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Evaluation of selected biochemical parameters in the serum of rats pretreated with simvastatin, doxepin or their combination

Ocena wybranych parametrów biochemicznych surowicy krwi szczurów poddanych działaniu simwastatyny, doksepiny lub ich kombinacji

INTRODUCTION

In the last years in many countries, including Poland, the cardiovascular diseases (the coronary disease, heart disease, arterial hypertension, stroke) are the most frequent cause of deceases. Mainly the lipid disorders, especially high cholesterol level, are responsible for development of these diseases. Statins are the most effective and the most frequently used lipid-lowering drugs. These medicines are generally well tolerated, however myopathy and asymptomatic increase in hepatic transaminases are the most important adverse effects associated with statins. Adverse effects are noted in approximately 3% of patients [8,11,18,21]. Because of the discovered pleiotropic effects of statins, their use has expanded to the treatment of many other conditions. For this reason statins are the most commonly used drugs in the world. Therefore, they are often taken by patients suffering from other illnesses, including depression. Depression is a psychiatric disorder that is reported to affect 5-18 % of the population at some stage during their lives. Although a wide range of antidepressants is now available, tricyclic antidepressants (TCAs) are still regarded as the first-line standard therapy for severe major depression [19, 23]. The side-effects (urine retention, tachycardia, sedation, liver dysfunction) and interactions with other drugs are major disadvantages of TCAs [20]. Because in bibliography there is no sufficient data on the simultaneous usage of simvastatin (one of the most frequently used statins) and doxepin (most used as tricyclic antidepressant agent), our study was aimed to evaluate whether, and in what degree both drugs affect the serum biochemical parameters indicating liver function in rats.

MATERIALS AND METHODS

Drugs and chemicals. In this study the following substances and commercial test kits were used: simvastatin (SIM) (Vastan, ICN Polfa Rzeszów S.A., Poland), doxepin hydrochloride (DOX) (Sigma-Aldrich GmbH, Germany), aqua pro injectione (Polfa, Lublin, Poland), aspartate aminotransferase (AST) – Liquick Cor-AST-60, alanine aminotransferase (ALT) – Liquick Cor-ALT-60, α-fetoprotein – AFP-ELISA (DIMA GmbH, Goettingen, Germany) and total protein - Liquick Cor-TOTAL PROTEIN 120.

Animals. The study was carried out on male Wistar rats weighing initially 200-250 g (purchased from licensed breeding farm of Brwinów, Poland). 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. The studies were approved by the Ethical Committee on Animal Experimentation of the Medical University of Lublin.

Treatments. SIM and DOX (suspended in distilled water with one drop of Tween 80) were injected intraperitoneally (i.p.) in volumes of 0.5ml/100g. The rats received SIM (10 or 20 mg/kg), DOX (10 or 20 mg/kg) and the combination of SIM and DOX once a day for 14 days. The drugs studied were applied to rats in effective doses [16, 22]. The control animals received identical volumes of the solvent (placebo).

Experimental protocols. Each experimental group consisted of eight animals. The rats were decapitated 24 h after the last injection and blood from each animal was taken. The blood was allowed to clot; 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 $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Monotherapy with statin has a proven record of safety and efficacy [12]. The less frequent but more important serious adverse events associated with the use of statins include, among others, hepatotoxicity [11]. Statins are biotransformed in the liver primarily by cytochrome P450 (CYP) 3A4 and clinical experience has shown that the risk of adverse effects, such as hepatotoxicity, increases with concomitant use of statins with drugs competing as substrates of the izoenzyme, for example, doxepin [1, 2, 5, 14]. The aminotransferases activities are basic diagnostic parameters supplying important information on the liver condition. The levels of AFP and total protein in blood serum can also be affected in the case of liver damage.

Our research indicated significant changes of biochemical parameters in rats pretreated with SIM and DOX. The 14 days application of SIM in the doses of 10 or 20 mg/kg in the combination with DOX in the doses of 10 or 20 mg/kg significantly increased the activity of AST in comparison to DOX [Fig.1]. Significant increase of AST activity has been also observed in the groups of rats treated with SIM for 14 days in both doses in comparison to the control group. The application of only DOX (10 or 20 mg/kg as well) did not have any influence on AST activity compared to the control group.

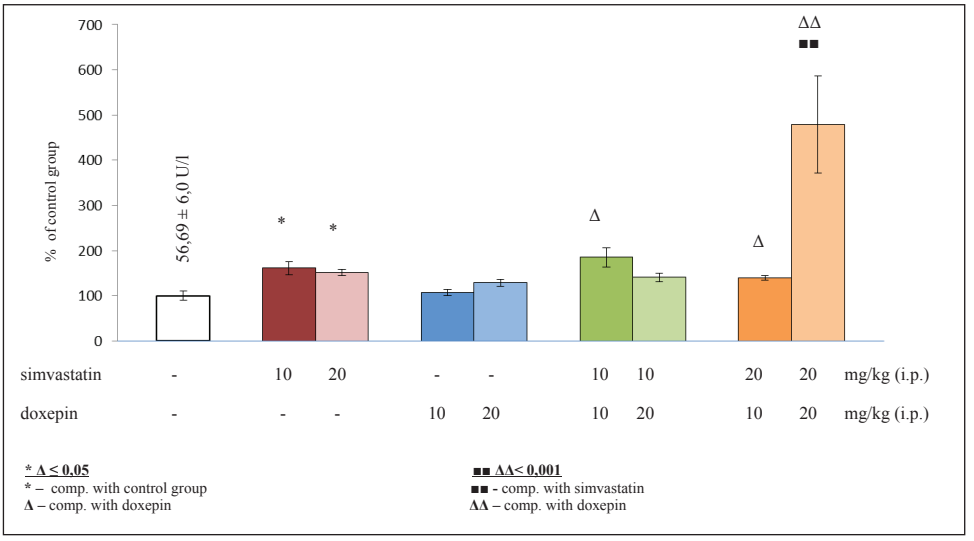

Figure 1. Effect of 14-day treatment with simvastatin, doxepin and their combination on the activity of AST in the serum of rats

SIM only in the dose of 20 mg/kg administered simultaneously with DOX only at a dose of 20 mg/kg for 14 day caused a significant increase of ALT activity in comparison to only SIM or DOX as well [Fig.2]. Significant increase of ALT activity has been also observed in the groups of rats treated with SIM only at a dose of 20 mg/kg or DOX only at a dose of 10 mg/kg.

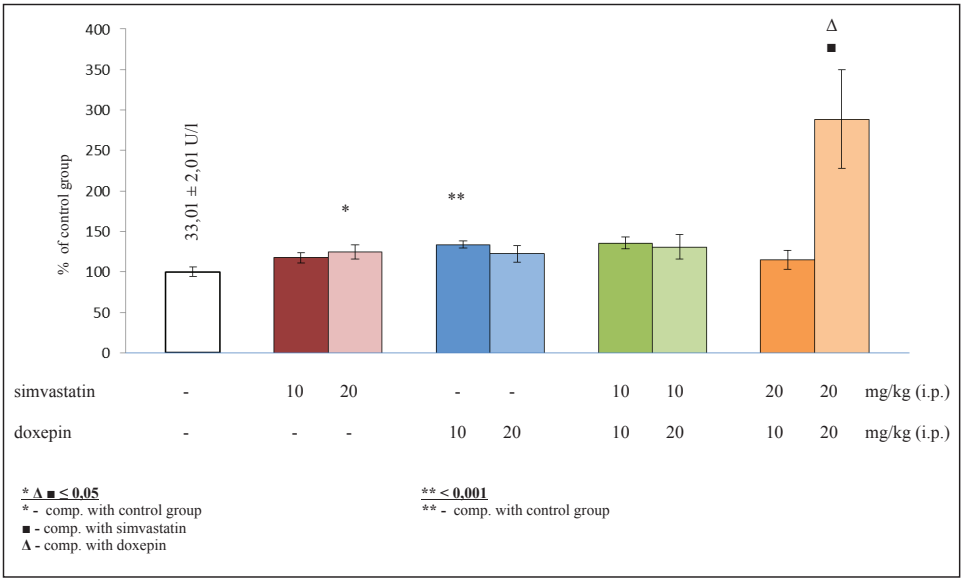

Figure 2. Effect of 14-day treatment with simvastatin, doxepin and their combination on the activity of ALT in the serum of rats

After 14 days of application of SIM (10 or 20 mg/kg) in combination with DOX (10 or 20 mg/kg), significant increase of the concentration of AFP has been noticed in comparison to only SIM (SIM 20 and DOX 10) and DOX as well [Fig.3]. On the other hand, 14-day treatment with SIM only at a dose of 20 mg/kg or doxepin in both doses significantly decreased the concentration of AFP in comparison to the control group.

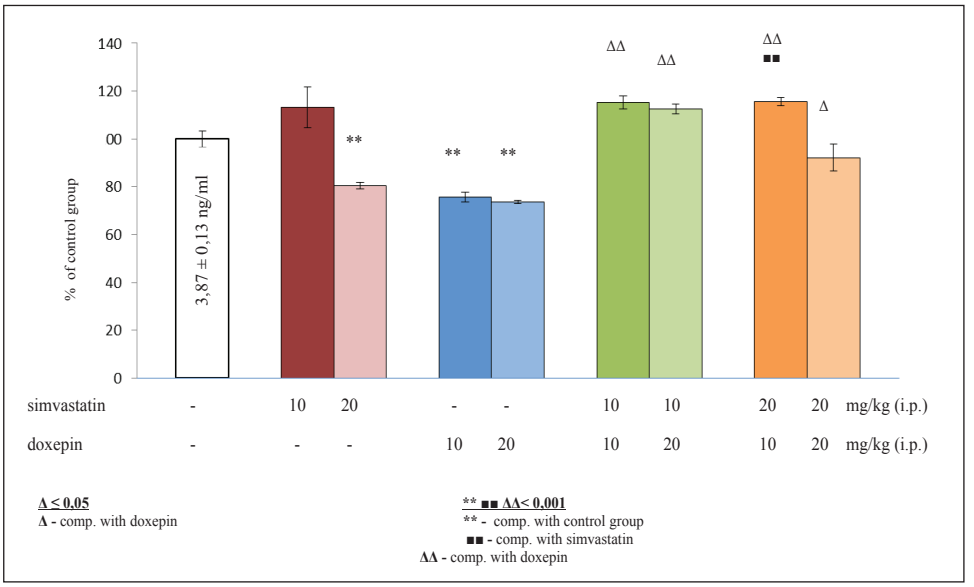

Figure 3. Effect of 14-day treatment with simvastatin, doxepin and their combination on the concentration of AFP in the serum of rats

The results showed the 14-day combined treatment with SIM (10 or 20 mg/kg) and DOX (10 or 20 mg/kg) caused generally the decrease of total protein concentration in comparison to only DOX [Fig.4]. In rats pre-treated with only SIM (10 or 20 mg/kg) or only DOX (20 mg/kg) this parameter was also significantly decreased. However, after treatment with DOX at a dose of 10 mg/kg the concentration of total protein was significantly increased.

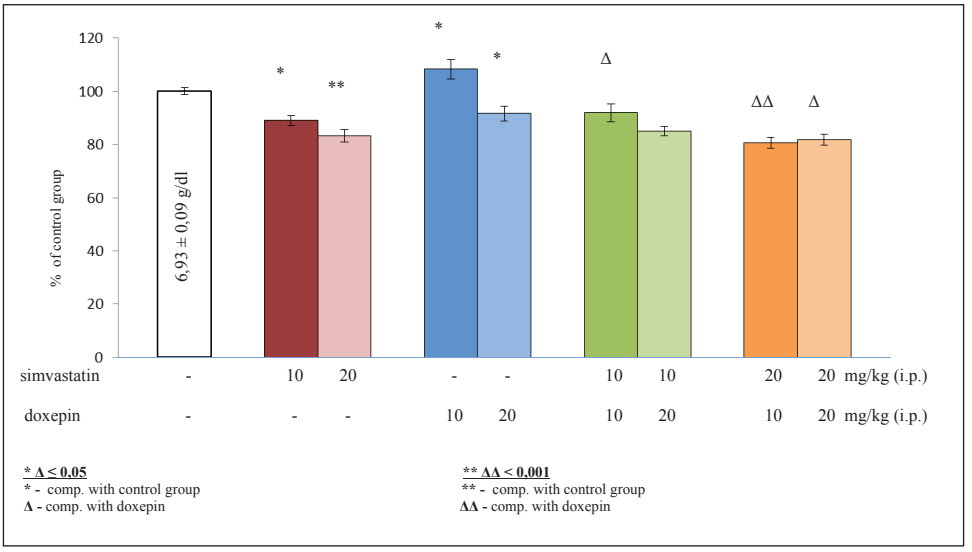

Figure 4. Effect of 14-day treatment with simvastatin, doxepin and their combination on the concentration of total protein in the serum of rats

Based on the literature data, it is known that SIM may increase aminotransferases activity [4, 7, 24]. However, according to many authors, the increase of this activity is reversible and dose-dependent [3, 7, 13]. It was found that 3-fold aminotransferases exceeded in two successive trials is an indication for statin withdrawal but you cannot ignore the occasional liver injury [9, 15, 17]. Increasing activities of these enzymes are also observed during treatment with antidepressants, including DOX [10].

In our study, we should pay particular attention to the significant increase of AST and ALT activity and decreased concentrations of total protein in the blood serum of rats treated simultaneously with SIM and DOX at doses of 20 mg/kg for 14 days. The observed changes maybe due to the fact, that both applied drugs used in this research are biotransformed in the liver primarily by cytochrome P450 [12, 21].

The results suggest that the combined use of SIM and DOX may lead to liver dysfunction, and this in turn can cause damage. Many years of clinical observations have proved the risk of functioning disorders of this organ applies to patients taking permanent statins which were recorded three times beyond the activity of transaminases. However, during the combined treatment with both examined drugs the monitoring of biochemical parameters indicating liver function is advisable. These data can be helpful in the treatment with these drugs.

SUMMARY

The aim of this study was to estimate the influence of 14-day simultaneous i.p. adminiatration of simvastatin and doxepin on some biochemical parameters indicating liver function in rats. The activity of AST, ALT and the concentration of total protein and α-fetoprotein was determined. The

results show that 14-day combined application with simvastatin and doxepin causes the increase of transaminases activities and AFP level but the decrease of total protein in comparison to the groups receiving these drugs individually. It seems that the combined treatment with simvastatin and doxepin is possible on the condition of the regular control of parameters indicating liver function. These data can be helpful in the treatment with these drugs.

Keywords: simvastatin, doxepin, biochemical parameters

STRESZCZENIE

W pracy oceniano wpływ łącznego 14-dniowego i.p. podawania szczurom simwastatyny i doksepiny na wybrane parametry biochemiczne w surowicy krwi świadczące o funkcji wątroby. Oznaczano aktywność AST i ALT oraz stężenie białka całkowitego i AFP. Na podstawie otrzymanych wyników stwierdzono, że 14-dniowe podawanie szczurom simwastatyny w kombinacji z doksepiną powoduje zwiększenie aktywności aminotransferaz, zwiększenie stężenia AFP oraz zmniejszenie stężenia białka całkowitego w odniesieniu do grup otrzymujących te leki pojedynczo. Na podstawie uzyskanych wyników wydaje się jednak, że łączne stosowanie simwastatyny i doksepiny wymaga systematycznej kontroli parametrów świadczących o funkcji wątroby. Dane te mogą być pomocne w terapii tymi lekami.

Słowa kluczowe: simwastatyna, doksepina, parametry biochemiczne

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