EVALUATION OF SELECTED BIOCHEMICAL PARAMETERS OF OXIDATIVE STRESS IN RATS PRETREATED WITH ROSUVASTATIN AND FLUOXETINE

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Abstract: The aim of this study was to assess the effect of a combined 14-day treatment with rosuvastatin (10 mg/kg) and fluoxetine (10 mg/kg) on selected biochemical parameters of oxidative stress in the blood of rats. The activity of glutathione peroxidase (GPX), glutathione reductase (GR) and the total antioxidant status (TAS) were determined. A combined 14-day treatment with rosuvastatin and fluoxetine significantly increases glutathione peroxidase and glutathione reductase activity and decreases the level of TAS. Rosuvastatin administered to rats caused a decrease in the glutathione peroxidase activity and an increase in the glutathione reductase activity but did not affect the level of TAS. A 14-day treatment with fluoxetine exerted practically no effect on the investigated parameters of oxidative stress in rats. The abovementioned changes in the biochemical parameters after the combined treatment with rosuvastatin and fluoxetine may imply an imbalance in prooxidant and antioxidant levels in the combined treatment with rosuvastatin and fluoxetine.

Keywords: rosuvastatin, fluoxetine, rats, oxidative stress parameters

Cardiovascular diseases, such as hypertension, coronary heart disease, heart failure and stroke, are the leading cause of mortality worldwide and their incidence rises. Reactive oxygen species (ROS) and reduced antioxidant enzymatic defense are important factors in the pathogenesis of cardiovascular disorders. Oxidative stress is defined as a disturbance in the balance between the production of ROS and antioxidant defense (1). ROS are highly reactive and may oxidize and damage important components such as proteins or DNA. They cause changes in biological molecules; these changes accumulate over time in the biological structures, which may lead to the molecular damage of tissue structure (2). Statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) are widely used in the clinical practice for reducing morbidity and mortality attributed to cardiovascular diseases. Experimental and clinical studies have shown that statins may also have important antiinflammatory effects through their ability to block the production and activity of reactive oxygen species (3). Rosuvastatin is a potent statin displaying pharmacologic and pharmacokinetic advantages. It has a superior efficacy in lowering LDL-C as well as improving HDL-C. Rosuvastatin cholesterol-independent effects include the modulation of vascular and inflammatory response and antioxidant activity (4-6). Rosuvastatin maintains the balance between oxidant generation and oxidant scavenging (7). Our previous studies conducted on rats also indicated a positive effect of rosuvastatin in this regard (8).

Depressive disorders are common for patients with cardiovascular diseases. Several studies have shown that depression and its associated symptoms are a major risk factor for both the development of cardiovascular disease and death after an index myocardial infarction (9). Therefore, the treatment of a depressed patient with a cardiovascular disease is necessary. The selective serotonin reuptake inhibitors (SSRIs) appear to be a relatively safe and effective treatment for depression in patients with a comorbid heart disease (10). Fluoxetine is a commonly chosen first-line medication because it is not associated with the cardiovascular effects (11). A combined treatment with rosuvastatin and fluoxetine may lead to the weakening of the antioxidant properties of rosuvastatin, which may be crucial in a therapy with this drug. Moreover, a long-term combined therapy can also cause oxidation-reduction imbalance and an increase in the generation of ROS that can cause oxidative stress.

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The purpose of the study was to evaluate some selected biochemical parameters of oxidative stress in the blood of rats pretreated with rosuvastatin and fluoxetine. After 14 days of intraperitoneal (*i.p.*) application of the drug, alone or in combination, the activity of glutathione peroxidase (GPX), glutathione reductase (GR) and also total antioxidant status (TAS) were determined. GPX appears in many tissues, first of all in the liver and blood. Its main role is to protect cells from oxidative stress, especially from hydrogen peroxide. GPX is closely connected with glutathione reductase (12). The determination of the total antioxidant status can serve for the establishment of the antioxidant potential of the drug and can indicate whether the treatment has any adverse effect on the antioxidation system.

MATERIALS AND METHODS

Animals

The study was carried out on male Wistar rats weighing initially 200-250 g and obtained from a licensed breeder. The animals were kept in room temperature ($20 \pm 1^{\circ}$C) under a natural day-night cycle in constant environmental conditions. The rats had access to food and water *ad libitum*. The study was approved by the Ethical Committee for Animal Experimentation of the Medical University of Lublin.

Drugs and chemicals

The following drugs were used in our study: rosuvastatin (Romazic tabl., Polpharma SA, Poland), fluoxetine (Fluoksetyna, Anpharm, Poland), *aqua pro injectione* (Baxter, Poland). Ready-made diagnostic kits (RANDOX Laboratories Ltd., Antrim, U. K.) were used to determine: GPX, GR and TAS.

Experimental protocols

Aqueous solutions of rosuvastatin (10 mg/kg) and fluoxetine (10 mg/kg) were prepared *ex tempore* and administered *i.p.* once daily for 14 days alone or in combination in the constant volume of 0.5 mL/100 g of body weight. The control groups were given the appropriate amounts of *aqua pro injectione*. The experimental groups consisted of eight animals each. Twenty four hours after the last injection, the animals were decapitated, and the blood was taken. One part of the blood was collected in heparin tubes (whole blood) and the other as clot. The whole heparinized blood was used to estimate the GP activity. The other part of the blood, as indicated above, was left to clot. The serum fraction was separated and taken in order to determine the GR activity and TAS.

Statistical analysis

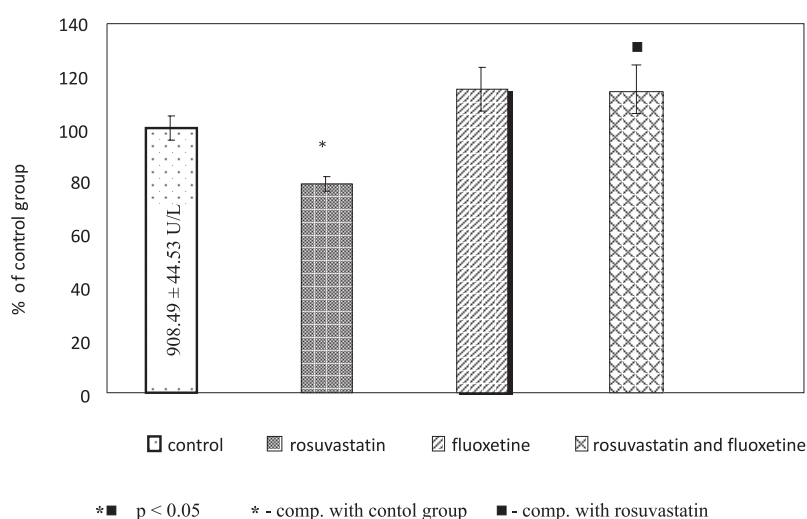All statistical calculations were carried out with the ANOVA test, and p-values less than 0.05

Figure 1. Effect of 14-day treatment with rosuvastatin (10 mg/kg) and fluoxetine (10 mg/kg) on glutathione peroxidase activity
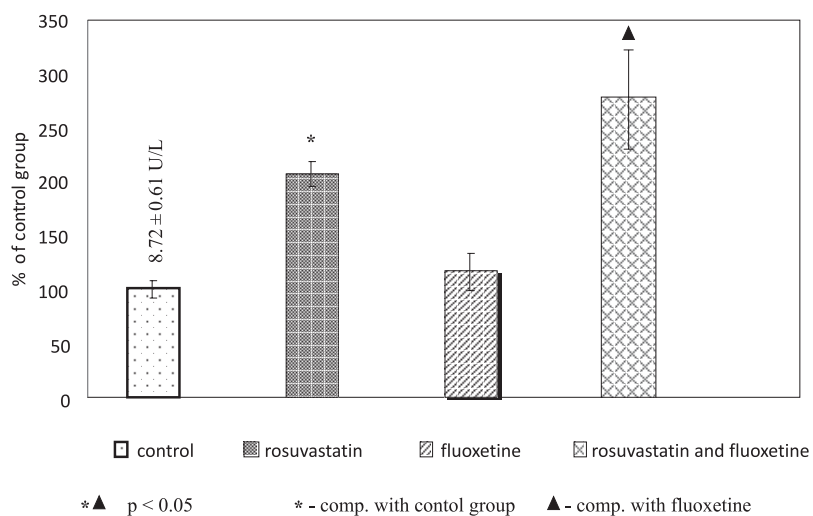
Figure 2. Effect of 14-day treatment with rosuvastatin (10 mg/kg) and fluoxetine (10 mg/kg) on glutathione reductase activity
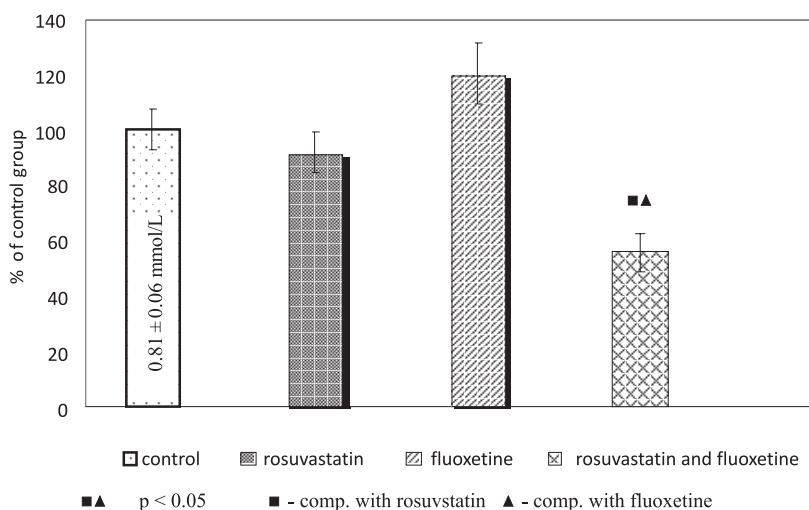

Figure 3. Effect of 14-day treatment with rosuvastatin (10 mg/kg) and fluoxetine (10 mg/kg) on total antioxidant status level

were considered significant. The results were expressed as the mean ± SEM.

RESULTS

We observed that rosuvastatin (10 mg/kg) administered to rats for 14 days causes a decrease of the GP activity compared with the control group (Fig. 1). Fluoxetine (10 mg/kg) administered to rats did not significant affect the activity of GP.

However, rosuvastatin administered for 14 day simultaneously with fluoxetine causes an increase of the GP activity in rat blood compared with the group of animals receiving only rosuvastatin.

A two-week administration of rosuvastatin to rats increases the activity of GR compared with the control group (Fig. 2). In the group of rats receiving only fluoxetine, any significant changes in the activity of GR were recorded and compared with the control group. The combined treatment of rats with

rosuvastatin and fluoxetine causes a significant increase of the activity of GR when compared with the groups of animals receiving only fluoxetine.

The group of rats receiving only rosuvastatin or fluoxetine did not demonstrate any significant changes of the level of TAS when compared with the control group (Fig. 3), whereas the combined treatment with rosuvastatin and fluoxetine results in the decrease of TAS in the serum of rats compared with the groups treated with these drugs alone.

DISCUSSION

Hyperlipidemia and elevated plasma low-density lipoprotein are considered relevant risk factors for the emergence of cardiovascular diseases, the major cause of mortality in Western populations. Also the intensification of the oxidative stress and decreased antioxidant capacity are likely to contribute to the increased risk of a cardiovascular disease (2).

As shown in clinical studies, a rosuvastatin therapy not only leads to a reduction of cholesterol but also significantly reduces oxidative stress and has further beneficial immunomodulatory and thus antiinflammatory effects, which may lead to the reduction of risk of atherosclerosis and cardiovascular diseases (4, 6, 13).

We observed that a two-week treatment with rosuvastatin reduces the activity of GP, increases the activity of GR, yet has no influence on TAS. Similar results were obtained in our previous study (8). GP is an enzyme present mainly in blood and liver, and its main task is to protect cells against oxidative stress, especially against hydrogen peroxide. This enzyme catalyzes the reaction of hydrogen peroxide and organic peroxides by reduced glutathione (14). The final product of the reaction is glutathione disulfide (GSSG). GSSG is harmful to cells because it oxidizes the thiol groups of proteins and leads to their inactivation. GP remains closely connected with GR which reproduces a reduced form of glutathione. A decrease in the activity of GP may confirm the beneficial effect of rosuvastatin consisting in restraining the formation of ROS. Also an increased activity of GR may suggest the protective effect of rosuvastatin aimed at maintaining an adequate level of the reduced form of glutathione and preventing the accumulation of hydrogen peroxide.

Some studies suggested a beneficial effect of fluoxetine in reducing oxidative stress (15, 16). In our research, fluoxetine administered to rats has no significant effect on the determined parameters. However, a 14-day combined treatment with rosuvastatin and fluoxetine yields significant changes in the assayed biochemical parameters. A combined treatment with these drugs significantly enhances the activity of GP in comparison with the group of rats receiving rosuvastatin, which may indicate a diminished antioxidant activity of rosuvastatin. A simultaneous application of rosuvastatin and fluoxetine causes an increased activity of GR compared with the group of rats receiving only fluoxetine, while causing no significant change compared with the group treated with rosuvastatin. A significant increase in the activity of GR in relation to the control group may indicate an increased production of hydrogen peroxide. Two weeks of a simultaneous treatment with rosuvastatin and fluoxetine proved a decrease of the total antioxidant status in comparison with the groups of rats receiving both drugs separately. The total antioxidant status, defined as an ability of the serum to quench free radical production, consists in a multicompartmental protection against molecular damage of the cell structure. TAS is sensitive to changes in the plasma antioxidant levels and degrees of oxidative stress (2). A decrease in the level of TAS suggests an increase in the generation of oxygen free radicals and a decrease in the antioxidant defense system (17, 18). Thus, the observations after the combined drug treatment covered by this study suggest increased oxidant stress and decreased antioxidant levels. In turn, our previous studies carried out on rats treated simultaneously with rosuvastatin and amitriptyline (tricyclic antidepressant (TCA)) indicated an increased total antioxidant status (8). These results are surprising because SSRIs seem to be relatively safer than TCAs. Perhaps, these differences result from the shared metabolism of rosuvastatin and fluoxetine. These drugs are biotransformed by cytochrome P450 izoenzyme CYP2C9, while this izoenzyme is not involved in the metabolism of amitriptyline.

CONCLUSIONS

Rosuvastatin (10 mg/kg) administered to rats for 14 days causes a decrease in the GP activity and an increase in the GR activity but does not affect the level of TAS.

A 14-day treatment with fluoxetine (10 mg/kg) has practically no effect on the investigated parameters of oxidative stress in rats.

A 14-day combined treatment with rosuvastatin and fluoxetine causes a significant increase in the glutathione peroxidase and glutathione reductase activity but reduces the level of TAS.

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The changes observed in the examined parameters may suggest an imbalance in the prooxidant and antioxidant levels in the combined treatment with rosuvastatin and fluoxetine.

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