EVALUATION OF SOME OXIDATIVE STRESS MARKERS IN DEPRESSIVE DISORDER AND INSOMNIA

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Abstract: We determined the specific activities of some enzymatic antioxidant defenses like superoxide dismutase (SOD) and glutathione peroxidase (GPX), as well as a lipid peroxidation marker (MDA-malondialdehyde) from the serum of patients with depression and insomnia, in comparison with a normal age-matched control group. There was an increased oxidative stress in patients with depression, which was even more pronounced in patients with insomnia. Our results could raise some important issues for therapeutics in depression and insomnia disorders, suggesting that one possible solution would be to associate antioxidants with the classical therapy.

INTRODUCTION

There are currently evidences that oxidative stress is implicated in several neuropsychiatric disorders such as dementia, schizophrenia or Parkinson’s disease (Hritcu et al., 2008, Padurariu et al., 2010).

Recently, some authors suggested that oxidative stress may also be involved in the etiopathogenesis of the depressive disorder (DD). However, only a few studies investigated the role of oxidative stress in DD and the results have been inconsistent. Generally, it was reported that during depressive episodes the concentration of some lipid peroxidation markers like malondialdehyde is increased (Galecki et al., 2009), the specific activities of some enzymatic antioxidant defenses like glutathione peroxidase (GPX) is decreased (Kodykova et al., 2009) and the total antioxidant capacity is lowered (Cumurcu et al., 2009). However, some studies reported an increased activity of some antioxidant enzymes like catalase (Galecki et al., 2009) and superoxide dismutase (SOD) (Kodykova et al., 2009) in acute depressive episodes. An important aspect of DD is insomnia. Additionally, it is considered that sleep deprivation represents an oxidative challenge for the brain and that sleep could have a protective effect against oxidative distress (Gopalakrishnan et al., 2004).

In this context, our objective was to determine some oxidative stress parameters (SOD, GPX and MDA) in patients with depression and in patients with insomnia, compared to a normal age-matched control group.

MATERIALS AND METHODS

The subjects of this study (30 patients) consisted of 15 individuals with DD, 5 with insomnia and 10 healthy age-matched controls. Patients were recruited from the Psychiatry University Hospital, Iasi, Romania. The psychiatric examination for depression was based on structured interview and ICD-10 (International Classification of Diseases) criteria. The sleep evaluation was assessed also by using psychiatric interview. Patients were under treatment with antidepressant and hypnoinductive drugs, respectively. Additionally, none of the subjects studied was taking antioxidant supplements.

The study was conducted according to provisions of the Helsinki Declaration and the local ethics committee approved the study. All the patients signed the consent for the participation in this study.

Biochemical estimations

Blood samples were collected in the morning, before breakfast, allowed to clot and centrifuged immediately. Sera were aliquoted into Eppendorf tubes and stored at -35°C until measurement.

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 min of reaction time at 37°C. The percent inhibition was normalized by mg protein and presented as SOD activity units.

The glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (Sigma Chemicals). 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.

Malondialdehyde (MDA) levels were determined by thiobarbituric acid reactive substances (TBARs) assay. 200 µL serum was added and briefly mixed with 1 mL of trichloroacetic acid at 50%, 0.8 mL of TRIS-HCl (pH 7.4) and 1 mL of thiobarbituric acid 0.73%. After vortex mixing, samples were maintained at 100 ºC 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 (Artenie et al., 2008, Ciobica et al., 2009).

Statistical analysis

Results were expressed as mean ± S.E.M. The results were analyzed statistically by means of the Student's "t" test (T- test: Paired Two Sample for Means). $p<0.05$ was taken as the criterion for significance (Georgescu and Dascalu, 2003).

RESULTS AND DISSCUSIONS

We observed a significant decrease in the specific activity of SOD and GPX in both depressed and insomniac patients, compared to the control group (figure 1 and 2). Moreover, in patients with insomnia we observed an additional decrease in the specific activity of the antioxidant enzymes, compared with the depressed group (figure 1 and 2).

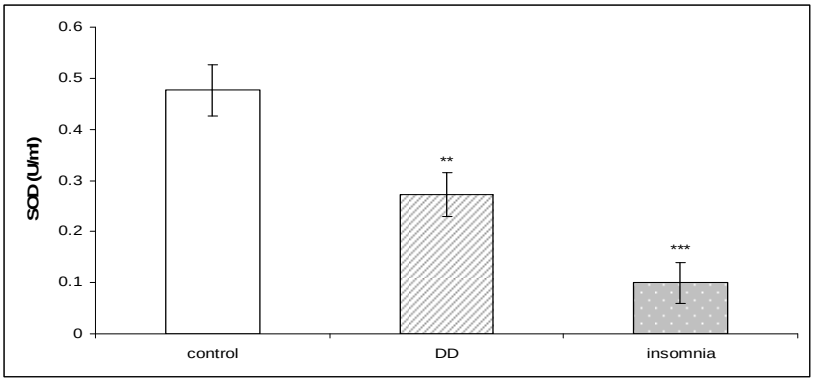

Figure 1. Superoxide dismutase specific activity in control, DD and insomniac patients serum. The values are mean ± SEM (n=10 for control group, n=15 for DD group and n=5 for insomnia group).**p<0,02 vs. control group, ***p<0,01 vs. control group.

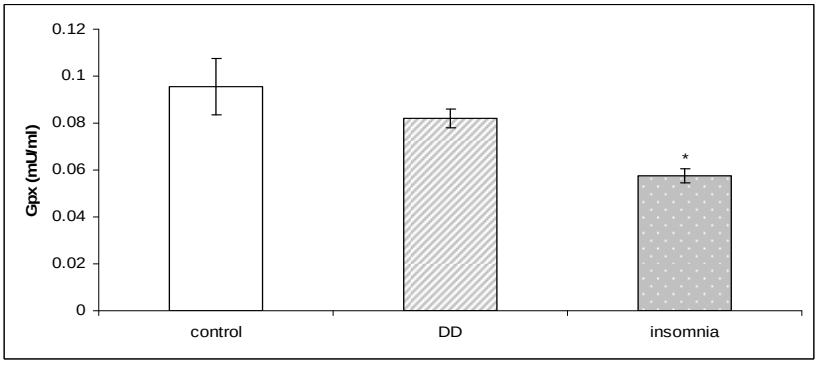

Figure 2. Glutathione peroxidase specific activity in control, DD and insomniac patients serum. The values are mean ± SEM. (n=10 for control group, n=15 for DD group and n=5 for insomnia group).*p<0,05 vs. control group.

Also, the concentration of serum MDA was significantly increased in patients with depression and in patients with insomnia, compared to the age-matched control group (figure 3). Moreover, we found that in the group with insomnia the level of MDA was increased, compared with the subjects with depression (figure 3).

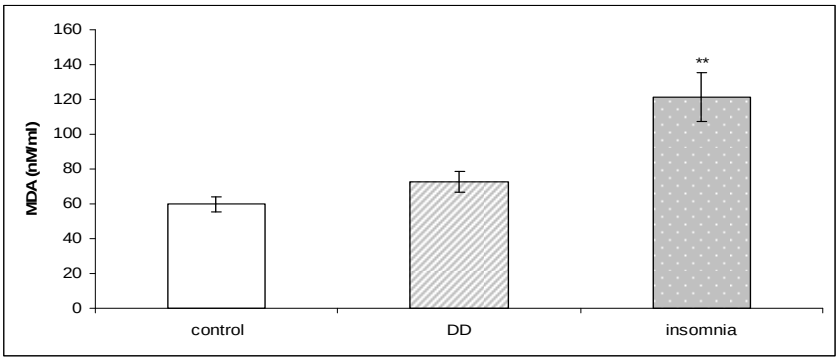

Figure 3. Levels of malondialdehyde in control, DD and insomniac patients serum. The values are mean ± SEM. (n=10 for control group, n=15 for DD group and n=5 for insomnia group).**p<0,02 vs. control group.

As we previously mentioned, it is considered that sleep deprivation represents an oxidative challenge for the brain and that sleep could have a protective effect against oxidative distress (Gopalakrishnan et al., 2004). This was demonstrated by some studies reporting increased thiobarbituric acid reactive substances (TBARS) in patients complaining of insomnia (Hachul de Campos et al., 2006). Also, protective effects of antidepressants like citalopram and desipramine were reported in sleep deprivation-induced behavioral alterations and oxidative damage in mice (Garg and Kumar, 2008). Anyway, the relation between DD and insomnia is controversial. Some authors hypothesized that sleep loss in some depressed patients might be an endogenous compensatory process that is therapeutic rather than pathological (Tufik et al., 2009).

In this paper we report an increased production of oxygen reactive species in patients with DD, which is even more pronounced in patients with insomnia. This could generate some controversies, considering that patients with DD have also sleep complains. However the results could be explained by the fact that some antidepressants have been reported for their antioxidant effects (Garg and Kumar, 2008). In addition, sleep deprivation leads to neurochemical and biochemical alterations in the body, like the accumulation of free radicals (Singh and Kumar, 2008; Tufik et al., 2009). However, it is also possible that an increased oxidative stress could affect the normal pattern of sleep.

CONCLUSIONS

Our results provide additional evidences that oxidative stress occurs in patients with depression and insomnia. This was demonstrated by a decrease of the main antioxidant enzymes (SOD and GPX) and an increase of MDA, as a lipid peroxidation marker. Moreover, the increased oxidative stress status from depression is even more pronounced in patients with insomnia. Our results could raise some important issues for therapeutics in depression and insomnia disorders, suggesting that one possible solution would be to associate antioxidants with the classical therapy.

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