Research article

CARVEDILOL MODIFIES ANTIOXIDANT STATUS OF PATIENTS WITH STABLE ANGINA

JAN KOWALSKI1, MACIEJ BANACH2*, MARCIN BARYLSKI1, ROBERT IRZMANSKI1 and LUCJAN PAWLICKI1

1Department of Internal Medicine and Cardiological Rehabilitation, Medical University of Łódź, Poland, 2Department of Cardiology, 1st Chair of Cardiology and Cardiac Surgery, Medical University of Łódź, Poland

Abstract: The aim of this study was to evaluate the effect of carvedilol on the enzymatic antioxidative defence and plasma antioxidative activity in patients with stable angina. The study comprised 30 patients, aged 37-49 years with stable angina. Patients received carvedilol in escalating doses of 12.5 mg/24 h, 25 mg/24 h, and 50 mg/24 h for 4 weeks each. The control group was matched for age and gender, and consisted of 12 healthy volunteers, aged 39-49 years. Blood samples were collected from the cubital vein before and 4, 8 and 12 weeks after the therapy from the patients and once from the control group. For all the subjects, the superoxide dismutase (SOD-1), glutathione peroxidase (GSH-Px), catalase (CAT) activities in the erythrocytes and the antioxidant activity of the blood plasma were determined. The enzymatic antioxidative defence was significantly decreased in patients with stable angina in comparison to the healthy subjects. During the carvedilol therapy, an increase in the SOD-1, GSH-Px and CAT activities was observed. Moreover, 8 and 12 weeks after carvedilol therapy, the GSH-Px activity did not differ significantly from that observed in the group of healthy subjects. Carvedilol also increased the plasma antioxidative activity in patients with stable angina, but its level remained significantly lower than in the control group. In conclusion, carvedilol enhances antioxidant defense mechanisms in patients with chronic stable angina pectoris.

* Author for correspondence: Department of Cardiology, 1st Chair of Cardiology and Cardiac Surgery, Medical University in Łódź, Poland. Sterlinga 1/3; 91-425 Łódź, Poland, e-mail: m.banach@termedia.pl, tel/fax: +48 42 636 44 71

Abbreviations used: catalase – CAT; CHD – coronary heart disease; GSH-Px – glutathione peroxidase; ROS – reactive oxygen species; SOD-1 – superoxide dismutase
**Key words:** Stable angina, Carvedilol, Superoxide dismutase, Peroxidase, Catalase, Plasma antioxidative activity

**INTRODUCTION**

There is growing evidence on the role of lower systemic antioxidant defense in the development of ischemic changes in patients with stable angina. Activated neutrophils are recognized as a major source of the free radicals that are responsible for lipid peroxidation, the inhibition of prostacyclin synthesis, and disturbance in the production of nitric oxide, the fundamental biological function of which is relaxation of the smooth muscles and inhibition of platelet aggregation [1].

Beta-blockers are one of the basic groups of drugs used in coronary heart disease (CHD). A particular place in this group belongs to carvedilol, a beta-blocker with vasodilating and antioxidative activity. It is thought that its antioxidative activity is connected with the cardioprotective effect of the drug [1-3]. Moreover, unlike other beta-blockers, carvedilol is supposed to modify the lipid profile of the plasma beneficially [4, 5].

Although the benefits of carvedilol are well documented, its antioxidative activity in patients with stable angina is still not well understood. Therefore, we conducted our study in order to estimate the effect of carvedilol on erythrocyte superoxide dismutase (SOD-1), glutathione peroxidase (GSH-Px) and catalase (CAT) activities and on plasma antioxidative activity in patients with stable angina.

**MATERIALS AND METHODS**

**Study population**

Patients who smoked, had been diagnosed with diabetes mellitus, liver or kidney failure, or systemic diseases, or were taking steroids and/or antioxidants (vitamins C, A, E, angiotensin-converting enzyme inhibitors, statins, angiotensin receptor blockers) were excluded from the study. The rationale for these exclusion criteria was that these conditions could influence the antioxidant status of the patients and yield incorrect results. After applying these exclusion criteria to 75 patients, a group of 30 patients with diagnosed stable angina were qualified for investigations.

The group comprised 20 men and 10 women, aged 37-49 years (mean 45.6 ± 4.2 years), with stable angina defined as typical chest pain precipitated by exertion and subsiding at rest within 3-5 min. or after short-acting nitrates, with transient ST segment depression during the chest pain. The symptoms of angina had appeared during the preceding 12 months, and were similar in frequency of occurrence and time of ceasing. All the patients in the study group had carvedilol administered orally, twice a day in doses increasing every 4 weeks: 12.5, 25 and 50 mg/24 h, respectively. In addition, all the patients had
acetylsalicylic acid administered in a daily dose of 75 mg and temporarily short-acting nitrates 2 months prior to and during carvedilol therapy. The control group consisted of 12 clinically healthy subjects (8 men and 4 women), aged 39-49 years (mean 45.7 ± 3.7 years). They were healthy volunteers, without coronary heart disease. These subjects did not smoke cigarettes and did not take any drugs, including antioxidative vitamins, and they were qualified for the study at the same time as the patients. There were no significant differences in terms of the presence of risk factors for atherosclerosis between the group of healthy subjects and the patients with stable angina (i.e. genetic predispositions, elevated total cholesterol in the serum, bad nutritional habits, obesity, high body weight, low physical activity). Detailed patient characteristics are shown in Tab. 1.

Tab. 1. Patients’ detailed characteristics.

|                        | Study Group | Control Group |
|------------------------|-------------|---------------|
| Number of Pts. (n)     | 30          | 12            |
| Sex M/F (n [%])        | 20 [66.7%]/10 [33.3%] | 8 [66.7%]/4 [33.3%] |
| Age - range [yrs]      | 37-49       | 39-49         |
| - mean [yrs]           | 45.6 ± 4.2* | 45.7 ± 3.7    |
| BMI (kg/m²)            | 29.2 ± 3.7* | 28.7 ± 4.1    |
| TC (mg/dl)             | 212.24 ± 30.98* | 207.45 ± 3.67 |
| HDL (mg/dl)            | 44.59 ± 11.82* | 43.07 ± 12.58 |
| LDL (mg/dl)            | 137.14 ± 27.05* | 131.09 ± 3.15 |
| Other drugs during the study | Acetylsalicylic acid in a daily dose of 75 mg | no drugs |
|                        | Short-acting nitrates 2 months prior to and during carvedilol therapy. | no drugs |

* P > 0.05, not significant

Blood samples (5 ml) were collected from the cubital vein in the early morning, about 12 h after the previous meal. They were collected immediately before drug administration, and 4, 8 and 12 weeks after its administration to the patients, and once from the control group. All the determinations were performed within 2 h of blood collection from the examined subject, according to the applied method. The laboratory staff who performed the determinations were blind to the group the sample came from. All the study subjects signed the informed consent form before their inclusion in the study, which was approved by the local Ethics Committee. The Helsinki Declaration recommendations were observed by the physician collecting the blood samples.
Antioxidative enzymes
Erythrocyte SOD-1 (EC 1.15.1.1) activity was determined according to the method of Misra and Fridovich [6]. Erythrocyte GSH-Px (EC 1.11.1.9) activity was measured according to the method of Little et al. using cumene hydroperoxide as a substrate [7]. Erythrocyte catalase (EC 1.1.11.6) activity was measured according to Beers et al. [8]. The antioxidative activity of the blood plasma was determined via the method of Bartosz et al. [9], based on the reduction of the preformed cation radical of 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) by the blood plasma.

Statistical analysis
The data was analyzed using Student’s t-test for independent groups. The comparisons between each measurement were performed with the t-test for paired data. Values are expressed as a mean ± SD. The probability value p < 0.05 was considered significant.

RESULTS
Erythrocyte superoxide dismutase activity
The SOD-1 activity in the erythrocytes was markedly lower (p < 0.05) in patients with stable angina qualified to carvedilol therapy (627.0 ± 60.5 U/gHb) than in healthy subjects (1068.0 ± 78.2 U/gHb) (Fig. 1). The activity increased to 712.5 ± 75.0 U/gHb after 4 weeks of carvedilol therapy (12.5 mg/24 h) and to 830.0 ± 70.5 U/gHb after the next 4 weeks (25 mg/24 h) (Fig. 1), and did not change after a further 4 weeks of treatment with carvedilol (50 mg/24 h), when it was 837.0 ± 75.05 U/gHb (Fig. 1). The increase in SOD-1 activity in erythrocytes was observed during carvedilol therapy, but its value remained significantly lower (p < 0.05) than in healthy subjects.

Figure 1. The effect of carvedilol on superoxide dismutase (SOD-1) activity in erythrocytes. Values are expressed as means ± SD (# means significant changes between pts. without and with carvedilol therapy).
Comparing the results of SOD-1 activity in erythrocytes in the groups of patients with stable angina qualified to carvedilol therapy, we did not observe significant changes between the groups. However, markedly different results were obtained for the comparison of patients before carvedilol therapy and patients who received carvedilol in doses of 25 and 50 mg/24 h (p < 0.05) (Fig. 1).

**Erythrocyte glutathione peroxidase activity**

The GSH-Px activity in the erythrocytes was significantly lower (p < 0.05) in patients with stable angina qualified to carvedilol therapy than in healthy subjects (2.36 ± 1.02 U/gHb vs. 3.65 ± 0.64 U/gHb) (Fig. 2). The activity increased to 2.90 ± 1.10 U/gHb after 4 weeks of carvedilol therapy (12.5 mg/24 h) but was still significantly lower (p < 0.05) than in the healthy subjects (Fig. 2).

![Fig. 2. The effect of carvedilol on glutathione peroxidase (GSH-Px) activity in erythrocytes. Values are expressed as means ± SD (# means significant changes between pts. without and with carvedilol therapy).](image)

After another 4 weeks of carvedilol therapy (25 mg/24 h), the GSH-Px activity in erythrocytes increased to 3.05 ± 1.10 U/gHb and did not differ significantly from that observed in the healthy subjects (Fig. 2). Similarly results were obtained after a further 4 weeks of the drug therapy (50 mg/24 h): erythrocyte GSH-Px activity increased to 3.15 ± 1.28 U/gHb and did not differ from that observed in the healthy subjects (Fig. 2). Comparing the results of GSH-Px activity in erythrocytes in the groups of patients with stable angina qualified to carvedilol therapy, we did not observe significant changes between the groups, but markedly different results were obtained during the comparison of patients before carvedilol therapy and patients who had carvedilol administered in doses of 25 and 50 mg/24 h (p < 0.05) (Fig. 2).

**Erythrocyte catalase activity**

CAT erythrocyte activity was significantly lower (p < 0.05) in patients with stable angina qualified to carvedilol therapy than in healthy subjects (2.90 ± 0.82 U/gHb vs. 6.22 ± 1.19 U/gHb) (Fig. 3). The activity increased to 3.20 ± 0.76 U/gHb
after the next 4 weeks (12.5 mg/24 h) and to 4.01 ± 0.85 U/gHb after a further 4 weeks of the carvedilol therapy (25 mg/24 h) (Fig. 3). The CAT activity in the erythrocytes did not change after the next 4 weeks of the treatment (50 mg/24 h) (4.5 ± 0.90 U/gHb) (Fig. 3). An increase in CAT activity in the erythrocytes was observed during carvedilol therapy, but it was still lower (p < 0.05) than in the healthy subjects. Comparing the results of the CAT erythrocyte activity in the groups of patients with stable angina qualified to carvedilol therapy, we did not observe significant changes between the groups (Fig. 3).

**Plasma antioxidative activity**

Plasma antioxidative activity was significantly lower (p < 0.05) in patients with stable angina qualified to carvedilol therapy than in healthy subjects (327.65 ± 70.1 vs. 544.2 ± 50.12 μmol Trolox equivalents/litre of plasma) (Fig. 4). After four weeks of carvedilol therapy (12.5 mg/24 h), an increase (p < 0.05; 349.24 ± 76.20 μmol Trolox equivalents/litre of plasma) in plasma antioxidative activity was found compared to the initial value (Fig. 4). An increase (p < 0.05) in plasma antioxidative activity (388.8 ± 81.52 μmol Trolox equivalents/litre of plasma) was also observed after the next 4 weeks of carvedilol therapy (25 mg/24 h) (also as compared to the initial value) (Fig. 4). The activity did not change after a further 4 weeks of carvedilol therapy (50 mg/24 h) (391.74 ± 82.8 μmol Trolox equivalents/litre of plasma) (Fig. 4). The plasma antioxidative activity increased during carvedilol therapy, but was still markedly lower (p < 0.05) than in healthy subjects.

Comparing the results of the plasma antioxidative activity in the groups of patients with stable angina qualified to carvedilol therapy, we did not observe significant changes between the groups, but markedly different results were obtained during the comparison of patients before carvedilol therapy and patients who had carvedilol administered in doses of 25 and 50 mg/24 h (p < 0.05) (Fig. 4).
**DISCUSSION**

In our study, we observed an increase in the activities of antioxidative enzymes (SOD-1, GSH-Px and CAT) in the erythrocytes during carvedilol administration to patients with stable angina pectoris. Furthermore, during carvedilol administration, an increase in plasma antioxidative activity was observed. Red blood cells are particularly exposed to free radical action as they carry molecular oxygen which are a potential source of ROS (reactive oxygen species). To prevent a peroxidation reaction, red blood cells are equipped with various enzymatic and non-enzymatic defense mechanisms, which become exhausted under conditions of oxidative stress.

In our study, the baseline values of SOD-1, GSH-Px and CAT activity were significantly lower in patients with stable angina pectoris as compared with those for the group of healthy subjects. During carvedilol therapy, an increase in SOD-1 and CAT activities was found in the erythrocytes, but their activity remained markedly lower than in the healthy subjects. Also, over the whole course of the therapy, an increase in GSH-Px activity was observed in the red blood cells. In experimental and clinical studies, decreased activity of antioxidative enzymes was indicated in CHD and other diseases [10-15].

The observed increase in antioxidative enzyme activity (SOD-1, GSH-Px and CAT) in the erythrocytes during carvedilol administration to patients with stable angina pectoris points to an additional mechanism of action of this drug. Antioxidative enzymatic system strengthening in angina pectoris patients has great importance considering our own and other authors’ studies, indicating a decrease in the level of general antioxidative defense in these patients [10, 16, 17].
Increased production of free radicals induces adaptative reactions in the organism. Initially, an increase in enzymes synthesis is observed. Then there is a decrease in their activity, which can indicate exhaustion of the antioxidative defense mechanisms.

Based on published reports, it may be concluded that the intensity of antioxidative enzyme biosynthesis depends on the intensification of ROS formation [18, 19]. In the case of long-lasting and intensive exposure to ROS, a decrease in the activity of these enzymes comes about. The decrease in their activity indicates deep disturbances in the oxidation-reduction balance in the blood of patients with stable angina. The observed increase in SOD-1, GSH-Px and CAT activity in the erythrocytes of patients treated with carvedilol may indicate its antioxidative action.

In our studies, we found that patients with stable angina have decreased plasma antioxidative activity. During carvedilol administration, an increase in plasma antioxidative activity was observed, but throughout the whole course of therapy, it remained significantly lower than that seen in healthy subjects. The demonstrated carvedilol antioxidative activity speaks for an additional mechanism of its action. Most probably, it is associated with the fact that carvedilol, by causing a decrease in neutrophil \( \mathrm{O}_2^+ \) generation, indirectly affects lower consumption of anti-oxidative enzymes and thus, their activity increases.

Furthermore, there is an increased possibility of their regeneration owing to the substrate decrease (\( \mathrm{O}_2^+ \)). The observed changes may also result from carvedilol having a protective effect on endothelium oxidative and nitrosative stress.

In physiochemical, biochemical and cellular assays, carvedilol and several of its metabolites inhibited lipid peroxidation, scavenged ROS, inhibited the formation of ROS and prevented the depletion of endogenous antioxidants such as vitamin E and glutathione [20-24]. Since the elements of the non-enzymatic component of the system protecting against the effects of the excessive activity of ROS may, as the exposure proceeds, become exhausted during the adaptative mechanism, their functions are gradually adopted by antioxidative enzymes. Their activity may temporarily increase, and a decrease in their activity evidences deep disturbances of oxidation-reduction balance. Extensive disturbances in the antioxidative defense mechanism observed in angina patients are the result of atherosclerosis developing for many years [25-27].

In this study, we observed that carvedilol influences the antioxidant status of patients with stable angina. However it is important to emphasize that the study had also some limitations: the absence of placebo group, the limited study population, and the absence of long-term follow-up. Thus, it would be necessary to continue the study in order to unequivocally confirm the obtained results.

In conclusion, we showed that carvedilol intensifies the antioxidant barrier in patients with stable angina by increasing the plasma antioxidative activity and antioxidative enzyme activity in erythrocytes. In patients with stable angina, carvedilol shows equally effective antioxidant activity at 25 and 50 mg/24 h.
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