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FORUM REVIEW ARTICLE

Magnetic Resonance Spectroscopy Study of the Antioxidant Defense System in Schizophrenia

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Abstract

Accumulating evidence suggests that oxidative stress associated with impaired metabolism of the antioxidant glutathione (GSH) plays a key role in the pathophysiology of schizophrenia. Magnetic resonance spectroscopy (MRS) is one of the brain-imaging techniques that can quantitatively measure bioactive substances such as GSH in the intact human brain. Four different measurement sequences including double quantum coherence (DQC) filtering, MEscher-GArwood Point-RESolved Spectroscopy (MEGA-PRESS), Stimulated Echo Acquisition Mode (STEAM), and PRESS have been used to evaluate the ^{1}H-MRS measurement of GSH in the brains of patients with schizophrenia. Although the results of these studies were somewhat diverse, a negative correlation between brain GSH levels and the severity of negative symptoms in schizophrenia patients suggests that increasing the brain GSH levels might be beneficial for schizophrenia patients with negative symptoms. Moreover, a recent double-blind, placebo-controlled study demonstrated that add-on of *N*-acetyl-L-cysteine (NAC), a precursor of GSH, to antipsychotics improved the negative symptoms and reduced the side effects (akathisia) in patients with chronic schizophrenia. MRS study of the antioxidant defense system in schizophrenia still remains in the infantile stage; future studies are needed to examine the brain GSH level before and after NAC treatment, and thereby to provide direct evidence of the induced production of GSH in the living brain. *Antioxid. Redox Signal.* 15, 2057–2065.

Introduction

Accumulating evidence suggests that oxidative stress plays a role in the pathophysiology of schizophrenia (10, 31, 41, 59). Reactive oxygen species (ROS), such as super oxide, hydroxyl ions, and nitric oxide, are generated under physiologic aerobic metabolism, and excess levels of ROS or impairments to the antioxidant defense system can cause cell injury in the central nervous system (CNS). Dopamine (DA) is also a major source of ROS in the CNS, as excess DA can easily autooxidize to produce DA-quinone (4). Thus, the detoxification of ROS is of substantial importance in protecting neural tissues from oxidative stress (33).

Previous studies have reported the occurrence of impairments in the antioxidant glutathione (GSH) levels and in the activities of the antioxidant enzymes in patients with schizophrenia (14). Currently, GSH is the only antioxidant that has been measured with magnetic resonance spectroscopy (MRS) in schizophrenia patients (15, 32, 50, 56). Therefore, in this article, we provide a brief overview and recent findings on the MRS studies of antioxidants in the brains of patients with schizophrenia.

Glutathione and Schizophrenia

Glutathione (GSH: L-glutamyl-L-cysteinyl-glycine) is the most abundant thiol in mammalian cells, with intracellular concentrations as high as 1 to 3 m*M*. GSH is known to be a nucleophilic scavenger and an enzyme-catalyzed antioxidant, and plays an important role in protecting the brain against ROS and harmful xenobiotics (16, 17, 26, 27). GSH is synthesized *in vivo* by the consecutive action of two enzymes. γ-Glutamylcysteine synthetase (glutamate cysteine ligase (GCL)), the rate-limiting enzyme for GSH synthesis, uses L-glutamate and L-cysteine as substrates and forms the dipeptide γ-glutamylcysteine, which is combined with glycine in a reaction catalyzed by the glutathione synthetase to generate GSH. The balance of cellular synthesis and the consumption of GSH are regulated by feedback inhibition of the GCL reaction by the end-product GSH (16, 17, 26, 27).

Several lines of evidence suggest that impaired GSH metabolism has substantial importance in the pathogenesis of schizophrenia. Decreased activity of glutathione peroxidase (GSH-Px) in red blood cells (1, 6) and plasma (60) of patients with schizophrenia has been reported. Plasma GSH-Px levels

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have been significantly and positively correlated with psychosis-rating scores in schizophrenia patients (58). Furthermore, a study using postmortem brain samples demonstrated that the levels of GSH, GSSG, GSH-Px, and GSH reductase in the caudate region of brains from schizophrenia patients were decreased (57). It has also been shown that differences in gene polymorphisms imply the negative effect on GSH metabolism in patients with schizophrenia. The activity of GCL, the rate-limiting enzyme for GSH synthesis, as well as the expressions of the catalytic GCL subunit (GCLC) and GCL modifier subunit (GSLM) in cultured skin fibroblasts from schizophrenia patients, were significantly decreased compared with those in control subjects (22, 23, 52). The distributions of polymorphisms in the genes of these subunits were also different between the schizophrenia subjects and controls (22, 52). Taken together, these findings provide functional evidence that an impaired capacity to synthesize GSH under oxidative stress is a vulnerability factor for schizophrenia (14).

^{1}H-MRS Technique

Among the *in vivo* molecular-imaging techniques, MRS provides direct information on the living biochemistry without using radioactive sources, and thus is widely used for its noninvasiveness. Because the same magnet is used for magnetic resonance imaging (MRI) and MRS, morphologic information can be acquired simultaneously, combined with single-voxel localization technique, Point RESolved Spectroscopy (PRESS) (8), and STimulated Echo Acquisition Mode (STEAM) (19, 20). Thereby, one can place the region of interest (ROI) on the specific brain region. Subtle neurobiologic abnormalities that can be detected with MRS often precede anatomic abnormalities; a good example is a previously reported case of multiple sclerosis, in which a discrepancy was observed between the symptoms and anatomic abnormalities on MRI (11).

MRS can be used to study the resonance spectra of compounds in the brain that contain odd-numbered (paramagnetic) nuclei, such as proton (^{1}H), lithium (^{7}Li), carbon (^{13}C), oxygen (^{17}O), fluorine (^{19}F), sodium (^{23}Na), magnesium (^{25}Mg), and phosphorus (^{31}P). Among those nuclei, ^{1}H-MRS has been the most widely used, because ^{1}H is the most abundant nucleus and provides the best access to a large group of compounds in the brain associated with major metabolic pathways, such as neurotransmitters, fatty acids, carbohydrates, and free amino acids (34). More than 30 compounds can be quantified with ^{1}H-MRS (21), although the proton has a narrow range of chemical shifts (15 ppm range) (9), which leads to unmanageable overlap of metabolite spectra (2), particularly at low field strength (<3.0T), and a requirement that the dominant water signals be suppressed. Because of their substantial concentration in the brain and singlet resonances, several metabolites have been used as the main markers for ^{1}H-MRS; these include *N*-acetyl aspartate (NAA, 2.0 ppm), creatine (Cre, 3.03 ppm; composed of creatine and phosphocreatine), and choline [Cho, 3.2 ppm; composed of four membrane/myelin-related chemicals: phosphorylethanolamine (PE), phosphorylcholine (PC), glycerophosphorylethanolamine (GPE), and glycerophosphorylcholine (GPC)]. In particular, NAA has been used as an *in vivo* marker of neuronal density (39, 44, 45). Some other metabolites of interest are not easily accessible.

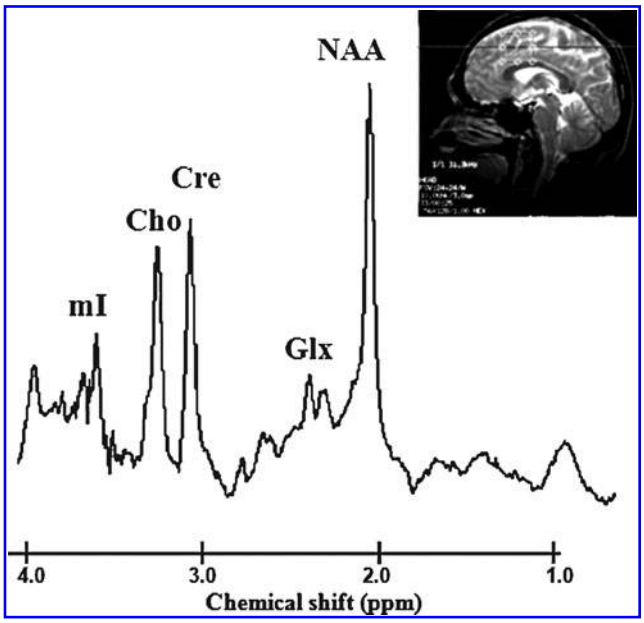

FIG. 1. Typical ^{1}H-MRS spectrum of human brain acquired with normal PRESS sequence (TE = 30 ms, TR 5,000 ms, NEX = 128). NAA, *N*-acetyl aspartate; Glx, complex of glutamate and glutamine; Cre, creatine; Cho, choline; mI, myoinositol. Note that NAA is the most distinct at 2.0 ppm. The spectrum was acquired from the ROI placed in the posterior medial prefrontal cortex (24×22×30 mm, upper right).

For example, Glx has been commonly used as a description of combined signals of glutamate, glutamine, and γ-aminobutyric acid (GABA) because methylene protons of glutamate [Glu, 2.04- to 2.35-ppm range; GABA, 2.28 ppm; and glutamine (Gln, 2.12 to 2.46 ppm) resonate as complicated and overlapped peaks. A typical ^{1}H-MRS spectrum of human brain acquired by normal PRESS sequence is shown in Fig. 1.

Detection of GSH with ^{1}H-MRS

Glutathione (GSH) is a tripeptide made up of glutamate, cysteine, and glycine (Fig. 2a), and protons of each moiety resonate differently under a magnetic field. Figure 2b shows a simulated spectral pattern of GSH at a field strength of 3.0 T, and as shown, GSH spectra show a complicated pattern because of the strong J-coupling between some of its hydrogen nuclei. From the glutamate moiety (GSH-Glu), the methylene protons give two separate multiplets at approximately 2.15 and 2.55 ppm, and the methine proton gives a doublet-of-doublets at 3.77 ppm. The cysteine moiety (GSH-Cys) gives a doublet-of-doublets at 4.56 ppm (α-CH), 2.93 and 2.978 ppm (β-CH_2), and the methylene proton of the glycine moiety (GSH-Gln) gives a singlet at 3.77 ppm (21). Until recently, measurement of GSH with ^{1}H-MRS had been elusive mainly for two reasons. First, the concentration of GSH in the brain is lower than the cerebral concentrations of other major metabolites, such as NAA, Cre, and Cho. Second, the resonances of other metabolites are overlapped with those of GSH. For example, the protons of the glutamyl moiety of the GSH resonance are heavily overlapped with those of free glutamate (Glu), glutamine (Gln), aspartate (Asp), and the aspartate group of NAA. Therefore, it is likely that clear dissociation of

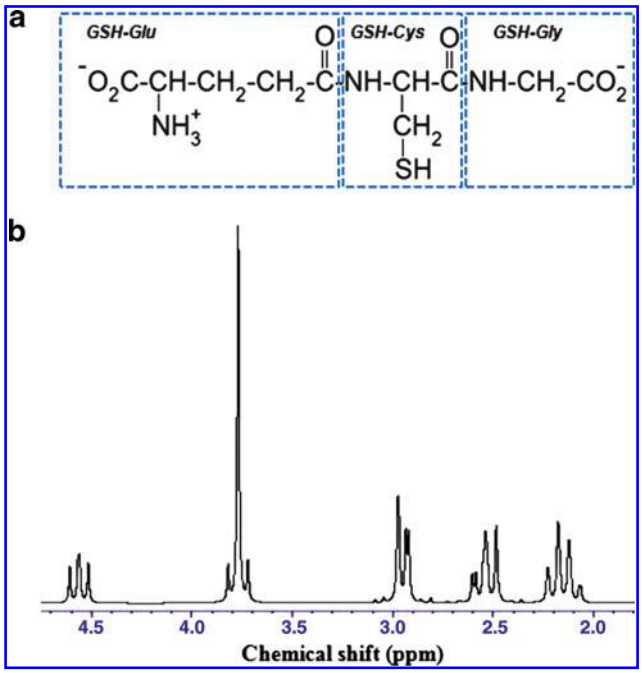

FIG. 2. Spectra and structure of glutathione. (a) Structure of glutathione (GSH), composed of three amino acid moieties. **(b)** Simulated spectra of glutathione under 3 T. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

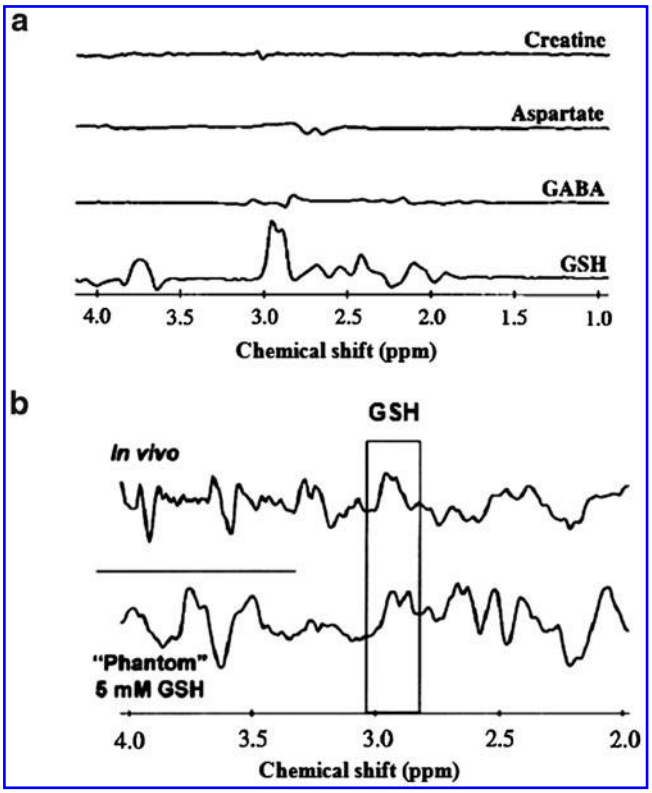

FIG. 3. Filtered spectra. (a) DQC filtered spectra of phantom containing Cre, GSH, aspartate, and GABA (7:3:1:1). Filtered spectra show that the signal of the 2.9-ppm region from GSH-cys and the other are well suppressed. **(b)** Representative *in vivo* (top) schizophrenic brain and *in vitro* (containing 5 m*M* GSH) DQC filtered spectra. Reproduced from (15, 53).

the resonance of GSH from other overlapped resonances is crucial for GSH measurement.

Spectral editing is one of the techniques used to reduce the information content of the spectral acquisition to enhance or suppress signals of certain nuclei (2, 55). Two spectral editing techniques, double quantum coherence (DQC) filtering (53) and MEGA-PRESS (MEscher-GArwood-Point RESolved Spectroscopy) (50), have been applied in the schizophrenic brain. The detailed theoretic aspects of these editing techniques are outlined elsewhere (2, 47, 53, 55). The common point in these techniques is that they focus on the cysteinyl moiety of GSH (GSH-Cys), which forms a strongly coupled ABX spin system. Two protons from β-CH_2 of GSH-Cys resonate at 2.93 and 2.97 ppm, and the resonances of these protons are overlapped by those of Cre (3.03 ppm), Asp (2.82 ppm), and GABA (3.01 ppm); thus the two editing techniques separate the GSH-Cys signal from these other signals, particularly the Cre signal, which is more hazardous because of high concentration of Cre in the brain.

The detection of GSH in the human brain was first reported by Trabesinger *et al*. (53) under 1.5 T, and this method was subsequently applied to patients with schizophrenia (15). GSH spectra were acquired by a DQC filtering sequence, which consists of the filtering of two radiofrequency (RF) pulse sequence with two field gradients integrated into a localizing PRESS sequence. *In vivo* spectra were acquired in healthy volunteers (53) and in schizophrenia patients and controls (15) from a 17.4 ml (24×22×33-mm) volume of ROI placed at the ventral medial prefrontal cortex. As shown in Fig. 3a, DQC filtering successfully suppressed the signals of other metabolites, and *in vivo* GSH signals comparable with the one from phantoms were obtained from both the controls and schizophrenia patients (Fig. 3b).

Studies using MEGA-PRESS sequences to detect GSH *in vivo* in schizophrenia patients were first reported by Terpstra *et al*. (47), and then by our group under 4 T and 3 T (32), respectively. In brief, two selective editing pulses that targeted the cysteinyl α proton of GSH (4.56 ppm) enabled its J-coupled cysteinyl β-CH_2 signal to appear inverted, and thus, subtraction of the editing pulse "on"-scans from "off"-scans resulted in the exclusive detection of the cysteinyl β-CH_2 GSH signal. Figure 4 shows the typical spectra from a phantom solution containing (NAA, Cre, GSH) acquired with normal PRESS (a), and MEGA-PRESS (b) sequences, and the ones from the human brain acquired with normal PRESS (c) and MEGA-PRESS (d) sequences. As is shown, a sufficiently strong GSH signal appears in the human brain, but it should be noted that the aspartyl resonances of NAA at 2.45 and 2.67 ppm are coedited (47). The spectra-acquiring parameters, such as TE and TR, differ among the three studies, and they are experimentally set at optimal values in each study.

A major advantage of DQC filtering over MEGA-PRESS editing is its shorter acquisition time (<5 min) because of the spectra acquisition with a single shot (53, 55). The measurement time is critically important because the motion of subjects severely affects the quality of MRS spectra acquisition. Motion artifacts are observed as an increase in line width, a diminished quality of water suppression, or a doubling of peaks (28). MEGA-PRESS editing requires twice as much time

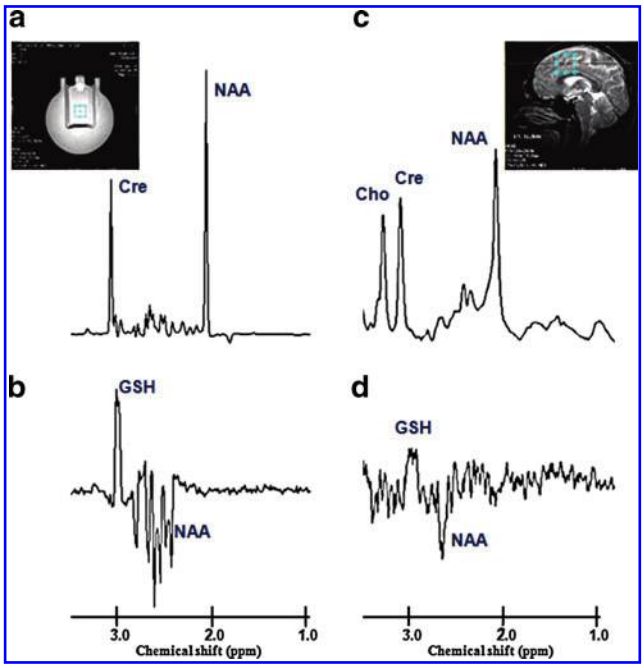

FIG. 4. Representative spectra of GSH from a phantom (containing GSH, 15 m*M*; NAA, 10 m*M*; and Cre, 8 m*M*) and a human subject. Spectra shown are not edited **(a, c)** and edited by MEGA-PRESS **(b, d)**. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

(or more) for acquisition as does DQC filtering, and thus it is more susceptible to artifacts; however, one of the advantages of MEGA-PRESS is its high S/N ratio, as shown in Fig. 4b and d. The recent shift from 1.5-T MRI to 3-T or higher magnetic field strength MRI is that it shortens the necessary measurement time (5, 24) in addition to allowing dissociation of overlapped signals. Thus, the high field strength can highlight the advantages of MEGA-PRESS editing by allowing the acquisition of GSH spectra with a sufficient number of excitations (NEX).

Very recently, Kaiser *et al*. (29) reported ^{1}H-MRS detection of the glycine residue of reduced glutathione (29). In their study, they focused on the glycine residue (GSH-Gly) whose protons resonate as a singlet at 3.77 ppm (21). The editing method is based on J-difference editing, and the quantification requires a rather complicated process compared with the GSH-Cys editing. Most of the data validation is achieved by phantom and simulation under a very high magnetic field (4 T to 14.0 T); *in vivo* data were acquired under 4 T only for three subjects. This method would be an attractive option for detecting GSH *in vivo* because of the high S/N, although the same method must be shown as applicable under a more clinically available magnetic field (3 T or less).

^{1}H-MRS Measurement of GSH in the Brains of Patients with Schizophrenia

To date, four reports measured GSH in the schizophrenic brain *in vivo* with ^{1}H-MRS (15, 32, 50, 56) (Table 1). The measurement sequences used were DQC filtering (15), MEGA-PRESS (32, 50), STEAM (50), and PRESS (56), and the quantification was done by using water (15) or NAA (50) as an internal reference, GSH in phantom (32), and LCModel (50, 56).

In the first study by Do *et al*. (15), they applied the same method as in their previous study (53) with 14 male patients (nine had schizophrenia, and five had schizophreniform disorder), including five drug-naïve patients. Compared with that in 14 normal controls, the GSH level in schizophrenics was significantly lower (52%; $p = 0.0012$; Mann–Whitney U test), and this was the first result showing that GSH is low in the schizophrenic brain *in vivo*. This result was strengthened by their subsequent finding of a significant, 27% decrease in the level of total GSH ($p < 0.05$) in the cerebrospinal fluid of drug-naïve schizophrenia patients ($n = 26$) (15). These results were a milestone for the GSH hypothesis in schizophrenia, because previously no report of *in vivo* GSH measurement existed for the schizophrenic brain. However, the next *in vivo* study by Terpstra *et al*. (50) with MEGA-PRESS did not show a significant difference in the GSH level between normal controls ($n = 9$) and schizophrenia patients ($n = 11$). Moreover, no significant difference was found in other metabolites quantified by LCModel, such as NAA, Cre, mI, Glu, and Gln. In their study, the primary objective was to validate the reliability of two different GSH-quantification methods, MEGA-PRESS editing and LCModel by the STEAM sequence, as described earlier, and the clinical characteristics of subjects were not a main concern.

Thus, when our group started its ^{1}H-MRS study, conflicting results were noted about GSH in the schizophrenic brain. We investigated GSH in schizophrenia, not within the context of a simple case–control study, but rather with the goal of examining the correlations between the GSH level and clinical features, including the severity of clinical symptoms (positive symptoms, negative symptoms, and cognitive deficits), and genetic aspects (32) of subjects, which had been mostly ignored in the two previous studies (15, 50). For this aim, clinical severity was assessed with the Scale for the Assessment of Negative Symptoms (SANS) and Brief Psychiatry Rating Scale (BPRS). Subjects enrolled in our study were 16 controls (four women) and 20 schizophrenia outpatients (eight women), with a mean 7.3 years duration of illness. The result was that no significant difference in the overall GSH level was found between patients (0.808 ± 0.26 m*M*) and normal controls (0.928 ± 0.24 m*M*) (Table 1). However, a significant negative correlation ($r = -0.68$; $p < 0.001$; Pearson correlation coefficients) was seen between the GSH level and the SANS total score, as well as the SANS subscales (affective flattering or blunting, alogia, avolition-apathy, and anhedonia-asociality) in schizophrenia patients (Fig. 5). To examine the genetic aspects, we analyzed the polymorphisms of several genes involved in GSH metabolism and, although we did not find significant associations, we did find trends toward significance in the difference in the GSH level in specific genotypes of *GSTT2* and *GCLM* between the patients and controls ($p = 0.058$ and 0.099, respectively), although a further detailed study using a larger sample will be necessary. Interestingly, it has been reported that the GSH concentration dramatically changes according to the induced oxidative stress at the cellular level, and the increase is regulated by the expression level of the *GCLM* or *GCLC* gene (51, 54). In the cultured human skin fibroblast study by Gysin *et al*. (22, 23), specific *GCLC* gene polymorphisms alter the production of GSH. Thus, the results of GSH measurement with ^{1}H-MRS might reflect those genetic differences in gene polymorphisms or transient expression level in the brain.

Quantification of GSH was accomplished by those editing studies (Table 1). Trabesinger *et al*. (53) used tissue water

TABLE 1. ^{1}H-MRS STUDY IN DETECTING GLUTATHIONE IN SCHIZOPHRENIA OR NORMAL CONTROL

Study	*Subject* (n)	T	*Measurement*	*Quantification method or reference*	*Acquisition parameters (TE/TR/NEX)*	*ROI/size*	*Estimated (GSH), mean ± SD (mM)*	*Other findings*
Do *et al.* (15)	N.C. (14) vs. Sz (14)	1.5	PRESS/DQC filtering	Water	75 ms/2,000 ms/128	Ventral mPFC/17.4 ml (24×22×33 mm)	Relative intensity	52% lower of GSH signal in Sz patients
Terpstra *et al.* (47)	N.C.(9) vs. Sz (13)	4	STEAM/MEGA-PRESS	NAA, LCModel	5 ms/4,500 ms/256 (STEAM) 68 ms/4,500 ms/512 (MEGA-PRESS)	mPFC 8 ml (STEAM) 17 ml (MEGA-PRESS)	1.6 ± 0.4 (STEAM) 1.4 ± 0.4 (MEGA-PRESS)	No difference
Matsuzawa *et al.* (32)	N.C. (16) vs. Sz (20)	3	MEGA-PRESS	GSH in phantom	94 ms/1,500 ms/512	Posterior mPFC/18.4 ml (22×30×27 mm)	0.93 ± 0.2 (N.C.) 0.81 ± 0.3 (Sz)	Negative correlation with SANS score
Wood *et al.* (56)	N.C. (18) vs. FEP (30)	3	PRESS	LCModel	30 ms/3,000 ms/128	Temporal cortex, bilateral 8 ml	Relative intensity	22% higher of GSH signal in FEP group

DQC, double quantum coherence; FEP, fist episode psychosis; GSH, glutathione; MEGA-PRESS, Mescher-Garwood Point-Resolved Spectroscopy; mPFC, medial prefrontal cortex; NAA, *N*-acetyl aspartate; N.C., normal control; NEX, number of excitations; ROI, region of interest; SANS, Scale for the Assessment of Negative Symptoms; SD, standard deviation; STEAM, Stimulated Echo Acquisition Mode; Sz, schizophrenia; TE, echo time; TR, repetition time.

FIG. 5. Correlation between GSH levels and the severity of negative symptoms in schizophrenia. A significant negative correlation ($r = 0.68$; $p < 0.001$) was found between GSH levels and SANS total scores of schizophrenia patients ($n = 20$). Reproduced from (32).

content as an internal standard, but they estimated the GSH concentration only as roughly 2 to 5 m*M* in the brain because of the limited information on the relaxation time of the two compounds. In the study by Terpstra *et al*. (47), co-edited aspartyl NAA was basically used as an internal standard, and then the concentration values were obtained by applying the edited spectra to the LCModel fitting. Our group applied the areas of *in vivo* GSH spectra to a reference linear concentration curve from GSH phantom solutions (containing GSH, 0.3 to 30.0 m*M*; NAA, 10 m*M*; and Cre, 8 m*M*) (Fig. 6a).

The fourth study was reported by Wood *et al*. (56); the GSH data were quantified by LCModel. LCModel analyzes *in vivo* spectra as a linear combination of *in vitro* model spectra acquired from a set of phantom metabolites under comparable *in vivo* conditions, and thus it provides well-separated values of overlapped metabolite peaks, such as Glx (35, 36). Subjects were normal controls ($n = 18$) and patients with first-episode psychosis, which included schizophrenia ($n = 9$), schizophreniform psychosis ($n = 11$), schizoaffective disorder ($n = 3$), major depression with psychotic symptoms ($n = 4$), and psychosis NOS ($n = 3$). Of the total 30 patients, 13 were neuroleptic-naïve, and the remaining 17 had received at least one atypical antipsychotic. By using 3-T MRS, they placed an 8 cm^3 ROI on the temporal lobe, including the hippocampus, and used a standard PRESS sequence with a short echo (TE 30 ms) and TR of 3 s. The results were inconsistent with the previous three studies (15, 32, 50), in that the GSH level was, surprisingly, 22% higher ($p = 0.035$) in the patient group than in the healthy control subjects. They also evaluated the skin-flush response to topical niacin as an indirect indicator of oxidative stress. A higher GSH level was more evident in

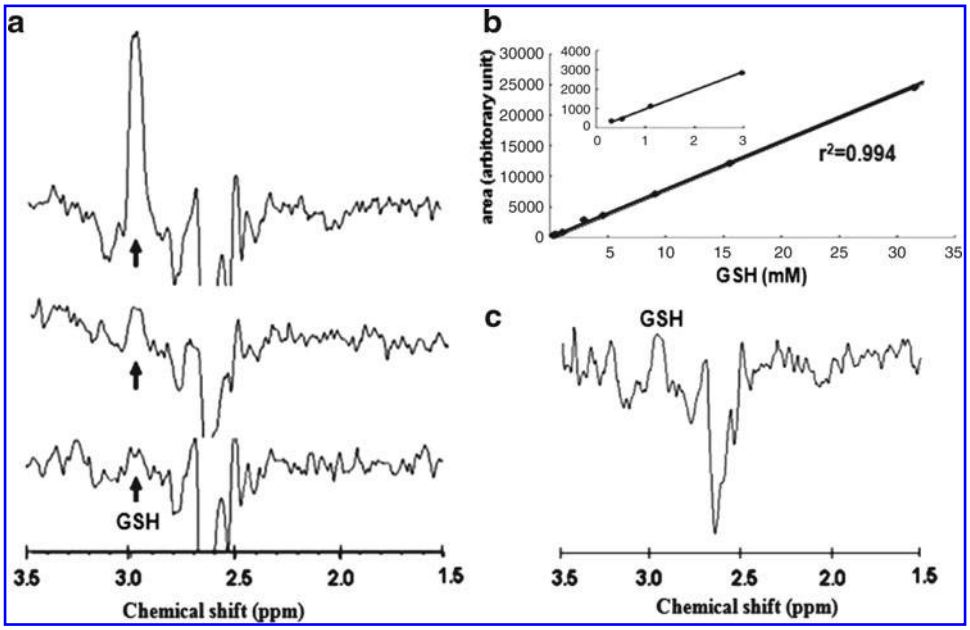

FIG. 6. Correlations between negative signal and phantom concentration. (a) Representative data of reference phantom spectra of GSH (0.5, 1.0, and 3.0 m*M*). Note that the GSH signal increases according to the phantom concentration. **(b)** Quantification of GSH. Plots showing a linear correlation ($r^2 = 0.994$) between the GSH signal area at 2.95 ppm and the concentration of GSH. **(c)** Representative GSH signal from the posterior medial frontal cortex of a human subject. The GSH level was calculated as 0.735 m*M* by applying the linear concentration curve on **(b)**. Pictures in **(a)** and **(c)** represent the ROIs placed in phantom and human, respectively. Reproduced from (32) with permission.

nonresponders to niacin treatment, possibly indicating that GSH is increased in a compensatory manner. Although their result was very intriguing, it should be noted that the mean percentage standard deviation (%SD) of the fit of the obtained data was higher than 20%. According to the manufacturer's manual, it is demanded that the %SD (also called Cramer-Rao lower bonds) of the estimated concentration be lower than 20% (37). A rather broad (>11 Hz) mean full-width half-maximum spectrum also represents insufficient uniformity in the local magnetic field. Meanwhile, Terpstra *et al*. (50) reported *in vivo* GSH data from LCModel analysis by using 4-T MRS with reliable exclusion criteria for %SD (data with >15% SD were disregarded). The results were well comparable with the data from MEGA-PRESS editing with low %SD (mean, 14%).

Effect of GSSG Signal to GSH Signal

In addition to the difficulty of detecting GSH signals *in vivo*, one should consider that GSH actually is present in two forms, reduced (GSH) and oxidized (GSSG) under physiologic conditions (18). The contribution of GSSG signals to GSH signals was conducted with a MEGA-PRESS sequence under 3 T (40) and 4 T (49). In the study by Satoh and Yoshioka (40), spectra were acquired by MEGA-PRESS editing, and Fig. 7 shows the signals from phantoms containing GSH/GSSG = 100:1, 10:1, and 1:1 and 10% BSA as a negative control. As can be seen, the 100:1 solution had almost no effect on the GSH signals, and the 10:1 solution gave a very small reverse signal of GSSG, which was almost negligible. Under the normal physiologic conditions, GSSG exists only at the 1/100 GSH level (12), and even under oxidative stress, GSSG could be increased to the 1/10 GSH level (3), which means GSH is much more abundant, even under severe oxidative stress. Aging affects the GSSG level in plasma, but it remains at the 1/100 GSH level as well (18). Therefore, GSSG signals would be negligible when obtaining GSH signals *in vivo* with ^{1}H-MRS with MEGA-PRESS editing. In a recent study of Terpstra and colleagues (48) in which simultaneous detection of GSH and vitamin C (ascorbic acid) was achieved, the GSSG signal was shown to be negligible. In addition, our preliminary data suggest that this is true for the GSH spectra obtained with DQC filtering and LCModel as well.

^{1}H-MRS Detection of Ascorbic Acid

Recently, another antioxidant, ascorbic acid (vitamin C) in the human brain was quantified with ^{1}H-MRS (42, 46). Ascorbic acid is well established as an essential nutrient that functions as an antioxidant. The concentration of ascorbic acid in the human brain is about 1.0 m*M* (38). This concentration is

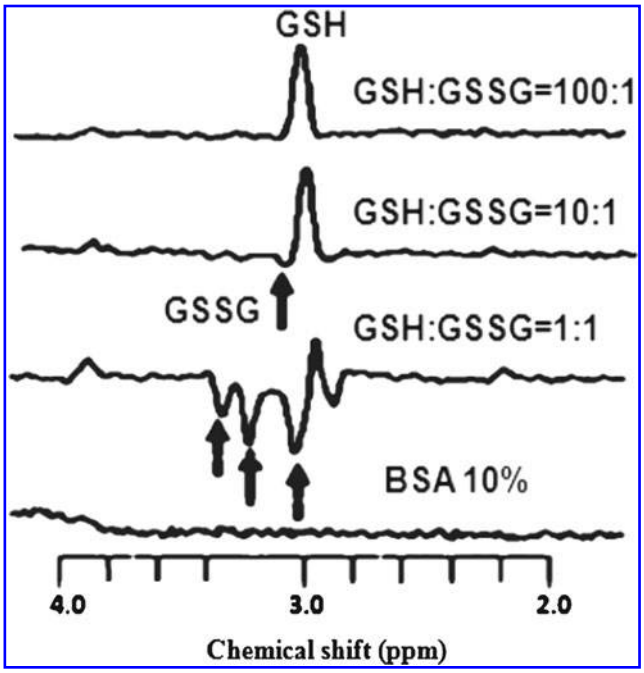

FIG. 7. The MEGA-PRESS–edited spectra from phantoms containing GSH/GSSG, 100:1, 10:1, and 1:1, and 10% BSA. *Bold arrows*, reversed signals that originated from GSSG. Reproduced from (40) with permission.

sufficiently high to be detectable with MRS. Ascorbic acid resonates at 3.73, 4.01, and 4.50 ppm, with overlapped resonances with Glu (3.75 ppm). By applying MEGA-PRESS editing for both ascorbic acid (targeted the proton at 4.01 ppm) and GSH alternatively in one examination, Terpstra and Gruetter (48) successfully quantified both of them simultaneously in the human brain. Shih *et al.* (42) demonstrated that the ascorbic acid in the brain could be quantified by LCModel with a PRESS sequence under 3 T when sufficiently narrow full-width half-maximum values were obtained. Because the supplementation of antipsychotic drugs with agents such as ascorbic acid has beneficial effects on the positive and negative symptoms of schizophrenia (13, 43), it will be of interest to examine whether levels of ascorbic acid are altered in the brains of patients with schizophrenia.

Summary and Future Perspectives

In this review, we summarized the currently available methods for ^{1}H-MRS detection of the major antioxidant GSH. To date, four articles report measuring GSH levels in the brains of patients with schizophrenia (15, 32, 50, 56), but the results of these studies have not been congruent with respect to whether GSH is decreased in the schizophrenic brain compared with normal controls. The discrepancy is partly explained by the methodologic differences in magnetic field strength, reference signal, and the location of the ROI. Tissue segmentation would be demanded in the future studies to evaluate partial-volume effects due to the composition of cerebrospinal fluid in the ROI. Furthermore, although the GSH level *in vivo* exists at a sufficient concentration to be detected by the editing sequence methods, these methods are too complicated for use in routine examinations. LCModel quantification after the signal acquisition by a normal PRESS or STEAM sequence is a much easier option (47, 48, 56), but this method will require further verification in terms of its reliability at magnetic fields of 3 T or lower. In addition, it remains to be elucidated whether the GSH level in the brain reflects a transient increase of oxidative stress or a prolonged increase, or whether it changes dynamically in relation to the brain function, psychotic state, and medication. From the data currently available, we cannot conclude that decreased or increased GSH levels in schizophrenia patients constitute trait level (*i.e.*, static) differences, rather than being a reflection of transient oxidative stress. Further evidence should be accumulated with more homogeneous patient profiles in diagnosis as well.

Interestingly, we found a negative correlation between GSH levels and negative symptom severity (32), suggesting that increasing the brain levels of GSH should be considered a potential therapeutic approach for negative symptoms in schizophrenia. Recently, Lavoie *et al.* (30) reported that treatment of schizophrenia patients with *N*-acetyl-L-cysteine (NAC), a precursor of GSH, significantly improved impaired mismatch negativity, which is an auditory evoked potential component related to *N*-methyl-D-aspartate (NMDA)-receptor function. Furthermore, a multicenter double-blind, placebo-controlled trial of NAC showed improvement of negative symptoms after 6 months of treatment with NAC (7). These findings suggest that NAC has potential as a therapeutic drug for negative symptoms in schizophrenia. This is encouraging, because the present antipsychotic treatments are rather ineffective against cognitive and negative symptoms of patients with schizophrenia. It would be an intriguing issue to examine the brain GSH level before and after NAC treatment, and thereby provide direct evidence of the induced production of GSH in the living brain (25).

Finally, further detailed studies of the antioxidant defense system in the brain by using MRS technology would provide novel biomarkers for the early detection of schizophrenia as well as possibly a novel therapeutic approach for prevention of this disease.

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Abbreviations Used

Asp = aspartate
BPRS = Brief Psychiatry Rating Scale
Cho = choline
CNS = central nervous system
Cre = creatine
Cys = cysteine
DA = dopamine
DQC = double-quantum coherence
GABA = γ-aminobutyric acid
GCL = glutamate cysteine ligase
Gln = glutamine
Glu = glutamate
GSH = glutathione
GSH-Px = glutathione peroxidase
GSSG = glutathione disulfide
GSTT2 = glutathione-*S*-transferase T2
LCModel = linear combination model
MEGA-PRESS = Mescher-Garwood point-resolved spectroscopy
MRI = magnetic resonance imaging
MRS = magnetic resonance spectroscopy
NAA = *N*-acetyl aspartate
NAC = *N*-acetyl-L-cysteine
NEX = number of excitations
NMDA = *N*-methyl-D-aspartate
PRESS = point-resolved spectroscopy
ROI = region of interest
ROS = reactive oxygen species
SANS = Scale for the Assessment of Negative Symptoms
STEAM = stimulated echo-acquisition mode
TE = echo time
TR = repetition time

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