The Relevance of Oxidative Stress Status in Type 2 Diabetes and the Chronic Consumption of Alcohol

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Abstract

To this date, the relevance of oxidative stress status in type 2 diabetes patients and in those with chronic alcohol consumption has been subject to numerous controversies in the literature, since various modifications of the oxidative stress markers have been reported in these disorders. In this context, we assessed in the present paper the oxidative stress status, by determining the specific activities of the main antioxidant enzymes (superoxide dismutase-SOD and glutathione peroxidase-GPX) and the levels of a lipid peroxidation marker (malondialdehyde-MDA), in 80 patients diagnosed with diabetes type 2 and chronic alcohol consumption, and 14 controls, who were also chronic alcoholics, but with no diagnosis of diabetes, in order to determine alcohol influence on oxidative stress in diabetes. In this way, we observed a significant decrease for the specific activities of SOD and GPX in the group of diabetic chronic consumers of alcohol, when compared to the control group. Also, significant increases for the levels of MDA, as a main marker of the lipid peroxidation processes, were recorded for the group of diabetic chronic consumers of alcohol, as compared to the control group. These aspects suggest an increased oxidative stress status as a result of type 2 diabetes deficiencies and further open the field for future studies regarding the relevance of antioxidant treatment in this area of research.

Keywords: oxidative stress, type 2 diabetes, alcohol

1. Introduction

Polyphenols Oxidative stress is considered a biochemical event consisting in the disruption of the oxidants/antioxidants balance and resulting in the overcoming of the defence capacity of the antioxidant component. Some adverse extracellular events, as well as intracellular events, represented by cellular physiological processes alterations may lead to an increased production of toxic molecules, known as reactive oxygen species (ROS), various types of radical compounds, such as the superoxide radical (O2-), hydroxyl radical (OH•), nitric oxide (NO), and also non-radical compounds, including hydrogen peroxide (H2O2), ozone (O3), oxygen (O2) or reactive aldehydes (ROCH) [1]. Thus, in order to minimize the negative effects of free radicals, the organism is endowed with an antioxidant defense system which is highly effective in physiological conditions. Within this defense system, several components were identified, which were classified in terms of their biochemical structure into an enzymatic and a non-enzymatic component. The antioxidant enzymes play a special role in
the processes of neutralization of the ROS. In this way, superoxide dismutase (SOD) catalyzes the dismutation of the superoxide radical (O$_2^-$), while glutathione peroxidase (GPX) and catalase reduce hydrogen peroxide to water [2]. As mentioned, the balance between pro- and antioxidant processes is relatively fragile, and the maintenance of it is particularly important in ensuring the cellular functioning in optimal conditions [1,2]. Thus, changes to this balance brought by increases of the pro-oxidizing component that exceed the body's antioxidant capacity can lead to oxidative damage. Thus, due to their high reactivity, the free radicals exhibit numerous harmful effects on the organism, being involved in numerous pathologies including cancer, atherosclerosis, chronic inflammation, neuropsychiatric disorders or diabetes [1-9]. In this way, with regards to diabetes, the harmful effects of chronic hyperglycemia on the secretion and action of insulin are considered to be at least partially mediated by chronic oxidative stress, as a result of increased generation of reactive oxygen species (ROS) [10]. Moreover, the generation of ROS and increased oxidative stress are essential for the numerous vascular disturbances in diabetes, such as endothelial dysfunction, vascular permeability changes, the inducing of leukocyte adhesion deficiency, cell type-dependent apoptosis, vascular cell cycle regulation abnormalities or the altered vasomotor tonus [11]. Furthermore, there is increasing evidence that hyperglycemia environment coupled with a compromised blood intake, overloads metabolic possibilities of the mitochondria leading to oxidative stress. This is also important, since the injury of mitochondria occurs because of the excess of ROS and reactive nitrogen species (RNS) [12]. Moreover, besides their metabolic role, mitochondria are involved in cell viability [13]. Also, evidence for the intensity of oxidative stress can be indirectly obtained through the measurement/study of the antioxidant status. Thus, plasma level of ascorbic acid (vitamin C) is low, both in the diabetic patient and in the diabetes experimentally induced animal, while its primary oxidation product, called the dehydroascorbate, is increased [14]. Still, when it comes to the levels of the main oxidative stress markers in the diabetic patients, there are a lot of controversies in the literature. In this way, some authors have found higher erythrocyte SOD activity in diabetic patients with complications; while also the erythrocyte GPX activity was higher in patients with diabetic nephropathy, while other studies have found reduced activities of SOD and CAT in diabetics compared to non-diabetic patients and as well as in the patients with retinal microangiopathy vs. those without complications [11, 15, 16]. Additionally, most studies found an inadequate antioxidative defense in diabetic patients, in terms of the non-enzymatic antioxidants, as well as the enzymatic ones (SOD, CAT, GPX), presumably due to their exaggerated consumption in the course of the intense oxidative processes [11, 14, 16]. Moreover, in other studies low values were found for SOD and CAT activity in diabetic patients with retinal microangiopathy [17]. Also, when it comes to the concentration of the oxidative stress markers related to alcohol consumption, there are similar controversial results. In this way, for all the markers of the oxidative stress, as in the case of the main antioxidant enzymes (SOD and GPX), there are previous reports stating both increased and decreased activities [18-20], while also, the other side of the oxidative stress balance, which is represented by the reactive oxygen species, is reported to suffer controversial modifications: increased levels (especially in patients with alcohol withdrawal – [21]), as well as authors stating clear reductions in MDA levels following alcohol withdrawal for example [20]. In this context, in the present paper we have assessed the status of oxidative stress, by determining the specific activities for the main antioxidant enzymes (SOD and GPX) and the levels of a lipid peroxidation marker (malondialdehyde -MDA), in 80 patients diagnosed with diabetes and chronic alcohol consumption, and 14 controls, who were also
chronic alcoholics, but for whom the diagnosis of diabetes was excluded, in order to determine alcohol influence on oxidative stress in diabetes.

2. Materials and methods

The subjects of this study were 80 patients (39 females and 41 males; age: 61.2 years±4.7) with diabetes and 14 healthy age-matched controls (8 females and 6 males; age: 59.5 years±5.8). Diabetes was diagnosed when fasting glycemia was ≥126 mg/dl [22]. They were all recruited from the University Hospital of Psychiatry “Socola”, Iasi, Romania and they were all chronic alcohol abusers, fulfilling DSM diagnostic criteria of alcohol dependence. The healthy control subjects had diabetes excluded by blood analysis. Also, the demographic data of the controls were chosen in order to match the patients with diabetes. This study was conducted according to provisions of the Helsinki Declaration. Exclusion criteria for all participants were represented by malignancies, hypothyroidism, psychiatric comorbidities and supplementation by vitamins, polyunsaturated fatty acids and/or antioxidants.

Biochemical estimations

Blood samples were collected in the morning, before breakfast, allowed to clot and centrifuged immediately. Serum was aliquoted into Eppendorf tubes and stored at −40°C until measurement. All samples were measured in duplicate and averaged.

Determination of SOD

Superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 substrate (a water soluble tetrazolium dye) and xanthine oxidase using a SOD Assay Kit (FLUKA, 19160) according to the manufacturer’s instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance wavelength for the colored product of WST-1 reaction with superoxide) after 20 minutes of reaction time at 37°C. The percent inhibition was normalized by mg protein and presented as SOD activity units.

Determination of GPX

The glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (SIGMA). This kit uses an indirect method, based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The decrease in NADPH at 340 nm during oxidation of NADPH to NADP is indicative of GPX activity.

Determination of MDA

Malondiadehyde levels were determined by thiobarbituric acid-reactive substances (TBARs) assay. 200 µL of serum was added and briefly mixed with 1 mL of trichloroacetic acid at 50%, 0.9 mL of TRIS-HCl (pH 7.4) and 1 mL of thiobarbituric acid 0.73%. After vortex mixing, samples were maintained at 100 °C for 20 minutes. Afterwards, samples were centrifuged at 3000 rpm for 10 min and supernatant read at 532 nm. The signal was read against an MDA standard curve and the results were expressed as nmol/ml.

Data Analysis

The results for antioxidant enzymes activity and MDA level were analyzed using one-way ANOVA. All results are expressed as mean ± SEM. F values for which P<0.05 were regarded as statistically significant.
Results

In this way, for the SOD specific activity, which, as mentioned above, is the first antioxidant enzyme in the way of the free radicals, a significant decrease (F(1,93)=14, p = 0.002) was registered in the case of the diabetic alcoholics group, as compared to the control group, consisting in chronic alcoholics, but with the diagnosis of diabetes excluded (Figure 1).

![Figure 1. Superoxide dismutase (SOD) specific activity in the serum of control and diabetes patients. The values are mean ± SEM (n = 14 in control group, n = 80 in the diabetes group). **p = 0.002.](image1)

Also, as in the case of SOD, a very significant decrease (F(1,93)=887, p < 0.0001) was also noticed for the GPX specific activity, within the group of chronic alcoholic patients with type 2 diabetes, when compared to the control group, consisting of chronic alcoholics, but with the diagnosis of diabetes ruled-out (Figure 2). Moreover, statistically significant increases (F(1,93)=442, p < 0.0001) for the levels of MDA, as a main marker of the lipid peroxidation processes were also recorded for the group of diabetic chronic consumers of alcohol, as compared to the control group (no diabetes!) (Figure 3), suggesting the installation of a pronounced oxidative stress for the subject group.

![Figure 2. Glutathione peroxidase (GPX) specific activity in the serum of control and diabetes patients. The values are mean ± SEM (n = 14 in control group, n = 80 in the diabetes group). ***p < 0.0001.](image2)

![Figure 3. The levels of malondialdehyde (MDA) in the serum of control and diabetes patients. The values are mean ± SEM (n = 14 in control group, n = 80 in the diabetes group). ***p < 0.0001.](image3)

3. Discussions

Our results provide additional evidences of increased oxidative stress in type 2 diabetes, as demonstrated in this case by the decrease of the specific enzymatic activity of both SOD and GPX, as well as an increased in the MDA, as a main lipid peroxidation marker, in the group of chronic alcoholic patients with type 2 diabetes, when compared to the control group, consisting in chronic alcoholics, but with the diagnosis of diabetes excluded. As previously
mentioned, the oxidative stress seems to have a special relevance in the context of the undertaken studies regarding the chronic ingestion of alcohol in diabetes.

In fact, these arguments arise from both in vitro and in vivo previous experiments. In this way, it was previously demonstrated that the administration of some antioxidants, such as N-acetylcysteine or aminoduanidine in P cells culture (e.g. HIT-T 15) could exert some protective effects, especially after chronic or glucose increased concentration [23]. As the mechanism involved, it seems that the increased concentration of ROS, as a result of glucose administration could include glyceraldehydes, pyruvate, with the Krebs cycle and the formation of ATP and ROS.

Moreover, it was previously demonstrated that the levels of nytrotyrosine and 8-hydroxy-2-deoxyguanosine (other important markers of the oxidative stress status) are elevated in the pancreatic islets of the subjects with type 2 diabetes, when compared to non-diabetics. Additionally, it was showed that the concentrations for the nytrotyrosine and 8-hydroxy-2-deoxyguanosine were inversely correlated with the glucose-stimulated release of insulin. Moreover, the addition in the culture of glutathione, a well-known antioxidant, reduced the level of nytrotyrosine and partially restored the secretory function of the beta cells [24].

Also, information regarding the oxidative mechanism involvement in generating diabetes are additionally coming from the studies involving rat models of diabetes, such as in the case of alloxan and streptozotocin, since both of these selectively destroying the Langerhans islets by oxidative stress-induced mechanisms [25, 26]. Very relevant, in the case of these models, their toxicity can be inhibited by chelating agents of metal ions, hydroxyl radical cleaners and liposoluble antioxidants.

Similar aspects were also observed in vivo, as in the case of the ZDF (Zucker diabetic fatty) obese rats, which spontaneously develop diabetes. Thus, in the case of this type 2 diabetes model the administration of various antioxidants also resulted in a significant decrease for the main markers of oxidative stress status (ROS), which was also combined with beneficial effects on insulin secretion [27].

As a matter of fact, in some studies there is a well-known cited relative 'poverty' of SOD and GPX specific activity of the Langerhans islets, since might be the result of an intense peroxidic activity that is necessary in the prostaglandin metabolism (involved in the insulin secretion regulation) [11,14].

In fact, as mentioned before, in our study we could observe a very significant decrease of the specific enzymatic activity of both SOD and GPX, as well as an increased in the MDA, as a main lipid peroxidation marker, in the group of chronic alcoholic patients with type 2 diabetes, when compared to the control group, consisting in chronic alcoholics, but with the diagnosis of diabetes excluded.

In this way, pathological conditions associated with intense tissue oxidation could be responsible of the isles altered functions, even by interfering with the prostaglandins metabolism [16]. Likewise, numerous studies have demonstrated that the plasma levels of lipoperoxides are significantly higher in patients with diabetes, when compared to non-diabetics. Also, in patients with diabetes mellitus type 2, complicated by retinopathy, the level of the Thiobarbituric Reactive Substances (such as MDA) is increased, as compared to those without retinopathy [28].

Moreover, it seems that the chronic complications of diabetes, retinal and glomerular microangiopathy, neuropathy and macroangiopathy, with their 3 main locations (coronary arteries, cerebral and lower limbs arteries) constitute another very important field of research for the relevance of the ROS in the production of these lesions [29].
Thus, it seems that oxidative stress can be a common pathway that connects the various pathogenetic mechanisms of the complications in diabetes. In this way, the processes that contribute to the intensification of oxidative stress in diabetes include not only non-enzymatic glycation and oxidative glycosylation, but also the resulting metabolic stress from changes in energy metabolism, imbalances on the sorbitol pathway, changes in the level of the inflammation mediators and of course, the depressed status of the antioxidant system [30].

In addition, it seems that in diabetes the low-density lipoproteins (LDL), which are playing a central role in the mechanism of atherogenesis, are undergoing intense oxidation, which eventually leads to an increase in their collection by the macrophagic specific 'scavenger' receptors, initiating in this way cytotoxicity against endothelial cells. Moreover, in response to these oxidative modified LDL, antibodies are formed, which have been proposed as biological markers of the LDL \textit{in vivo} oxidation and which, very importantly, seem to be an independent predictor of the carotid atherosclerosis progression [31].

Also, in regards to the involvement of oxidative stress in diabetic pathology, we could also add the fact that the increased oxidation of the plasma lipoproteins in patients with diabetes can be actually reduced under antioxidant treatment with Probucol or vitamin E [32].

Moreover, these aspects regarding the oxidative stress status in the selected individuals for this study, which were all chronic alcohol consumers, are consistent with previous reports stating that the antioxidant activity is lower in chronic alcoholics. This was generally demonstrated for the antioxidant enzymes such as SOD, GPX and CAT [19, 21], but also for the classic antioxidants such as glutathione [33]. In addition, the lipid peroxidation processes, as evaluated through the various levels of thiobarbituric acid reactive substances, were reported to be significantly decreased in the chronic alcohol consumers [34, 35]. In this way, it is generally believed that the mechanistics responsible for these effects are mainly represented by the mobilization of Fe$^{3+}$ ions, acetaldehyde, as well as an alcohol-induced increase in NADPH-oxidases [36]. Also, the alcohol-inducible cytochrome P450 seems to play a very important role in chronic alcohol consumers, by generating an increased rate of ROS formation [35, 37].

4. Conclusions

Our results provide additional evidence of increased oxidative stress status in type 2 diabetes, as shown by a significant decrease of serum SOD and GPX specific activities, as well as increased concentrations of MDA, as a lipid peroxidation marker, in the group of chronic alcoholic patients with type 2 diabetes, when compared to the control group, consisting in chronic alcoholics, but with the diagnosis of diabetes excluded.

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References

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34. N. YUKSEL, I. UZBAY, H. KARAKILIC, Increased serum nitrite/nitrate (NOx) and malondialdehyde (MDA) levels during alcohol withdrawal in alcoholic patients. Pharmacopsychiatry. 38, 95–6 (2005).

