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No evidence of exogenous origin for the abnormal glutathione redox state in schizophrenia

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ABSTRACT

Schizophrenia has been associated with low glutathione (GSH), one of the most important substrates for natural defense against oxidative stress. This abnormality is often attributed to genetic or other pathological causes. However, low GSH in schizophrenia could also be due to insufficient antioxidant consumption or other exogenous factors. We evaluated GSH in relation to diet, smoking, and medication status in schizophrenia patients. We recruited 54 participants (29 schizophrenia patients and 25 normal controls). The Antioxidant Dietary Source Questions was used to estimate the total antioxidant capacity (TAC) from participants' diet. GSH and the oxidized form of glutathione (GSSG) were assayed. We found that GSH was significantly lower ($p<0.001$) while %GSSG was 2 to 5 fold higher ($p=0.023$) in patients compared with controls. No evidence for lower TAC dietary intake was found in schizophrenia patients compared with controls; rather nominally higher TAC level was found in the patients diet ($p=0.02$). Analysis of consumption of individual food categories also failed to find evidence of reduced dietary antioxidant intake in schizophrenia patients. Smoking and medications did not significantly predict the GSH deficit either. However, there was a significant smoking by diagnosis interaction on GSH ($p=0.026$) such that smoking was associated with higher GSH level in controls while smoking in patients was not associated with this effect. Schizophrenia patients may have an impaired upregulation of GSH synthesis that normally occurs due to smoking-induced antioxidative response. Lower GSH was independently present in patients on clozapine ($p=0.005$) and patients on other antipsychotics ($p<0.001$) compared with controls. In conclusion, none of the exogenous sources played a major role in explaining abnormalities in the glutathione pathway in patients. The state of abnormal glutathione redox may therefore be a part of schizophrenia pathophysiology.

1. Introduction

Schizophrenia has been associated with increased oxidative stress (Do et al., 2009; Kano et al., 2012). In aerobic cells, reactive oxygen species (ROS) and free radicals are byproducts of oxidative metabolism. The defense against oxidative stress is complex, involving multiple enzymes and antioxidant compounds. One of the major mechanisms for sequestration of ROS and free radicals is through the glutathione pathway where the reduced form of glutathione (monomeric glutathione or GSH) is converted to its oxidized form (glutathione disulfide or GSSG). Glutathione is present mainly (~99%) in GSH form in the body (Lenton et al., 1999). Although there are multiple defense systems and several of them have shown to be abnormal in schizophrenia, low GSH concentration is perhaps among the most consistently reported in schizophrenia. Low GSH has been found in post-mortem brain samples (Yao et al., 2006; Gawryluk et al., 2011) and in-vivo magnetic resonance spectroscopy studies (Do et al., 2000; Wood et al., 2009). GSH levels were decreased in the blood of antipsychotics-free and antipsychotics-treated patients (Raffa et al., 2009) and in cerebrospinal fluid of drug-naive patients (Do et al., 2000) suggesting that it is not secondary to antipsychotics. Taken together, low GSH level in schizophrenia provides a good index of an abnormal redox state associated with this illness, which is relatively consistently observed and not attributed solely to treatment with antipsychotics. Administration of the glutathione precursor n-acetylcysteine (NAC) improved clinical symptoms in patients (Berk et al., 2008), further supporting the association of oxidative stress with schizophrenia. However, it is unclear whether the mechanism that produces such effects is a direct result of pathophysiology associated with schizophrenia, an indirect result of other related pathology, or arising from sources outside the primary disease process that may differ between patients and controls. Hence, we examined the potential role of three exogenous factors in mediating

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oxidative stress, as indexed by abnormal GSH and GSSG levels, in schizophrenia: diet, smoking and antipsychotics.

First, diet is an important source of variance in oxidative stress in the general population (Jenkinson et al., 1999; Watson et al., 2005). We assessed whether there is a deficit in intake of antioxidants in food in schizophrenia, as dietary antioxidant contents provide the substrate and also regulate enzymes in the redox pathways (Bagchi et al., 1997; Huber et al., 2002). One prior study found no evidence of reduced antioxidant intake in schizophrenia (Strassnig et al., 2005). Another study showed that lower plasma antioxidant levels were not correlated with body mass (Reddy et al., 2003). However, to date no study has assessed whether dietary antioxidant intake affects the reported glutathione deficit in schizophrenia.

Second, smoking can contribute to oxidative stress by increasing lipid peroxidation (Solak et al., 2005). However, smoking also induces a potent antioxidant response and increases systemic production of glutathione (Gould et al., 2011). One study found increases in antioxidant enzymes in schizophrenic patients who smoke (Zhang et al., 2007). More data are needed to clarify if smoking contributes to the decreased glutathione in schizophrenia.

Third, the oxidative profile of antipsychotics remains unclear; some studies show antioxidant properties (Parikh et al., 2003; Miljevic et al., 2010; Stojković et al., 2012; Zhang et al., 2012) or improvement of peripheral glutathione and antioxidant enzymes by antipsychotics (Raffa et al., 2009; Zhang et al., 2012). On the other hand, patients on clozapine have shown higher superoxide dismutase and lower glutathione peroxidase, which was interpreted as secondary to increased oxidative stress by clozapine (Miljevic et al., 2010). Hence, we tested if clozapine versus non-clozapine antipsychotics were correlated with glutathione abnormalities in schizophrenia.

2. Methods

2.1. Participants

We recruited 54 participants (age range 18–62 years): 29 medicated patients and 25 normal controls, frequency-matched on age, gender and smoking status (Table 1). Patients were recruited from outpatient clinics of Maryland Psychiatric Research Center and neighboring community clinics. Controls were recruited using local media advertisements. Exclusion criteria included major medical and neurological illnesses, history of head injury with cognitive sequelae, mental retardation, substance dependence within the past 6 months or current alcohol or illicit drug abuse. Participants taking dietary supplements with antioxidant contents or drugs that inhibit beta-oxidation including acetaminophen and ibuprofen (Pessayre et al., 1999) on regular basis were excluded. The Structured Clinical Interview for DSM-IV (SCID) was administered to all subjects to obtain DSM-IV diagnoses. Controls were interviewed with SCID and had no DSM IV Axis I diagnosis and no family history of psychosis in 3 generations. Patients were clinically stable individuals with DSM-IV schizophrenia on antipsychotics. The dose of the antipsychotics was converted to chlorpromazine equivalent (CPZ). Among these patients, 3 were on first generation antipsychotic medications (CPZ of daily dose = 117.1 ± 23.4 mg) and the rest was on second generation antipsychotics: including 12 on clozapine (368.8 ± 137.5 mg), 3 on aripiprazole (18.3 ± 10.4 mg), 3 on risperidone (6.7 ± 4.6 mg),2 on olanzapine (10.0 ± 7.1 mg), 2 on quetiapine (800.0 ± 282.8 mg) and the remaining on 2 or more antipsychotic medications. All subjects gave written informed consent in accordance with local Institutional Review Board guidelines.

2.2. Measurement of GSH and GSSG

Participants were instructed to avoid strenuous physical exercise 24 h before, to fast overnight and not to smoke in the morning on the day of the blood draw. Venous whole blood sample was drawn between 8:30 AM to 10:30 AM. The key step of sample preparation was to prevent GSH, the predominant form of glutathione, to be oxidized into GSSG (so that GSSG can be accurately measured). GSSG samples were mixed with a pyridine derivative thiol-scavenging reagent before freezing. This redox scavenger overcomes shortfalls of other methods associated with undesirable enzyme reactivity or slow rate reaction (Güntherberg and Rost, 1966; Griffith, 1980). The scavenging reagent is a proprietary product by Oxford Biomedical Research Inc., Rochester Hills. MI, USA. It is related to the M2VP (1-methyl-2-vinylpyridinium trifluoromethane-sulfonate) described in the literature (Tietze, 1969), an approach with the advantage of complete scavenging of GSH in less than 1 min, compared to other methods that could take 60 min to remove GSH in the sample during which time oxidation of GSH may occur, resulting in overestimation of the GSSG concentration. Scavenger-processed (for GSSG) and unprocessed (for GSH) whole blood samples were immediately stored at −80 °C to avoid in vitro oxidation by room temperature and cellular lysation (blood draw occurred in the freezer room). At the end of the study, all samples were shipped using a secured dry ice container to Oxford Biomedicals, who performed GSH and GSSG assays in one batch blind to subject information. The reaction of GSH with Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid (DTNB)) gave rise to a product quantified spectrophotometrically at 412 nm. This reaction was used to measure the reduction of GSSG to GSH and its rate is proportional to GSH and GSSG concentrations (Iwasaki et al., 2009). The assay uses an eight-point standard curve for both total GSH and GSSG determinations. Given the predominant form is GSH (Lenton et al., 1999), GSSG is more meaningful when expressed as %GSSG

Table 1
Demographic, clinical characteristics, and dietary total antioxidant capacity (TAC) intake.

	Normal controls (N = 25)	Schizophrenia patients (N = 29)	Statistics	P values
Age (years)	38.76 ± 13.72[1]	41.10 ± 13.81	0.009	0.55
Sex (male:female)	11:14	20:9	2.48	0.12
Smoker:Nonsmoker	7:18	8:21	0.00	1.00
Education (year)	13.4 ± 1.6	12.1 ± 2.0	5.77	0.020
Age of illness onset (year)	n/a	21.4 ± 8.4	n/a	n/a
Duration of illness (year)	n/a	20.0 ± 13.4	n/a	n/a
BPRS score	n/a	43.28 ± 9.93	n/a	n/a
UPSA-2	102.9 ± 10.3	90.7 ± 10.5	17.62	<0.001
Fasting duration (hours)	12.18 ± 2.81	12.48 ± 3.64	0.11	0.74
GSH (μmol/L)	800.50 ± 201.73	516.50 ± 238.40	21.06	<0.001
GSSG (μmol/L)	19.96 ± 24.82	27.94 ± 23.19	8.56	0.005
%GSSG	2.40 ± 3.02	12.43 ± 24.18	2.28	0.023
TAC intake/week (μmol FRAP/week)	12,620.6 + 1046.2	15,257.7 + 1800.0	1.48	0.23

BPRS: Brief Psychiatric Rating Scale total score. UPSA-2: the University of California San Diego Performance-based Skills Assessment, 2nd edition total score (the data for controls was from 23 subjects because 2 controls did not complete UPSA-2).

[1] Values are mean ± sd.