

EVALUATION THE RELATIONSHIP BETWEEN OXIDATIVE STRESS PARAMETERS AND LIPIDIC PROFILE IN STABLE ANGINA

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ABSTRACT. Oxidative stress can be considered an imbalance between generation of reactive oxygen species and antioxidant defense system. Cellular ROS formation is a physiologic process in the vasculature and occurs in endothelial and smooth muscle cells as well as fibroblasts. Although ROS are small, structurally simple molecules with high and random reactivity, they appear to be subtle physiologic modulators of biochemical processes involved in intracellular signaling. Stable angina as cardinal manifestation of ischemic heart disease, has attracted our attention, causing initiation of a study to track the benefits of pharmacological treatment in this condition. We investigated variations of antioxidant status and oxidative stress parameters in correlation with modifications of lipidic profile under antianginal drugs treatment. The association of beta blockers with Ca antagonists reduced the oxidative stress and has a positive effect on the lipidic profile.

Keywords: antioxidant status, oxidative stress, stable angina, antianginal drugs

INTRODUCTION

The reduction of the molecular oxygen occurs within the cellular mitochondria. If there is sufficient amount of molecular oxygen this process allows the continual synthesis of ATP. ROS are generated during the reduction of oxygen and comprise two groups of molecules: free radicals with short biological half-lives, such as superoxide (O₂⁻), hydroxyl (OH), and nitric oxide (NO), and nonradicals, such as singlet oxygen (1O₂), hydrogen peroxide (H₂O₂), hypochlorous acid (HOCl), peroxynitrite (ONOO⁻), and lipid hydroperoxides (LOOH), which are more stable and have longer half-lives than free radicals (Vass L., & Bud I., 2010).

These free radicals release themselves from the respiratory chain having a harmful effect on organelles. Free radicals first attack fatty acids, because the C:C double bonds in these molecules are sensitive to oxidative damage. In a cell, where free oxygen radicals accumulate, the fatty acids, which would normally be subjected to betaoxidation within the mitochondria, will be subjected to lipid peroxidation, production of toxic and reactive aldehyde metabolites such as malondialdehyde (MDA) which is commonly used as an index of lipid peroxidation. The presence of large amounts of reactive oxygen species indicates an intense but less efficient metabolic activity of the mitochondria as well as the perturbation of lipid metabolism by lipid peroxidation. Antioxidant activity of the human serum has been attributed to the presence of transferrin and ceruloplasmin (CP). It has been suggested that CP might be the major antioxidant in plasma as a scavenger of oxygen radicals.

Cellular ROS formation is a physiologic process in the vasculature and occurs in endothelial and smooth muscle cells as well as fibroblasts. Although ROS are small,

structurally simple molecules with high and random reactivity, they appear to be subtle physiologic modulators of biochemical processes involved in intracellular signaling (Wolf G., 2000, Poli G. et al, 2004, Antje R. et al, 2010).

During the past decade, a large body of evidence identified oxygen reaction products, as important molecules in signal transmission and regulation of vascular function. However, excessive reactive oxygen species (ROS) production leads to oxidative stress, which has been determined to play a key role in the pathophysiology of cardiovascular diseases such as endothelial dysfunction, atherosclerosis, hypertension, and myocardial infarction (Păduraru I. et al, 2008, Antje R. et al, 2010).

The role of oxidative stress in cardiovascular disease was evaluated by both experimental and clinical studies. In this context the most accepted in atherosclerosis is hypothesis of endothelial dysfunction because of multiple aggression factors. Endothelial dysfunction could be produced by mechanic, viral or bacterial factors, toxic exposure, high level of metabolites, turbulent blood flow, all this aggression are followed by atherosclerosis (Buege J.A. et al, 1990, Bourasa M.G. et al 2006, Chiorescu R. et al, 2008).

These atherosclerotic lesions could be agravated by metabolic stress hyperglycemia and hypercholesterolemia and oxidative stress.

Mediators produced in response to major cardiovascular risk factors including advanced glycation end-products, LDL and ox-LDL, angiotensin II and cytokines such as TNF- α stimulate generation of reactive oxygen species at endothelial level by a variety of enzymatic and non-enzymatic sources. Oxidative stress biomarkers are biomarkers of antioxidants depletion and specific markers: lipid peroxidation products, protein and

and ADN oxidative degradation products.

Lipid peroxidation is a complex process, started from the beginning of atherosclerosis. Peroxide assay could be loss of substrate and total or partial peroxide measurements. Impossibility to obtain an ideal assay to determinate lipid peroxidation was followed by complex evaluation of oxidative stress and development of total antioxidant activity assays (Bourasa M.G. et al 2006, Bobescu E., 2007).

Stable angina as cardinal manifestation of ischemic heart disease, has attracted our attention, causing initiation of a study to track the benefits of pharmacological treatment in this condition. It is interesting to note that part of the pharmacologic activity of long-established cardiovascular drugs might be a result of their antioxidant properties (Antje R. et al, 2010).

It has been shown that antianginal drugs can inhibit lipid peroxidation, like several β -blockers dose-dependently inhibit membrane lipid peroxidation. By comparing the various classes of Ca^{2+} antagonists, studies showed that the dihydropyridines (eg, nifedipine, nisoldipine) were particularly effective in protecting against H_2O_2 -induced decreases in contractile function of rat hearts and low-density lipoprotein oxidation (Antje R. et al, 2010). Our research focusing on well established and widely used treatment.

We investigated variations of antioxidant status and oxidative stress parameters in correlation with modifications of lipidic profile.

MATERIAL AND METHODS

Our research was performed on plasma and total blood, from three groups:

Group 1 - treated with selective β_1 -adrenergic antagonists (Bisoprolol, Atenolol, Betaxolol, Nebivolol) (47 patients)

Group 2 - treated with dihydropyridine, Ca-channel antagonists (Nifedipina, Amlodipina, Felodipina) (35 patients)

Group 3 - treated simultaneous with selective β_1 - beta blockers and dihydropyridine Ca-channel antagonists (30 patients)

Blood samples were obtained at the start of treatment and after 6 months.

Cholesterol, triglycerides, HDL and LDL were measured using enzymatic methods (Mihele D., 2002).

Non-enzymatic antioxidants determined parameters were reduced glutathione (GSH), serum ceruloplasmine levels (CP) and plasma concentration of malondialdehyde (MDA), as lipid peroxidation index.

Colorimetric determination of malondialdehyde

Malondialdehyde is one of the products of lipid peroxidation. Its determination in the blood represents a standard method of assessing the oxidative stress. The dosage method is based on the reaction with thiobarbituric acid (TBA). The biological sample is heated with TBA, in acidic medium. As a result of the reaction, one molecule of MDA reacts with two

molecules of TBA, with the production of a pink pigment, with a measured optical density at 530 nm using Pharmacia LKB Ultraspec. III spectrophotometer."

Normal values of the MDA serum levels are between 0,27 - 1,02 nmol/ml. Increased values of the MDA serum levels confirm the presence of the oxidative stress (Muresan M. et al, 2003, Sies H., 1997).

Determination of ceruloplasmin serum level

Ceruloplasmin (ferroxidase) is a 150 kD, blue α_2 -glycoprotein that is synthesized in the liver and it is accumulated in the matrix and in the inner membrane of the mitochondria (Macynire G et al, 2004).

Ceruloplasmin acts mainly as a ferroxidase, catalyzing the oxidation of Fe (II) to Fe (III) and as a Fe (II) carrier in the plasma in association with transferrin, the only protein which can carry iron in this state. Beside its detoxifying activity in the blood, ceruloplasmin also presents a dismutase-like activity (lower than that of the superoxide dismutase), it inhibits the peroxidation of polyunsaturated fatty acids (in vitro demonstration) and it has immunologic activity. Ceruloplasmin limits the quantity of free radicals, acting as a plasmatic antioxidant. Normal ceruloplasmin serum levels are between 0,20-0,40 mg/ml (11-24 pM). Abnormal ceruloplasmin level impedes the mitochondrial respiratory process (Muresan M. et al, 2003, Sies H., 1997).

Determination of serum glutathione activity

Glutathione plays important roles in antioxidant defense of cells (Townsend, D. M. et al, 2003).

The GSH Assay Kit utilizes a carefully optimized enzymatic recycling method, using glutathione reductase, for the quantification of GSH. The sulfhydryl group of GSH reacts with DTNB (5,5'-dithio-bis-2-(nitrobenzoic acid), Ellman's reagent) and produces a yellow colored 5-thio-2-nitrobenzoic acid (TNB). The mixed disulfide, GSTNB (between GSH and TNB) that is concomitantly produced, is reduced by glutathione reductase to recycle the GSH and produce more TNB. The rate of TNB production is directly proportional to this recycling reaction which is in turn directly proportional to the concentration of GSH in the sample. Measurement of the absorbance of TNB at 405-414 nm provides an accurate estimation of GSH in the sample (Sies, H., 1999, Jones D. P., 2002).

RESULTS AND DISCUSSIONS

The results from figures 1, 2 and 3 suggest the positive effect of combination with beta blockers and Ca antagonists on the lipidic profile in sense of decrease for total cholesterol, LDL cholesterol and increase for HDL-cholesterol level, the values of triglycerides doesn't change compared with only beta-blockers or Ca antagonists treatment.

Figures 4, 5 and 6 showed ceruloplasmine (CP), serum glutathione (GSH) and malondialdehyde (MDA) activity.

CP and GSH activity are intercorrelated, their level

Increase both in samples from group 1 and 2. the most increases value we obtained at group 3 with concomitant administration of beta blockers and Ca antagonists.

In the same time has been a decrease of MDA serum level. The decrease of MDA level indicate a limitation of oxidative stress, through direct protection of endothelial cell membrane. The largest decrease we obtained in samples from simultaneous beta blockers and Ca antagonists treatment, compared with only beta-blockers or Ca antagonists treatment.

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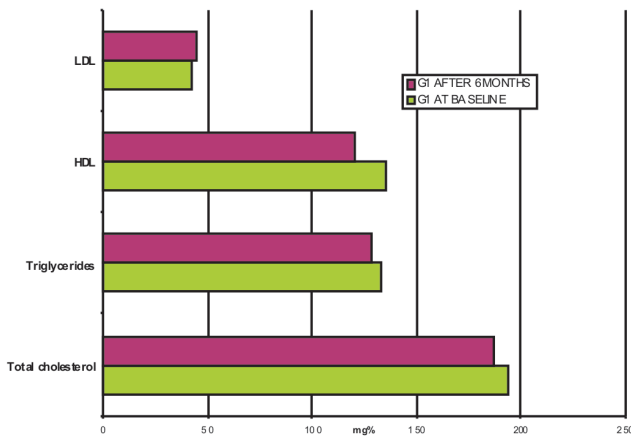


Fig. 1 Modifications of lipidic profile at group 1

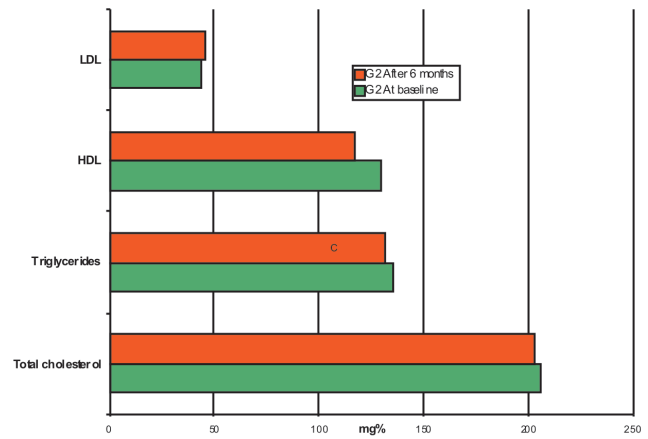


Fig. 2 Modifications of lipidic profile at group 2

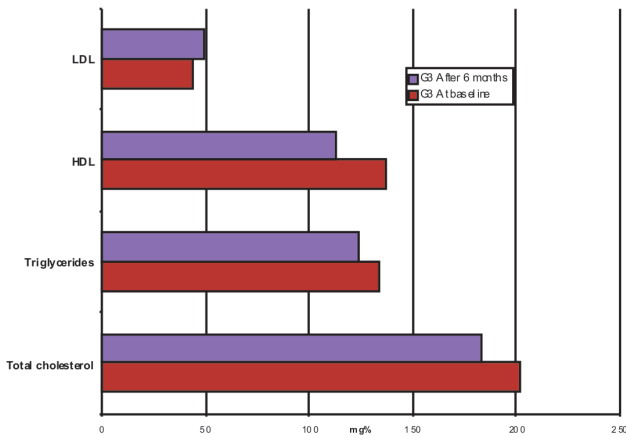


Fig. 3 Modifications of lipidic profile at group 3

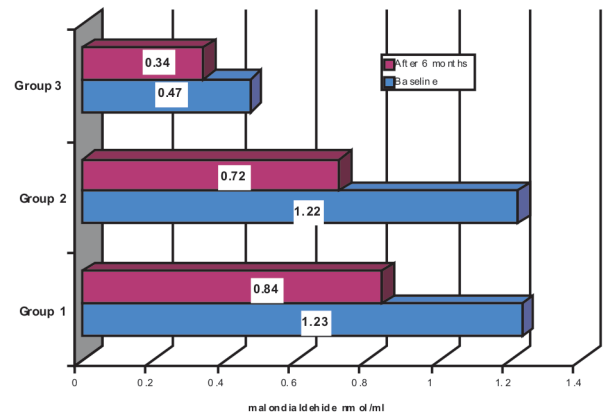


Fig. 4 Serum level of malondialdehyde modifications

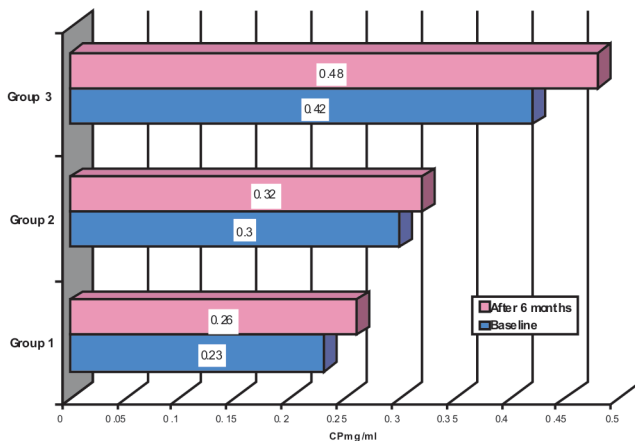


Fig. 5 Serum level of ceruloplasmine modifications

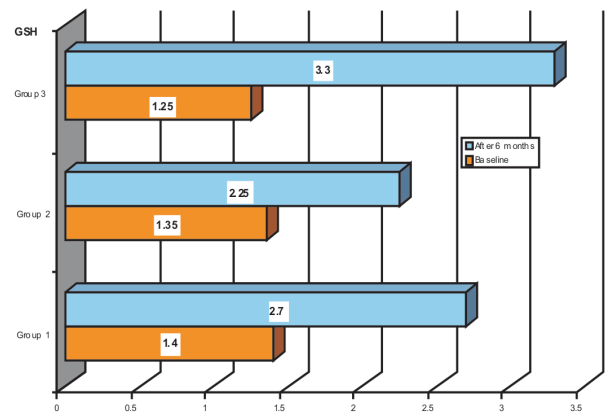


Fig. 6 Serum level of GSH modifications

CONCLUSIONS

Oxidative stress can be considered an imbalance between generation of reactive oxygen species and antioxidant defense system. The oxidative stress is correlated with a series of cardiovascular diseases such as stable angina and several already-registered cardiovascular-effective compounds possess significantly antioxidant activity, which may explain some of their therapeutic activity.

Simultaneous administration of beta blockers and Ca antagonists has a positive effect on the lipidic profile by decreasing total plasmatic cholesterol, LDL-cholesterol and by increasing HDL-cholesterol and the values of triglycerides does not change.

The association of beta blockers Ca antagonists reduced the oxidative stress, through the decrease of the level of malondialdehyde and through the increase of the level of reduced glutathione and ceruloplasmine.

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