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The influence of combined treatment of simvastatin and amitriptyline on some biochemical parameters in rat serum

Wpływ łącznego podawania simwastatyny i amitryptyliny na wybrane parametry biochemiczne w surowicy krwi szczurów

INTRODUCTION

From the scientific data it is known that statins (HMG-CoA -enzyme reductase inhibitors) are one of the most frequently used drugs in the world [26]. These drugs are effective in the treatment of hyperlipidemias and coronary heart disease and they are very widely prescribed because of their pleiotropic effects [18, 23, 24]. In general statin monotherapy the drug is well-tolerated and has a low frequency of adverse events. The most significant side-effect is myopathy, which can progress to rhabdomyolysis, the asymptomatic increase in hepatic aminotransferases and to the disorder of kidney functioning [16, 21, 22]. During the long-term therapy statins are used very often simultaneously with other medicaments which can cause possible interactions [1].

However, patients who are frequently treated by statins suffer from depression because in recent years there has been a notified growth of this disease. The traditional treatment of depression includes tricyclic antidepressants – drugs which are known to possess a lot of adverse events (e.g. liver dysfunction, urine retention) and interactions with other drugs [15].

There is some information of simultaneous usage of statins and antidepressants but in bibliography there is not sufficient data of a simultaneous usage of simvastatin (one of the most frequently used statins) and amitriptyline (most used as tricyclic – antidepressant agent) [10, 20]. Therefore, it seems crucial to conduct research to find out to what extend the simultaneous usage of the above drugs can influence some biochemical parameters in the rat serum.

MATERIALS AND METHODS

D r u g s a n d c h e m i c a l s . The following substances were used in the study: amitriptyline (Sigma-Aldrich GmbH, Germany), simvastatin (Simvacard, Zentiva), aqua pro injectione (Polfa,

Lublin, Poland) and commercial kits: alanine (ALT) and aspartate (AST) aminotransferases – Liquick Cor-ALT-60 or Liquick Cor-AST-60, total protein - Liquick Cor-TOTAL PROTEIN 120, creatinine– Liquick Cor-CREATININE 60, urea– Liquick Cor-UREA 120 – (all from CORMAY, Lublin, Poland), α-fetoprotein – AFP-ELISA – DIMA GmbH (Goettingen, Germany) and β_2-microglobulin (β_2-M) – Beta-2-Microglobulin ELISA (Immundiagnostic AG, Bensheim, Germany).

Animals. The study was carried out on male Wistar rats weighing initially 195–275 g (purchased from breeding farm of Brwinów, Poland). The animals were kept under standard laboratory conditions and maintained on a 12 h day/ 12 h night cycle with free access to food and water. Each experimental group consisted of eight animals. The studies were approved by the Ethical Committee on Animal Experimentation of the Medical University of Lublin.

Treatment. Simvastatin – SIM (1 or 10 mg/kg as suspensions in a 1% Tween 80) and amitriptyline – AMI (10 mg/kg) as aqueous solutions were prepared *ex tempore* and administered intraperitoneally (i.p.) to rats once daily for 14 days separated or combined in the constant volume of 5 ml/kg. The control animals received identical volume of the solvent. The drugs studied were applied to rats in an effective doses [13,17].

Experimental protocols. The rats were decapitated 24 h after the last injection and blood from each animal was taken. The blood was allowed to clot and the serum fraction was separated and subsequently stored at -20 °C until biochemical assays were performed.

Statistical analysis. Statistical significance among the groups was determined by Student's *t*-test and *p*-values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

From the literature data it is clear that the probability of liver functioning disorder while using statins is slight [4, 21]. The rare side effect of statin therapy is increased activity of aminotransferases in the serum but patients treated with statins do not have an increased risk of progression of liver functioning [14]. Literature data supply some information on the growth of aminotransferases activity to be reversible and be dependent on the dosage – the highest doses of statins generally have higher rates of these enzymes [3, 5, 9]. In addition, a lot of studies show the effectiveness of using these drugs in such illnesses as chronic hepatitis C, nonalcoholic steatohepatitis, infection and hepatocellular carcinoma [6, 8, 19].

The results showed that 14-day treatment with simvastatin only in a dosage of 1 mg/kg or amitriptyline (10 mg/kg) caused the decrease of activity of AST in comparison to the control group (Fig.1). However, the combined 14-day treatment with simvastatin only in a dosage of 10 mg/kg and amitriptyline (10 mg/kg) significantly increased the activity of AST in comparison to simvastatin itself as well as to amitriptyline.

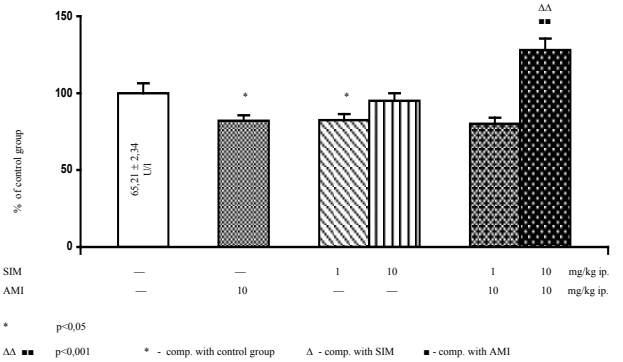

Fig 1. Effects of 14-days combined treatment with SIM and AMI on the activity of AST in the serum of rats

A significant increase in the activity of ALT was noted in the rat serum pre-treated with simvastatin only in a dose of 10 mg/kg or amitriptyline (10 mg/kg) in comparison to the control group (Fig. 2). The increased activity of ALT can imply the damage of liver cells. Clinical observations also showed that the risk of the functioning disorder of that organ applies to the patients who were recorded three times beyond the activity of aminotransferases [2]. A combined 14-day treatment with both doses of simvastatin and amitriptyline (10 mg/kg) had no influence on the activity of ALT.

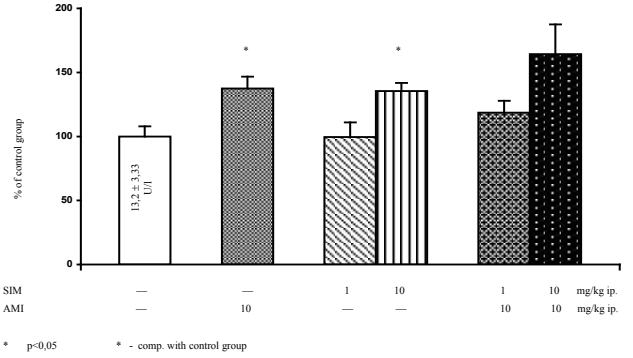

Fig 2. Effects of 14-days combined treatment with SIM and AMI on the activity of ALT in the serum of rats

A significant increase was observed of the concentration of AFP in the rat serum pre-treated with a 14–day action of amitriptyline (10 mg/kg) in comparison to the control group (Fig. 3). Simvastatin (1 or 10 mg/kg) did not change with the concentration of AFP in comparison to the control group. However, 14-day combined treatment with simvastatin (1 or 10 mg/kg) and amitriptyline (10 mg/kg) significantly decreased the concentration of AFP in the rats serum in comparison to the amitriptyline only.

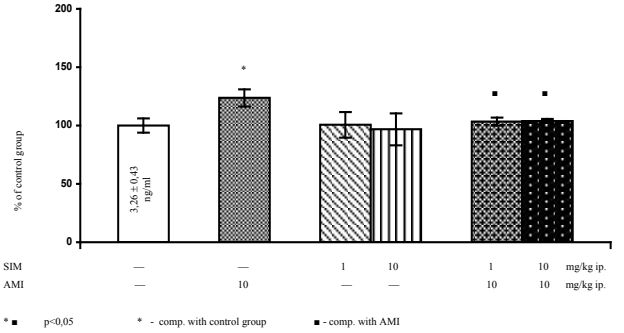

Fig 3. Effects of 14-days combined treatment with SIM and AMI on the activity of AFP in the serum of rats

In the blood serum of rats pre-treated with investigation the concentration of total protein, which can indicate the liver functioning, was determined. It was found that simvastatin only in a dosage of 10 mg/kg or amitriptyline (10 mg/kg) administered for 14 days caused a significant decrease of the concentration of total protein in comparison to the control group (Fig. 4). After the combined treatment with simvastatin only in a dosage of 1 mg/kg and amitriptyline (10 mg/kg), decrease of total protein in comparison to simvastatin was observed. However, a 14-day combined treatment with simvastatin (10 mg/kg) and amitriptyline (10 mg/kg) did not show any changes in the concentration of total protein.

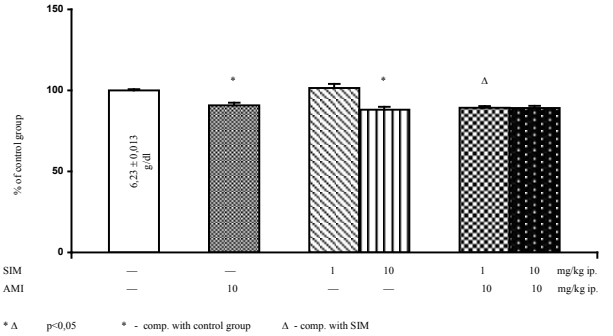

Fig 4. Effects of 14-days combined treatment with SIM and AMI on the concetration of total protein in the serum of rats

The common side effects of amitriptyline therapy are alimentary disorders. However, toxic liver injury is rare [26]. Literature data give information that amitriptyline therapy is associated with very rare idiosyncratic hepatotoxicity [25]. Urinary tract disorders are seldom notified, especially in men with benign prostatic hyperplasia [26].

From the literature data it is obvious that statin therapy does not retard the kidney functioning [8, 10]. These drugs sometimes are used in chronic kidney disease [12].

Our research shows that significant increase of the concentration of urea in comparison to the control group was observed only in the group of rats getting simvastatin (10 mg/kg). Simvastatin (1 or 10 mg/kg) treatment for 14 days in combination with amitriptyline (10 mg/kg) did not have any influence on the concentration of urea in the comparison to simvastatin and amitriptyline only (Table 1).

Table 1. Cumulative summary of evaluated means (x±SEM) of biochemical parameters indicating liver and kidney function in rats serum after 14-day combined treatment with simvastatin (SIM) and amitriptyline (AMI)

Treatment i.p. (mg/kg m.c.)	Urea (mg/dl) x±SEM	Creatinine (mg/dl) x±SEM	B_2-M (mg/l) x±SEM
-	42.24 ± 1.28	0.56 ± 0.03	0.205 ± 0.005
SIM 1	49.31 ± 2.65	0.64 ± 0.01 *	0.192 ± 0.003
SIM 10	55.35 ± 1.99 *	0.56 ± 0.05	0.160 ± 0.004 **
AMI 10	47.34 ± 3.12	0.52 ± 0.02	0.118 ± 0.004 **
SIM 1 + AMI 10	55.77 ± 4.00	0.52 ± 0.02 Δ	0.175 ± 0.006 Δ ■■
SIM 10 + AMI 10	61.61 ± 8.66	0.51 ± 0.04	0.118 ± 0.001 Δ

* Δ ■ $p<0.05$; ** ■■ $p<0.001$; * comp. with control group; Δ comp. with SIM; ■ comp. with AMI

It was found that simvastatin (1 mg/kg) caused the significant increase of the concentration of creatinine in comparison to the control group (Table 1) However, 14-day combined treatment with simvastatin (1 mg/kg) and amitriptyline (10 mg/kg) caused a significant decrease of the concentration of creatinine in comparison to simvastatin only.

The decrease of concentration of β_2-microglobuline was observed after 14 days of application with simvastatin only (10 mg/kg) or amitriptyline only (10 m/kg) in comparison to the control group. Simvastatin (1 or 10 mg/kg) given simultaneously with amitriptyline for 14 days significantly decreased the concentration of β_2-microglobuline in the serum, in comparison to simvastatin. Nevertheless, it increased in comparison to amitriptyline (only simvastatin in a dosage of 1 mg/kg with amitriptyline) (Table 1).

The present study indicates that a combined 14-day treatment with simvastatin and amitriptyline can cause a few changes in the biochemical parameters indicating liver and kidney functioning in rats. The observed changes cannot be ignored because both applied drugs used in this research are biotransformed in the liver primarily by cytochrome P450 [7, 16].

It seems that the combined treatment with simvastatin and amitriptyline is possible with the systematic examination of the above parameters which is very important.

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SUMMARY

The aim of this study was to estimate a 14-day simultaneous usage of simvastatin and amitriptyline on the some biochemical parameters indicating liver and kidney functions in rats. The estimation of the activity of AST, ALT and the concentration of total protein, creatinine, urea, α-fetoprotein and β_2-microglobuline was conducted. The results show that after 14 days of combined application with simvastatin and amitriptyline some changes were observed in the biochemical parameters examined. It seems that the combined treatment with simvastatin and amitriptyline is possible on condition of a regular control of parameters indicating liver and kidney functions.

Keywords: simvastatin, amitriptyline, biochemical parameters

STRESZCZENIE

W pracy oceniano wpływ łącznego 14-dniowego i.p. podawania szczurom simwastatyny i amitryptyliny na wybrane parametry biochemiczne w surowicy krwi, świadczące o funkcji wątroby i nerek szczurów. Oznaczano aktywność AST i ALT oraz stężenie AFP, białka całkowitego, mocznika, kreatyniny i β_2-mikroglobuliny. Na podstawie otrzymanych wyników stwierdzono, że 14-dniowe podawanie szczurom simwastatyny w kombinacji z amitryptyliną powoduje zmiany w oznaczanych parametrach biochemicznych. Wydaje się, że możliwa jest długotrwała łączna terapia simwastatyną i amitryptyliną, ale pod warunkiem systematycznej kontroli parametrów świadczących o funkcji wątroby i nerek.

Słowa kluczowe: simwastatyna, amitryptylina, parametry biochemiczne