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INFLUENCE OF COMBINED THERAPY WITH ROSUVASTATIN AND AMITRIPTYLINE ON THE OXIDATION-REDUCTION STATUS IN RATS

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Abstract: The aim of the present study was to assess the impact of combined therapy with rosuvastatin (10 mg/kg) and amitriptyline (10 mg/kg) on oxidation-reduction status in the blood of rats. After 2-week application of drugs alone or their combination, the activity of glutathione peroxidase (GPX), glutathione reductase (GR) and total antioxidant status (TAS) were determined. It was noticed that combined therapy with rosuvastatin and amitriptyline significantly increased the activity of GPX in comparison to the group receiving only rosuvastatin and decreased the activity of GR in comparison to groups receiving only rosuvastatin or amitriptyline. However, the activity of these enzymes as a result of combined therapy was placed in the level of the control groups. Our studies indicated that the combined therapy with both drugs caused an increase of TAS compared to the groups of animals receiving only one of these drugs. The results indicate on the oxidation-reduction balance and increasing the antioxidant status in rats treated with rosuvastatin and amitriptyline.

Keywords: rosuvastatin, amitriptyline, rats, oxidative stress

In recent years there has been increasing incidence of diseases of the cardiovascular system, such as atherosclerosis, coronary heart disease, hypertension. In Poland, myocardial infarction and stroke are the causes of over 50% of deaths. There are many factors that contribute to development of these diseases. One of them is high cholesterol level (1, 2). The most commonly used lipid lowering drugs in patients having high cardiovascular risk are statins - 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors, which reduce cholesterol synthesis. Statins beneficially alter the lipid profile of patients, improve endothelial function and they are anti-inflammatory, anti-proliferative, anti-thrombogenic and anti-proteolytic. These therapeutic effects decrease the incidence of cardiovascular (30–34%) and cerebrovascular events (19–37%) and are likely to attenuate such events when they occur (3). Rosuvastatin is more effective than other statins in reducing LDL cholesterol levels and produces significantly greater improvements in other elements of the lipid profile (4). Rosuvastatin therapy also reduces risk of coronary heart disease, ischemic stroke, vascular mortality, because lowers the level of CRP by approximately 30% (5). Rosuvastatin is a well-tolerated drug; most commonly reported side effects are nausea, dyspepsia and diarrhea. These symptoms are usually mild and transient (6, 7). The most serious adverse effect are related myopathy, liver toxicity and rhabdomyolysis (4, 6).

There is a connection between cardiovascular disease and depression (8-10). Depressive disorders are common for patients with ischemic heart disease and have serious consequences in terms of the risk of further cardiac events and cardiac mortality. Among survivors of acute myocardial infarction, up to one fifth meets diagnostic criteria for major depression, and the presence of major depression carries a more than 5-fold increased risk for cardiac mortality within 6 months (11). The risk of development of heart diseases for patients having depression is more or less 1.6 times higher than for healthy people (9). Appropriate treatment of depression in patients with cardiac disease is necessary. Of the available antidepressant agents, tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) are commonly chosen first-line medications (11). TCAs show good clinical efficacy against depression. Although, they are associated with anticholinergic side effects are still regularly used (12). Amitriptyline is a frequently prescribed tricyclic antidepressant used by psychiatrists for the

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therapeutic treatment of depression (13). The drug is associated with a number of side effects such as blurred vision, constipation, urination problems, dry mouth, delirium, vertigo and sedation (12).

Combined therapy of cardiovascular diseases and depression can lead to increased side effect of used drugs. It can also cause oxidation-reduction imbalance and generation increase of reactive oxygen species (ROS). ROS are highly reactive and may modify and inactivate proteins, lipids, DNA, and induce cellular dysfunctions (14). Defense against the toxic effect of ROS are two antioxidant systems: enzymatic (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase) and nonenzymatic (glutathione, plasma proteins, vitamins E, C, A) (14, 15).

The aim of the present study was to evaluate the influence of combined therapy with rosuvastatin and amitriptyline on the oxidation-reduction status in rats. After 14-days intraperitoneal application of drugs alone or their combination, the activity of glutathione peroxidase (GPX) was determined in whole blood and the activity of glutathione reductase (GR) and total antioxidant status (TAS) were determined in the serum.

MATERIALS AND METHODS

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.

Drugs and chemicals

The following drugs were used in the study: rosuvastatin (Romazic tabl., Polpharma SA, Starogard Gdański, Poland), amitriptyline hydrochloride (SIGMA-Aldrich, GmbH, Germany), *aqua pro injectione* (Baxter, Lublin, Poland). The following ready-made diagnostic kits were used: glutathione peroxidase (GPX), glutathione reductase (GR) and total antioxidant status (TAS) – all from RANDOX Laboratories Ltd., Antrim, UK.

Experimental protocols

Rosuvastatin and amitriptyline (suspended in *aqua pro injectione* with one drop of Tween 80) were injected intraperitoneally (*i.p.*) in volumes of 0.5 mL/100 g per rats. The animals received rosuvastatin (10 mg/kg), amitriptyline (10 mg/kg) or their combination once a day for 14 days. The control animals received identical volumes of the solvent (placebo). The experimental groups consisted of eight animals each. Twenty four hours after the last injection, the animals were decapitated and the blood was taken and divided, one part to heparin tubes (whole blood) and the second one to clot. The whole heparinized blood was used to estimate glu-

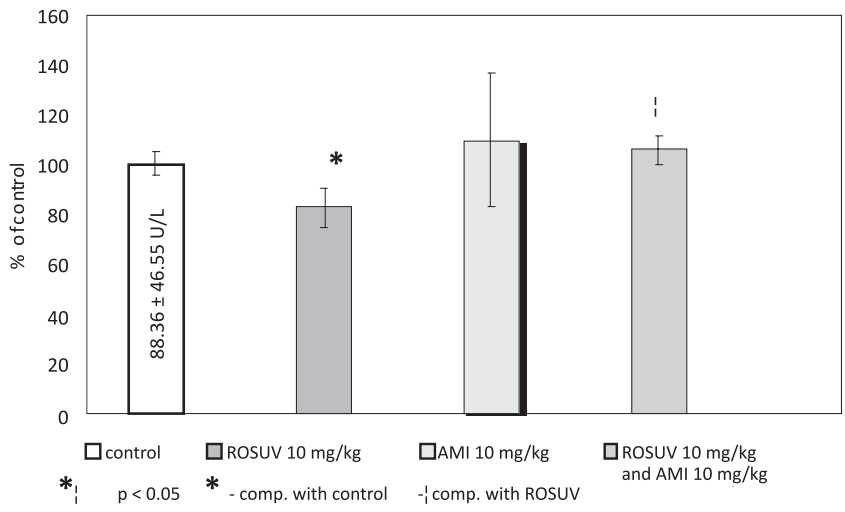

Figure 1. Influence of 14-day treatment with rosuvastatin and amitriptyline on glutathione peroxidase activity

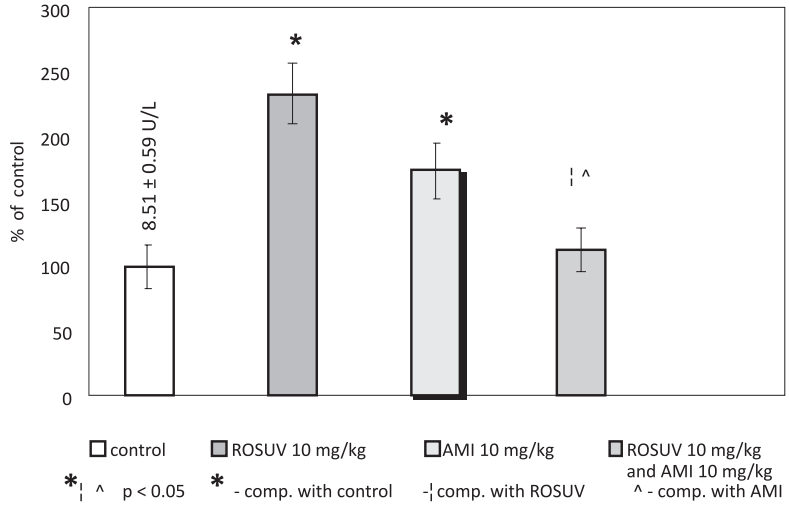

Figure 2. Influence of 14-day treatment with rosuvastatin and amitriptyline on glutathione reductase activity

tathione peroxidase activity. The second part of blood, was allowed to clot, the serum fraction was separated and was taken to determine glutathione reductase activity and total antioxidant status.

Statistical analysis

Results were expressed as the mean ± SEM. Statistical significance among groups was determined by ANOVA test and p-values less than 0.05 were considered significant.

RESULTS

The 14 days combined treatment with rosuvastatin (10 mg/kg) and amitriptyline (10 mg/kg) resulted in the increase of glutathione peroxidase activity in rat blood compared to the group of animals receiving only rosuvastatin (Fig. 1). However, in the blood of rats treated with rosuvastatin significant decrease of glutathione peroxidase activity was noted in comparison to the control group. No change of the glutathione peroxidase activity was observed in the blood of animals receiving only amitriptyline. The combined administration of rosuvastatin with amitriptyline to rats caused significant decrease of the activity of glutathione reductase compared to the groups of animals receiving only rosuvastatin or amitriptyline, respectively (Fig. 2). On the other hand, in a groups of rodents treated with only rosuvastatin or only amitriptyline the significant increase of the activity of glutathione reductase was observed in comparison to the control group. The combined treatment with rosuvastatin and amitriptyline resulted in the increase of total antioxidant status in the serum of rats compared with the groups treated with these drugs alone (Fig. 3). In the serum of rats pretreated for 14 days with rosuvastatin or amitriptyline significant changes of the level of total antioxidant status were not reported in comparison to the control group.

DISCUSSION

Oxidation-reduction balance disorder may lead to increased oxidative stress. Oxidative stress is associated with many diseases, among others atherosclerosis, hypertension, diabetes (16). Long-term studies on safety of using statins and tricyclic antidepressants have shown, that these drugs are in general well tolerated by patients, however, used chronically cause adverse effects (17-19). Treatment of depression in patients with heart disease demands long-term and simultaneous treatment with a few drugs, for instance, statins and tricyclic antidepressants. Long-term polypharmacotherapy causes the risk of intensification of side effects and increased oxidative stress, which may lead to many organs dysfunction.

The oxidative stress parameters were assessed in rats pretreated for 14 days with rosuvastatin and amitriptyline, alone or their combination. The activity of antioxidant enzymes such as glutathione peroxidase, glutathione reductase and total antioxidant status were determined in the blood. Glutathione

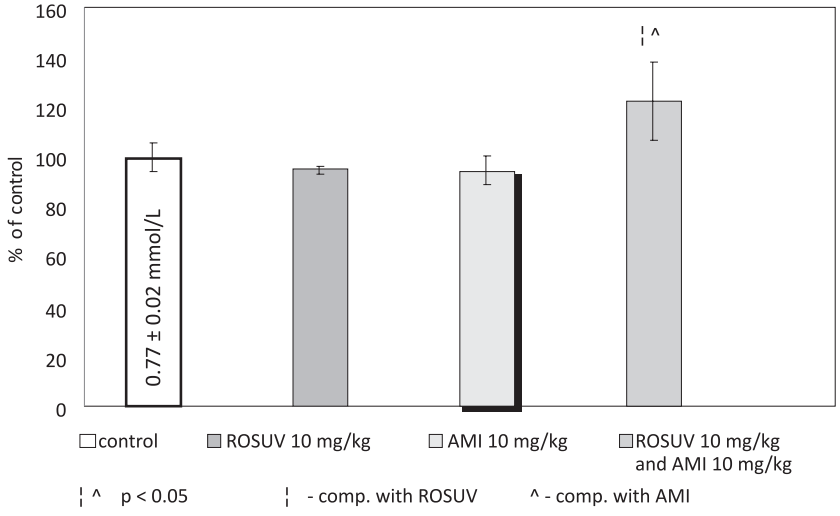

Figure 3. Influence of 14-day treatment with rosuvastatin and amitriptyline on total antioxidant status level

peroxidase appears in many tissues, first of all in the liver and blood. Its main role is to protect the cells from oxidative stress, especially from hydrogen peroxide. Glutathione peroxidase is closely connected with glutathione reductase (14). Determination of total antioxidant status provides an index of the sum of activities of all antioxidants.

Rosuvastatin has a beneficial safety profile and good tolerability. Serious adverse events with rosuvastatin therapy are rare and they include myopathy and liver toxicity (4, 6, 17, 18). According to some authors, rosuvastatin, like other 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, reduces oxidative stress and prevents from the formation of the oxygen free radicals (20, 21). The own studies have shown that 14-day treatment with rosuvastatin decreased the activity of glutathione peroxidase and increased the activity of glutathione reductase, whereas it did not have any influence on total antioxidant status. The decrease of the activity of glutathione peroxidase may suggest the beneficial effect of this drug, restraining the formation of reactive oxygen species. Also the increased activity of glutathione reductase may prove the protective effect of rosuvastatin, aimed at maintaining an adequate level of reduced form of glutathione and preventing the accumulation of hydrogen peroxide.

Amitriptyline in approximately 50% of patients receiving therapeutic doses causes side effects. Very rare but severe incidences of hepatotoxicity were also noticed (22). Several reports have demonstrated that reactive oxygen species (ROS) are implicated in the toxicity of amitriptyline through an increase of oxidative stress (23, 24). Bautista-Ferrufino et al. (24) have shown, that amitriptyline treatment induces oxidative stress in liver, lung, kidney, brain, heart, skeletal muscle but liver and lung seem to be the organs more predisposed to amitriptyline oxidative toxicity. Studies on people poisoned with antidepressant have shown, that the activity of glutathione reductase in their blood was lower than in the control group, which was manifested by the intensification of the oxidative stress (16). Our studies have shown, that after 14-day treatment with amitriptyline significant increase of the activity of glutathione reductase was noticed. However, it should be noted that in our research amitriptyline was administered at a dose usually used in similar studies, which does not cause toxic effects (25, 26). There have not been observed any changes in the activity of glutathione peroxidase and the level of total antioxidant status.

The increase of glutathione peroxidase activity during the decrease of glutathione reductase activity, and thus the weakening of the antioxidant system, were observed in aging process and neurodegenerative disorders (14). The conducted research showed that 14-day combined treatment with rosuvastatin and amitriptyline significantly increased the activity of glutathione peroxidase compared with the

group of rats receiving rosuvastatin and decreased the activity of glutathione reductase compared with groups of rats receiving only rosuvastatin or amitriptyline. The observed changes may suggest impairment of the oxidoreductive balance. However, it should be noted that simultaneous changes in the activity of both marked enzymes occur only in comparison to rosuvastatin, which has a protective effect. More to the point, the activity of these enzymes in groups of rats receiving simultaneously both drugs was placed in the level of the control groups.

Decrease of the level of total antioxidant status suggests the increase of oxygen free radicals generation and a decrease of antioxidant defense system (15, 16). Our studies have shown that combined treatment with rosuvastatin and amitriptyline increased total antioxidant status in the serum of rats in comparison to groups of rats receiving rosuvastatin or amitriptyline, respectively. Probably, the increase of the level of total antioxidant status is associated with metabolic mobilization of the organism treated with drugs and may indicate increasing amount of endogenous antioxidants.

CONCLUSIONS

The combined 14-day therapy with rosuvastatin (10 mg/kg) and amitriptyline (10 mg/kg) does not interfere with the oxidation-reduction status in the blood of rats.

After 2-week combined treatment with rosuvastatin and amitriptyline activities of glutathione peroxidase (GPX) and glutathione reductase (GR) were placed in the level of the control group. We noted significant increase of the activity of glutathione peroxidase compared with group of rats receiving rosuvastatin and decrease of the activity of glutathione reductase (GR) compared with groups of rats receiving only rosuvastatin or amitriptyline.

Our studies indicated that the combined treatment with both drugs caused an increase of total antioxidant status (TAS) compared to the groups of animals receiving only one of these drugs.

The lack of changes of determined enzymes activities in relation to the control group and an increase of total antioxidant status in aspect of the combined therapy with rosuvastatin and amitriptyline may indicate increasing amount of the endogenous antioxidants.

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