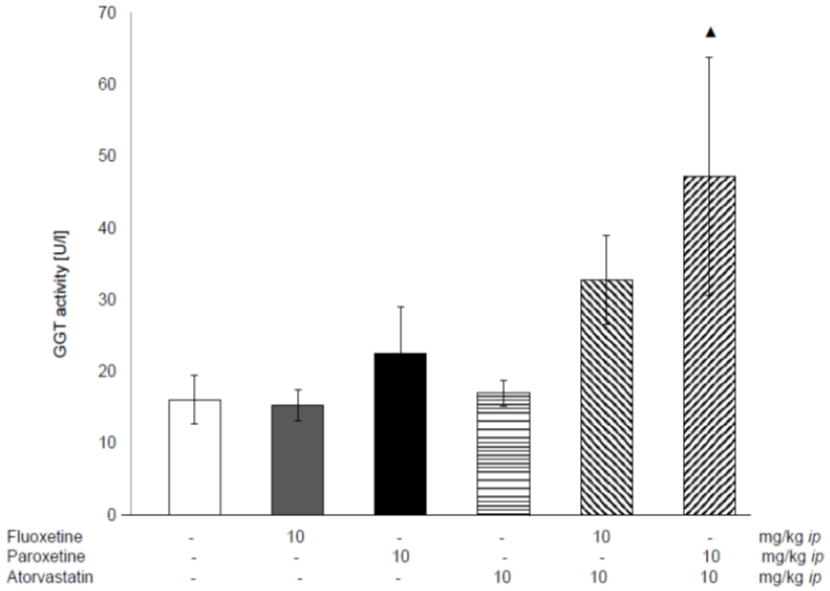

Figure 3.

Effect of 28 days administration of fluoxetine, paroxetine, atorvastatin and their combination on the activity of GGT in the serum of rats

Data are presented as mean ± SEM. Significance: * p < 0.05. Data was analysed by one-way ANOVA followed by Dunnett test (groups 2 - 4 *vs*. control) and two-way ANOVA with Tukey's post hoc test (group 5 *vs*. 2, 4; group 6 *vs*. 3, 4); ▲ – p < 0.05; ▲ - compared with atorvastatin.

In animals treated with atorvastatin and selected SSRIs, the increases of ALT and AST activity levels were also observed but with no statistical significance due to the high SEM values (Table II). The results also showed that the four-week combined administration of atorvastatin with paroxetine caused the increasing of the concentration of urea, which was not the case in the group of rats receiving only paroxetine. The aforementioned marker of the renal function was also significantly enhanced compared with the control group (Figure 4).

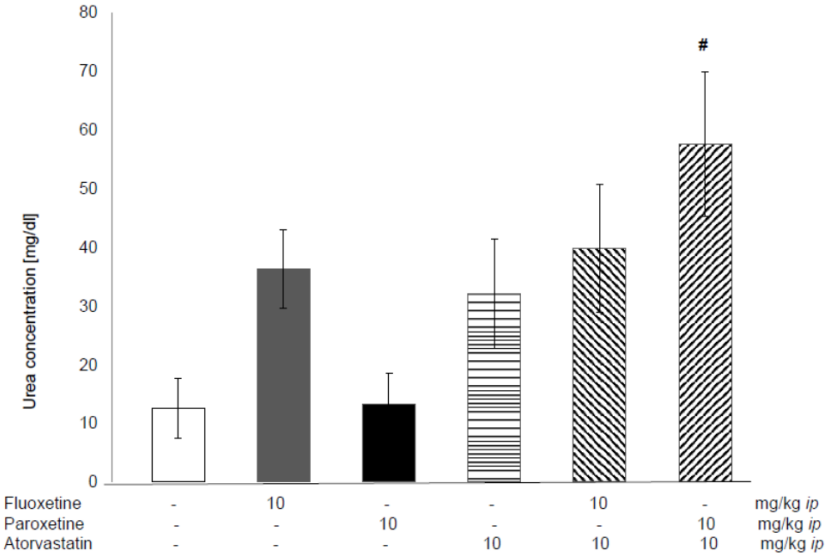

Figure 4.

Effect of 28 days administration of fluoxetine, paroxetine, atorvastatin and their combination on the concentration of urea in the serum of rats

Data are presented as mean ± SEM. Significance: $^{*}p < 0.05$. Data was analysed by one-way ANOVA followed by Dunnett test (groups 2-4 *vs*. control) and two-way ANOVA with Tukey's *post hoc* test (group 5 *vs.* 2, 4; group 6 *vs*. 3, 4); # - p < 0.05, # - compared with paroxetine.

Our experiment indicated (Table II) that the exposure to atorvastatin and both SSRIs, alone and in combinations, for 28 days did not significantly influence the concentrations of creatinine and β_2-microglobuline.

Table II

The assessment of the biochemical parameters in the serum of rats treated with atorvastatin and SSRIs

Applied (mg/kg bw)	AST (U/L) $\bar{x}$ ± SEM	ALT (U/L) $\bar{x}$ ± SEM	Creatinine (mg/dL) $\bar{x}$ ± SEM	β2 - microglobulin (mg/L) $\bar{x}$ ± SEM
Aqua pro injectione	205.45 ± 9.54	79.15 ± 2.78	0.19 ± 0.05	0.58 ± 0.09
Atorvastatin 10	184.49 ± 9.25	74.21 ± 4.20	0.44 ± 0.02	0.71 ± 0.17
Fluoxetine 10	222.03 ± 13.81	80.90 ± 10.51	0.25 ± 0.05	0.55 ± 0.1
Paroxetine 10	261.90 ± 22.09	114.94 ± 19.89	0.30 ± 0.09	0.78 ± 0.23
Atorvastatin 10 + Fluoxetine 10	263.94 ± 30.12	189.44 ± 73.01	0.34 ± 0.01	0.66 ± 0.09
Atorvastatin 10 + Paroxetine 10	297.69 ± 61.64	240.07 ± 105.34	0.23 ± 0.06	0.53 ± 0.16

The animal model studies showed that the 28-days combined administration of SSRIs and atorvastatin did not result in any essential changes to the basic biochemical parameters indicating the renal and also liver functioning. Based on the obtained results, it was generally found that the combined administration of fluoxetine with atorvastatin adversely affected neither the functioning of liver nor kidneys in rats. In turn, the increase of the GGT activity in the group of rats receiving paroxetine with atorvastatin may suggest the possibility of minor liver function disorder during the long-term treatment with these drugs. Additionally, the enhancement of urea concentration in the same experimental group could possibly indicate renal function impairments. However, further and more detailed studies are needed to prove this hypothesis, e.g. histopathological analyses of the aforementioned internal organs. It must be noted that the GGT activity is considered an early and one of the most sensitive serum markers employed in the diagnosis of liver functioning impairment [7, 9, 14]. ALT and AST are fundamental indicators of hepatocyte integrity loss, so some significant changes may occur at a later stage of liver impairment [15]. In turn, increased blood urea is generally associated with the impaired filtration process. However, creatinine concentration as basic parameter used in the assessment of glomerular filtration, is considered the most important indicator of renal function. Therefore, the above-mentioned increase in the concentration of urea with no concomitant changes in the concentration of creatinine indicates rather an alteration due to drugs used in therapy than the impaired kidney filtering ability. Nonetheless, the presented observations require further long-term animal model studies and prospective clinical analyses that would specifically focus on the mechanism underlying the adverse effects of such a treatment on the internal organs discussed above.

Conclusions

In conclusion, the *in vitro* study demonstrated that the combination of SSRI and atorvastatin had a very adverse effect on cell viability, particularly fluoxetine coupled with atorvastatin. Based on the results obtained in the *in vivo* experiment, it was found that the combined administration of fluoxetine with atorvastatin adversely affected neither the functioning of liver, nor kidneys in rats. In turn, paroxetine coupled with atorvastatin were less desirable for liver and kidney welfare. Nevertheless, the changes observed in the tested parameters were not generally disturbing and more studies are needed. Drug-drug interactions which are a common problem causing serious adverse events should be extensively studied. *In vitro* research on this issue is required during drug development, but is also important in the simultaneous use of many drugs from different classes in clinical practice. In the presented study, it was shown that the *in vitro* method using kidney cell culture for drug testing provides an alternative means, but only to a certain level. The correspondence between the results achieved in the GMK cell culture and the *in vivo* biochemical studies appeared to be elusive. The *in vitro* models do obvious not have regard to the complex compensation and defence mechanisms of a living organism. In view of the species difference, the GMK cells, while extremely well characterized, their responses to cytotoxic stimuli might not be the same with a rat kidney cell line. The *in vitro* techniques have some limitations, therefore the research on drug safety involving animals and specific scientific procedures are still necessary.

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References

1. Almeida J., Duarte J.O., Oliveira L.A., Crestani C.C., Effects of nitric oxide synthesis inhibitor or fluoxetine treatment on depression-like state and cardiovascular changes induced by chronic variable stress in rats. *Stress*, 2015; 11(4): 1-13.
2. Amodeo L.R., Greenfield V.Y., Humphrey D.E., Varela V., Pipkin J.A., Eaton S.E., Johnson J.D., Plant C.P., Harmony Z.R., Wang L., Crawford C.A., Effects of acute or repeated paroxetine and fluoxetine treatment on affective behavior in male

and female adolescent rats. *Psychopharmacology (Berl)*, 2015; 232(19): 3515-3528.
3. An L., Ravindran P.P., Renukunta S., Denduluri S., Co-medication of pravastatin and paroxetine-a categorical study. *J. Clin. Pharmacol.*, 2013; 53(11): 1212-1219.
4. Andrade C., Selective serotonin reuptake inhibitor drug interactions in patients receiving statins. *J. Clin. Psychiatry*, 2014; 75(2): e95-e99.
5. Crespo M.J., Quidgley J., Simvastatin, atorvastatin and pravastatin equally improve the hemodynamic status of diabetic rats. *World J. Diabetes*, 2015; 6: 1168-1178.
6. Doke S.K., Dhawale S.C., Alternatives to animal testing: A review. *Saudi Pharm. J.*, 2015; 23(3): 223-229.
7. Flehi-Slim I., Chargui I., Boughattas S., El Mabrouk A., Belaïd-Nouira Y., Neffati F., Najjar M.F., Haouas Z., Ben Cheikh H., Malathion-induced hepatotoxicity in male Wistar rats: biochemical and histopathological studies. *Environ. Sci. Pollut. Res. Int.*, 2015; 22(22): 17828-17838.
8. Gougol A., Zareh-Mohammadi N., Raheb S., Farokhnia M., Salimi S., Iranpour N., Yekehtaz H., Akhondzadeh S., Simvastatin as an adjuvant therapy to fluoxetine in patients with moderate to severe major depression: A double-blind placebo-controlled trial. *J. Psychopharmacol.*, 2015; 29(5): 575-581.
9. Herbet M., Gawrońska-Grzywacz M., Izdebska M., Piątkowska-Chmiel I., Jagiełło-Wójtowicz E., Impact of combined treatment with rosuvastatin and anti-depressants on liver and kidney function in rats. *Exp. Ther. Med.*, 2016; 11(4): 1459-1464.
10. Herbet M., Izdebska M., Piątkowska-Chmiel I., Poleszak E., Jagiełło-Wójtowicz E., Estimation of oxidative stress parameters in rats after simultaneous administration of rosuvastatin with antidepressants. *Pharmacol. Rep.*, 2016; 68(1): 172-176.
11. Izdebska M., Piątkowska-Chmiel I., Herbet M., Gawrońska-Grzywacz M., Natorska-Chomicka D., Evaluation of the cytotoxic effects of combined application of methotrexate and statins on green monkey kidney cells. *Int. J. Clin. Pharmacol. Toxicol.*, 2015; 4: 185-191.
12. Ludka F.K., Constantino L.C., Kuminek G., Binder L.B., Zomkowski A.D., Cunha M.P., Dal-Cim T., Rodrigues A.L., Tasca C.I., Atorvastatin evokes a serotonergic system-dependent antidepressant-like effect in mice. *Pharmacol. Biochem. Behav.*, 2014; 122: 253-260.
13. Mavrides N., Nemeroff C., Treatment of depression in cardiovascular disease. *Depress. Anxiety*, 2013; 30(4): 328-341.
14. Mohamed A.S., Soliman A.M., Marie M.A., Mechanisms of echinochrome potency in modulating diabetic complications in liver. *Life Sci.*, 2016; 151: 41-49.
15. Mohammed N.E.M., Messiha B.A.S., Abo-Saif A.A., Effect of amlodipine, lisinopril and allopurinol on acetaminophen-induced hepatotoxicity in rats. *Saudi Pharm. J.*, 2015 (in press).
16. Nadeem R.I., Ahmed H.I., El-Denshary E.E., Effect of Imipramine, Paroxetine, and Lithium Carbonate on Neurobehavioral Changes of Streptozotocin in Rats: Impact on Glycogen Synthase Kinase-3 and Blood Glucose Level. *Neurochem. Res.*, 2015; 40(9): 1810-1818.
17. Călina D., Roșu L., Roșu A.F., Ianoşi G., Ianoşi S., Zlatian O., Mitruț R., Docea A.O., Rogoveanu O., Mitruț P., Nicolae A.C., Drăgoi C.M., Gofiță E., Etiological diagnosis and pharmacotherapeutic management of parapneumonic pleurisy. *Farmacia*, 2016; 64(6): 946-952.
18. Newcomer J.W., Hennekens C.H., Severe mental illness and risk of cardiovascular disease. *JAMA*, 2007; 298(15): 1794-1796.
19. Paraskevaidis I., Palios J., Parissis J., Filippatos G., Anastasiou-Nana M., Treating depression in coronary artery disease and chronic heart failure: what's new in using selective serotonin re-uptake inhibitors?. *Cardiovasc. Hematol. Agents. Med. Chem.*, 2012; 10(2): 109-115.
20. Roest A.M., Martens E.J., de Jonge P., Denollet J., Anxiety and risk of incident coronary heart disease: a meta-analysis. *J. Am. Coll. Cardiol.*, 2010; 56(1): 38-46.
21. Sager J.E., Lutz J.D., Foti R.S., Davis C., Kunze K.L., 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.
22. Sanchez C., Reines E.H., Montgomery S.A., A comparative review of escitalopram, paroxetine, and sertraline: Are they all alike?. *Int. Clin. Psychopharm.*, 2014; 29(4): 185-196.
23. Yılmaz A., Elbey B., Yazgan Ü.C., Dönder A., Arslan N., Arslan S., Alabalık U., Aslanhan H., Protective Effects of Caffeic Acid Phenethyl Ester on Fluoxetine-Induced Hepatotoxicity: An Experimental Study. *Biomed. Res. Int.*, 2016; 2016: 1247191.