Biogenic Amines Vol. 25, issue 3 (2011), pp. 145–159
BIA250311A01

Lower whole blood glutathione peroxidase (GPX) activity in depression, but not in myalgic encephalomyelitis / chronic fatigue syndrome: another pathway that may be associated with coronary artery disease and neuroprogression in depression

Reprinted from: Neuroendocrinology Letters 2011; **32**(2): 133–140.

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Key words: glutathione peroxidase; inflammation; oxidative stress; antioxidants; cytokines; neuroprogression; coronary artery disease; depression; chronic fatigue syndrome

Abstract

Major depression and myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS) are two disorders accompanied by an upregulation of the inflammatory and oxidative and nitrosative (IO&NS) pathways and a decreased antioxidant status. Moreover, depression is accompanied by disorders in inflammatory and neuroprogressive (IN-PRO) pathways.

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This study examines whole blood glutathione peroxidase (GPX) in depression and in ME/CFS; GPX is an enzyme that reduces hydroperoxides by oxidizing glutathione and consequently protects the cells from oxidative damage. Blood was sampled in 39 patients with depression, 40 patients with ME/CFS and 24 normal volunteers. Whole blood was analysed for GPX activity using the Ransel assay (Randox). Severity of illness was measured by means of the Hamilton Depression Rating Scale (HDRS) and the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF scale).
We found that whole blood GPX activity was significantly (p=0.001) lower in depressed patients than in normal controls and that there were no significant differences between ME/CFS and controls. In depression and ME/CFS, there were significant and inverse relationships between GPX activity and the FF items, depressed mood and autonomic symptoms. In depression, there were significant and negative correlations between whole blood GPX and the HDRS score and autonomic symptoms.
The results show that lowered whole blood GPX activity contributes to the lowered antioxidant status in depression. Since GPX activity is a predictor of neuroprogression and coronary artery disease (CAD), lowered GPX activity in depression contributes to the IN-PRO pathways and the comorbidity between depression and CAD. Our results suggest that patients with depression would benefit from Ebselen or a supplementation with glutathione, N-Acetyl-L-Cysteine and selenium.

Abbreviations:

CMS	- chronic mild stress
FF scale	- Fibromyalgia and Chronic Fatigue Syndrome Rating Scale
GPX	- glutathione peroxidase
HDRS	- Hamilton Depression Rating Scale
IN-PRO	- inflammatory and neuroprogressive
IO&NS	- inflammatory and O&NS
LDL	- low density lipoproteins
ME/CFS	- myalgic encephalomyelitis / chronic fatigue syndrome
O&NS	- oxidative and nitrosative stress
PMN	- polymorphnuclear
TRD	- Treatment resistant depression

INTRODUCTION

In aerobic organisms different mechanisms are involved in scavenging free radicals, including enzymes, such as glutathione peroxidase (GPX) (Mill 1957; Joseph 1995). The GPX antioxidant enzyme family consists of different isoforms, two of which are present in whole blood, i.e. the cellular GPX1 expressed in

red blood cells and the extracellular GPX3 found in plasma and expressed in kidney, lung, heart, breast, placenta, and liver (Chu *et al.* 1992), but secreted from renal proximal tubular cells and parietal epithelial cells of Bowman's capsule (Avissar *et al.* 1994; Whitin *et al.* 2002). GPX1 and GPX3 are immunologically distinct forms which display differences in physical properties and show only a moderate amino acid sequence homology (Chambers *et al.* 1986; Takahashi *et al.* 1990). Both GPX1 and GPX3 enzymes contain selenium and are tetramers of four identical subunits each of which presents a selenocysteine in the active site (Flohe 1978; Margis *et al.* 2008).

The selenium-dependent GPXs reduce glutathion disulfide (GSSG), the oxidized form of glutathione, into the reduced sufhydryl form, i.e. GSH (Meister 1988; Mannervil 1987). The GPXs catalyse the reduction of hydrogen peroxide and lipid peroxides to water or the corresponding alcohols at the expense of GSH, which is used as the ultimate electron donor to regenerate the reduced form of selenocysteine (Meister 1988; Mannervil 1987; Ursini and Bindoli 1987). In resting conditions the selenocysteine site is in a Selenium(–) (Se–) form and will be oxidized by peroxides to SeOH which is then trapped by a GSH molecule to Se-sulfhydryl group (SG) and by another GSH molecule to Se(–) again. Thus, the GPXs are key antioxidant enzymes that scavenge free radicals and as such have a central role in the control of reactive oxygen species (ROS) (Herbette *et al.* 2007). This explains why selenium-dependent GPXs protect cells and enzymes from oxidative damage. GPX1 clearly acts as a strong antioxidant with a major protective role in coping with oxidative injury and death, as demonstrated in GPX1 knockout and transgenic mice (Lei 2002). There is now evidence that abnormal GPX1 and GPX3 expression is relevant to the etiology of different disorders, such as cancer, cardiovascular disease, including arteriosclerosis and stroke, neurodegeneration, or diabetes (Lei 2002; Voetsch *et al.* 2007).

Lower GPX may also be involved in the pathophysiology of depression. Kodydkova *et al.* (2009) found that women with depression had significantly lowered GPX1 activities. Oczan *et al.* (2004) detected that GPX activity was significantly lower in patients with affective disorders than in controls. In olfactory-bulbectomized rats, a depression model, GPX activity was decreased (Song *et al.* 1994). In male Wistar rats, chronic mild stress (CMS)-induced depression was accompanied by lowered brain cortex GPX activity (Eren *et al.* 2007a; 2007b). Other authors (Srivastana *et al.* 2002; Andreazza *et al.* 2009) were unable to detect significant differences in GPX activity in plasma or polymorphnuclear (PMN) leukocytes, between depressed or bipolar patients and controls.

The abovementioned changes in GPX activity take part in an overall decreased TAS in depression, which is characterized by lowered vitamin E, zinc, coenzyme Q10, vitamin C, tryptophan, tyrosine, albumin, glutathione, and catalase activity (Kodydková *et al.* 2009; Ozcan *et al.* 2004; Maes and Meltzer 1995; van Hunsel *et al.* 1996; Maes *et al.* 1994; 1997; 2000; 2009b; Owen *et al.* 2005; Khanzode *et al.* 2003). These decreased defences against oxidative and nitrosative stress (O&NS) play a role in the damage caused by O&NS in depression, which is evidenced by increased levels of malondialdehyde (MDA), 8-hydroxy-2-deoxyguanosine, and IgM responses against phosphatidyl inositol and NO-albumin (Forlenza and Miller 2006; Sarandol *et al.* 2007; Maes *et al.* 2007c; 2008). A lowered antioxidant status plays a role in the activation of inflammatory and O&NS (IO&NS) and neuroprogressive (IN-PRO) pathways in depression (Maes 2008; Maes *et al.* 2009c; Berk *et al.* 2011; Maes *et al.* 2011). Neuroprogression indicates decreased neurogenesis, neurodegenerative processes and increased neural apoptosis (Berk *et al.* 2011).

Another medical illness characterized by activated IO&NS pathways is myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS) (Maes 2009; Maes *et al.* 2007a; 2007b). ME/CFS is accompanied by a decreased antioxidant status, as evidenced by lower serum zinc, dehydroepiandrosterone sulfate and plasma COQ10 (Maes *et al.* 2005; 2006a; 2009a); increased O&NS, as evidenced by higher isoprostane; oxidized low density lipoproteins (LDL); LDL thiobarbituric acid reactive substances (TBARS) and protein carbonyl levels and damage by O&NS to functional proteins and membrane fatty acids (Vecchiet *et al.* 2003; Kennedy *et al.* 2005b; Smirnova *et al.* 2003; Jammes *et al.* 2005; Maes *et al.* 2006b; 2007c; 2008). Fulle ***et al.*** (2000) detected an increased GPX activity in the muscles of ME/CFS patients, but to the best of our knowledge no studies have examined the activity of GPX in whole blood of ME/CFS patients. Moreover, there is a strong comorbidity between depression and ME/CFS that is based on shared pathways, such as activated IO&NS pathways, dysfunctional mitochondria; lowered antioxidant levels; lowered omega-3 polyunsaturated fatty acid levels; and increased translocation of gram-negative bacteria (Maes 2010). Other pathways, however, may discriminate both disorders, e.g. induction of indoleamine 2-3-dioxygenase and neuroprogression that are more specific to depression, and the 2'-5' oligoadenylate synthetase / RNase L pathway that typically occurs in ME/CFS.

The present study has been performed in order to examine GPX activity in whole blood of major depressed and ME/CFS patients and in normal controls under the working hypothesis that GPX is lowered in both depression and ME/CFS.

SUBJECTS AND METHODS

Subjects

One-hundred and three subjects participated in this study, i.e. 39 major depression patients, 40 ME/CFS patients and 24 normal controls. The patients were all outpatients admitted to the Maes Clinics, Antwerp, Belgium. Major depression was diagnosed according to the DSM-IV-R criteria (APA 2000), using a semistructured interview. The Severity of depression was measured with the Hamilton Depression Rating Scale (HDRS) (Hamilton 1960). Staging of treatment resistance was based on prior treatment responsivity according to the criteria of Thase and Rush (1995). Treatment resistant depression (TRD) was diagnosed if the patients showed a nonresponse to at least two adequate trials with antidepressant agents from different classes, e.g. tricyclics (TCSs) or selective serotonin reuptake inhibitors (SSRIs). ME/CFS was diagnosed by means of the Centres for Disease Control and Prevention (CDC) criteria (Fukuda *et al.* 1994). The severity of illness was measured using the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (FF scale) (Zachrisson *et al.* 2002). This scale measures 12 symptoms, i.e. pain, muscular tension, fatigue, concentration difficulties, failing memory, irritability, sadness, sleep disturbances, autonomic disturbances, irritable bowel, headache, and subjective experience of infection. The total sum on this scale is employed as a measure of the severity of illness. This FF scale was also scored in the depressed patients.

We have excluded the following subjects or patients: a) subjects with medical illnesses, e.g. inflammatory bowel disorders, diabetes type 1 or type 2, hypertension, etc.; b) patients with abnormal blood tests, such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), thyroid stimulating hormone (TSH) and total protein; c) subjects with acute inflammatory or allergic reactions the last 2 months prior to the study; d) patients with life-time diagnoses of psychiatric DSM IV-R disorders, e.g. psychotic, substance use and organic mental disorders; e) ME/CFS patients who (previously) had been diagnosed with depression; f) subjects who had been treated with anti-psychotic drugs, anticonvulsants or mood stabilizers; and g) subjects who had taken dietary supplements with glutathione, N-acetyl-L-cysteine (NAC) and selenium. Patients and controls gave written informed consent after the study protocol was fully explained; the study has been approved by the local ethical committee.

Methods

Blood for the assay of GPX activity was taken in the morning hours (8.30–11.30) after an overnight fast. We assayed GPX in whole blood by employing the RANSEL kit (RANDOX, Randox Laboratories Ltd, 55 Diamond Road, Crumlin, Co Antrim, United Kingdom, BT29 4QY). The RANSEL method is based on the method developed by Paglia and Valentine (1967). GPX catalyses the oxidation of glutathione by cumene hydroperoxide; in the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The reaction is performed on an automated chemistry analyzer (Dayona from RANDOX) and the decrease in absorbance at 340 nm is measured. The GPX concentration may be calculated as U/L of hemolysate = 8412 × ΔA 340 nm/minute. Under our assay conditions, one unit of GPX is defined as the amount of enzyme that catalyses the transformation of 1 μmol of NADPH per minute. The activity of GPX is expressed in U/g Hb and is calculated as the activity of GPx in U/L divided by the concentrations of Hb

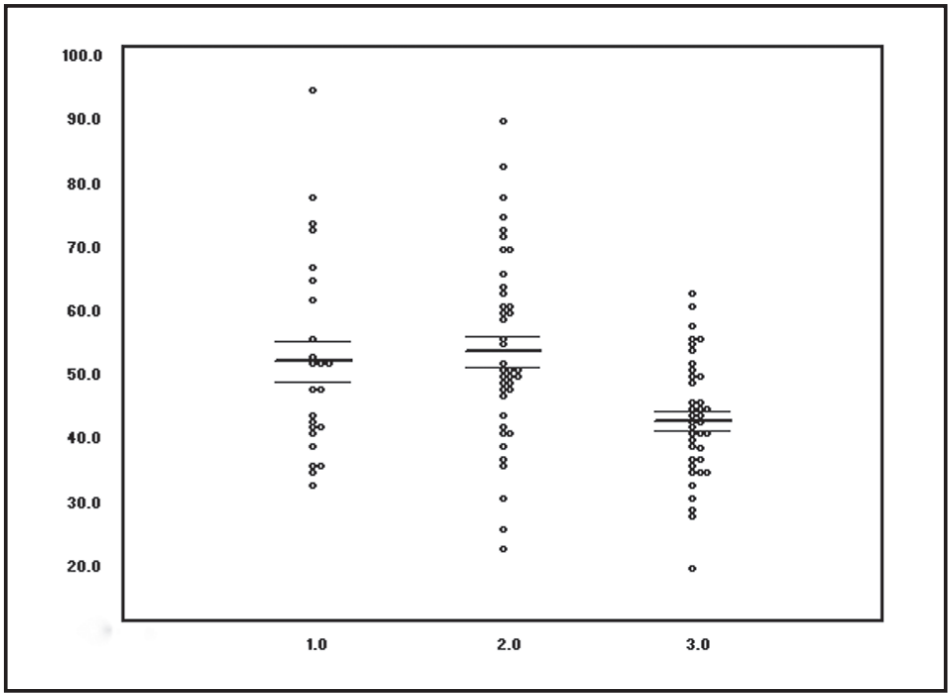

Fig. 1. Scatter plot of whole blood glutathione peroxidase (GPX) activity (expressed in U/g Hb) in normal controls (1.0), patients with myalgic encephalomyelitis / chronic fatigue syndrome (2.0) and major depression (3.0).

in g/L. The precision of our assays is checked with the controls provided by RANSEL. Our assay can be used to measure all of the glutathione-dependent peroxidases in whole blood. The inter-assay coefficient of variation is <7%.

Statistics

Differences between treatment means were analyzed by analysis of variance (ANOVA) or covariance (ANCOVA). Relationships between variables were ascertained by means of Pearson's product-moment correlation coefficients, regression analyses and multiple regression analyses with an p–to-enter of p=0.05. The independence of classification systems was checked by means of analysis of contingence Tables (χ^2-test) and Fisher's exact probability test. In order to assess the symptom profiles of different groups we used a stepwise discriminant multiple ANOVA (MANOVA) with an F-to-enter of p=0.05. The diagnostic performance of plasma CoQ10 for depression and TRD was checked by means of ROC (receiver operating characteristics) analysis with computation of the area under the ROC curve, sensitivity, specificity and predictive value of a positive test result (PV+) and with kappa statistics (Zweig and Campbell 1993). The significance was set at α=0.05 (two tailed).

RESULTS

Figure 1 shows that there are significant differences in whole blood GPX activity between the three study groups (F=7.2, df=2/100, p=0.001). The Dunn-Scheffe test showed that major depressed patients had significantly lower GPX values than normal controls (t=2.66, p=0.008) and ME/CFS patients (t=3.59, p=0.0008), while there were no significant differences in whole blood GPX between normal controls and ME/CFS patients (t=0.44, p=0.7). Patients with ME/CFS (mean age=37.5 ±14.5 years) were somewhat younger (F=4.4, df=2/100, p=0.01) than normal controls (mean age=45.5 ±9.9 years; t=2.47, p=0.015) and depressed patients (mean age=44.6 ±11.8 years; t=2.55, p=0.01), while there were no significant differences in age between depressed patients and controls (t=0.24, p=0.8). The male / female ratio was somewhat different (χ^2=7.9, df=2, p=0.02) between depressed patients (17/22), ME/CFS patients (5/35) and controls (7/17), with the only intergroup difference being a lower male/female ratio in ME/CFS than in depressed patients (χ^2=8.0, df=1, p=0.005). However, covarying for age and sex in an ANCOVA did not change the abovementioned results on the differences in GPX among the groups (F=6.4, df=2/98, p=0.003). Age (F=0.2, p=0.7) and gender (F=0.00, p=0.9) were not significant in this ANCOVA. Lower GPX yielded a significant albeit very weak diagnostic performance for major depression versus controls and

ME/CFS patients: at GPX < 40 U/g Hb we found a sensitivity = 51.3% and a specificity of 71.9% (κ=0.23, t=2.31, p=0.02).

In the combined patient group, we detected significant inverse correlations between whole blood GPX and the FF symptoms depressed mood (r=–0.38, p=0.0008) and autonomic symptoms (r=–0.24, p=0.03). We found that 19.8% of the variance in GPX was explained (F=9.5, df=2/76, p=0.0004) by the regression on depressed mood (F=13.2, p=0.0008) and autonomic symptoms (F=4.89, p=0.003). Discriminant MANOVA showed that two FF items significantly (F=8.8, df=1/77, p=0.004) discriminated patients with lower (< 40 U/g Hb) versus higher (> 40 U/g Hb) GPX activity, e.g. depression and autonomic symptoms (loadings on the discriminant score were 0.82 and 0.60, respectively).

In depressed patients we found a significant and negative correlation between whole blood GPX and the HDRS score (r=–0.36, p=0.02), but not with the FF score (r=0.28, p=0.08), although there was a significant and positive correlation between the HDRS and the FF score (r=0.58, p=0.00003). We found significant and negative correlations between whole blood GPX and two FF symptoms, i.e. autonomic symptoms (r=–0.42, p=0.007) and irritability (r=–0.35, p=0.03). GPX activity was not significantly different between TRD and non-TRD patients (F=0.00, df=1/37, p=0.9), nor between depressed patients who had been taking antidepressants and those who did not (F=0.00, df=1/37, p=0.9; 17 with versus 22 without). Thus, any possible effects of use of antidepressants on the results can be disregarded.

DISCUSSION

This study found that whole blood GPX activity was significantly lower in major depressed patients as compared with controls and ME/CFS patients and that there were no significant differences in GPX activity between patients with ME/CFS and controls.

The first major finding of this study is that depression is characterized by a lowered whole blood GPX activity and that it is significantly and inversely correlated to the severity of illness, as measured with the HDRS score and symptoms, such as depressed mood and irritability, another characteristic of depression. These findings corroborate those of Kodydková *et al.* (2009) and Oczan *et al.* (2004) who found a lowered GPX activity in depressed patients. In some other studies no significant differences could be found in GPX activity between depressed patients and controls (Srivastava *et al.* 2002; Andreazza *et al.* 2009).

However, Andreazza *et al.* (2009) examined bipolar disorder, while we examined major depressed patients in the acute phase of their illness. Srivastava *et al.* (2002) assayed GPX activity in PMNs, whereas we determined whole blood GPX activity. In any case, the findings on lower GPX activity are in agreement with translational studies which show lowered GPX activity in the bulbectomized rat and in Wistar rats with CMS-induced depression (Song *et al.* 1994; Eren *et al.* 2007a; 2007b). In addition, reduced concentrations of glutathione have been detected in animal models of stress-induced depression (Khanzode *et al.* 2003; Pal and Dandiya 1994). Lamotrigine and escitalopram, but not venlafaxine, significantly increased GPX activity in the brain of CMS-induced rats (Eren *et al.* 2007a). In another human study, it was found that GPX activity normalized during treatment (Ozcan *et al.* 2004). Taken together, those results show that lower GPX activity plays a role in the pathophysiology of depression.

The findings on lower whole blood GPX activity extent previous findings that the antioxidant capacity in the blood of depressed patients is diminished as has been evidenced by lowered vitamin E and C, serum zinc, tryptophan, tyrosine, albumin, glutathione, CoQ10 and catalase activity (see Introduction). The abovementioned results are also in agreement with other studies showing a decrease in the TAS in the blood of depressed patients (Galecki *et al.* 2009) and depressed patients with leukemia (Zhou *et al.* 2006).

The lower GPX activity and impaired antioxidative protection arguably predispose towards an induction of the IO&NS pathways and damage to membrane fatty acids and functional proteins and, by inference, to the neurotoxic damage that occurs in depression (Flohe 1978). Moreover, GPX has neuroprotective properties on its own. This may be deduced from studies using Ebselen (2-phenyl-1,2-benzisoselenazol-3[2H]-one), which mimics the activity of glutathione peroxidase (Muller *et al.* 1984) and which displays potent antioxidant and neuroprotective effects in vitro and in vivo (Posser *et al.* 2009). Ebselen displays neuroprotective properties against a number of different injuries, such as oxidative stress and oxidative damage of DNA that is involved in the delayed neuronal death in the brain regions; trimethyltin hydroxide-induced hippocampal injury; and neuronal damage and death caused by ischemic attacks and coronary artery occlusion (Satoh *et al.* 2004; Jean Harry *et al.* 2003; Seo *et al.* 2009; Li and Cao 2002; He *et al.* 2007). Ebselen prevents impaired neurogenesis within the dentate gyrus of the hippocampus as shown in a rat model of alcoholism (Johnsen-Soriano *et al.* 2007). The neuroprotective effects of Ebselen are obtained through different mechanisms, which are all relevant to major depression (Flohe 1978), such as increases in glutathione, ROS-scavenging activity; glutamate-related mechanisms; a decreased expression of nuclear factor kappa beta and inducible nitric

oxide synthase (iNOS); decreases in pro-inflammatory cytokines, such as interleukin-1 and tumor necrosis factor alpha; and inhibition of indoleamine 2,3-dioxygenase (Porciúncula *et al.* 2003; 2004; Yoshizumi *et al.* 2004; József and Filep 2003).

Our results that GPX is decreased in depression suggest that those patients would benefit from a treatment with antioxidants that modulate the GPX-glutathione pathways. Toward this end, not only Ebselen (see this Discussion), but also a combination of supplements with NAC, glutathione, cysteine and selenium is useful (Maes 2011). Recently, it has been shown that NAC is an effective augmentation strategy to treat depressive symptoms in bipolar disorder (Berk *et al.* 2008). Relevant to the IN-PRO pathophysiology of depression is the finding that the neuroprotective activity of Ebselen is augmented by NAC (Arakawa *et al.* 2007). Moreover, Ebselen exhibits antidepressant effects. In the rodent forced swimming test, Ebselen produces an antidepressant effect that seems to be dependent on its interaction with the noradrenergic and dopaminergic systems (Posser *et al.* 2009). In mouse, Ebselen prevents the biochemical effects of prolonged immobilization stress, including increased interleukin-1, cyclooxygenase-2 and nicotinamide adenine dinucleotide phosphate-oxidase in the brain, which eventually induce neuronal cell death in the cerebral cortex (Lee *et al.* 2006).

Another finding of this study is that lowered GPX activity is significantly correlated to the presence of autonomic symptoms, which occur in depression (Maes *et al.* 1993). There is ample evidence that gluthathione and GPX are involved in the function of the autonomic system. For example, GSH has an inhibitory whereas GSSH has a stimulatory effect on sympathetic nerve activity (Murakami *et al.* 1987). GSH decreases blood pressure (Kennedy *et al.* 2005a). Systemic depletion of GSH is found to be related to dysfunctions of the cardiac vagal system in hepatitis C patients (Barbaro *et al.* 1997). In stress-exposed rats, reduced glutathione levels correlate with increased sympathetic activity (Mercanoglu *et al.* 2008). The latter is the major contributing factor for the induction and prognosis of myocardial infarction. As such, lowered GPX and GSH may contribute to the comorbidity between depression and cardiovascular disorders which we have described in detail somewhere else (Maes *et al.* 2010).

In our study we were unable to detect any differences in whole blood GPX between ME/CFS patients and normal controls. Previously, increased GPX activity was detected in the muscles of patients with ME/CFS, indicating increased oxidative stress in muscles with consequently induced antioxidant enzyme activities (Fulle *et al.* 2000). The findings of our study, however, do not provide further evidence, either for disorders in GPX in the whole blood of patients

with ME/CFS, or for a role of GPX activity in the IO&NS pathophysiology of ME/CFS.

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