Elevated Plasma Lipid Peroxides at the Onset of Nonaffective Psychosis

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Background: *Impaired antioxidant defense and increased lipid peroxidation has been reported in chronic schizophrenic patients. Recently, we have reported an impaired antioxidant defense in never medicated first-episode schizophrenic and schizophreniform patients. We report now a concomitant increase in plasma lipid peroxides.*

Methods: *The plasma lipid peroxides [thiobarbituric acid reactive substances (TBARS)] were analyzed by chemical and high performance liquid chromatography procedures in 26 patients admitted for a first episode of schizophrenic (*N = *17) or schizophreniform psychosis (*N = *9) and 16 normal control subjects. The patients had a duration of 4.5 days (SD 2.8) of psychosis at the time of the study.*

Results: *Plasma TBARS levels were significantly higher in the patients than in normal controls (*$P < .002$*). TBARS levels were above the normal range in 16 of the 26 patients. Higher TBARS levels were associated with a greater severity of negative symptoms and lower red blood cell activity of the glutathione peroxidase.*

Conclusions: *The findings indicate ongoing oxidative injury at the very onset of psychosis. If valid, this would indicate the need for adjunctive antioxidant treatment from the beginning of the course of nonaffective psychoses. This might prevent a deteriorating course and development of the deficit syndrome.* Biol Psychiatry 1998; 43:674–679 © *1998 Society of Biological Psychiatry*

Key Words: Lipid peroxides, malondialdehyde, oxidative stress, psychosis, schizophrenia, antioxidants

Introduction

There is now substantial evidence for impaired antioxidant defense and increased oxyradical-mediated cellular injury in chronic schizophrenic patients (Cadet and Lohr 1987; Mahadik and Mukherjee 1996). Many studies have found peripheral activities of antioxidant enzymes to be altered (Glazov and Mamzev 1976; Abdalla et al 1986; Reddy et al 1991), and membrane lipid peroxidation products have been found to be elevated in both plasma (Peet et al 1993), as well as in cerebrospinal fluid (Pall et al 1987; Lohr et al 1990) of chronic schizophrenic patients, particularly those with involuntary movement disorders. These observations have led to therapeutic trials with antioxidants in patients with tardive dyskinesia, with initial evidence suggesting a modest beneficial effect (Lohr et al 1987; Scapicchio et al 1991; Adler et al 1993; Peet et al 1993). Because these studies were all conducted in chronic schizophrenic patients with many years of exposure to neuroleptic treatment, an implicit assumption is that the increased oxidative stress is a result of neuroleptic treatment. This notion is primarily based on the observation from studies in animals that neuroleptic treatment is associated with impaired activities of antioxidant enzymes and increased membrane lipid peroxidation (Cadet and Perumal 1990; Murthy et al 1989).

The possibility that an impaired antioxidant defense and increased peroxidative injury might be integral to the schizophrenic disease process has received less consideration. We recently found that the activity of an antioxidant enzyme—superoxide dismutase—was impaired in red blood cells (RBC) from never-medicated first-episode patients with a schizophrenic or schizophreniform psychosis (Mukherjee et al 1996). Considering the short duration of psychosis at the time of study (less than 5 days on average), this suggested that peroxidative injury may be involved at the very onset of nonaffective psychosis presenting as schizophrenia or schizophreniform disorder. Therefore, we directly examined plasma levels of lipid peroxidation products in patients experiencing a first episode of nonaffective psychosis.

Methods and Materials

Subjects

The sample comprised 26 patients (20 men and six women), all active duty personnel, who were admitted at the D.D. Eisen-

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hower Army Medical Center at Fort Gordon, GA, for the treatment of a first episode of nonaffective psychosis, and 16 normal control subjects who were recruited from the community. DSM-IIIR diagnosis in patients was based on a best estimate approach using information from the Structured Clinical Interview for DSM-IIIR (SCID), Patient Version, information from a well parent, review of medical records, and monitoring the course of illness during the first 6 months since onset. DSM-IIIR diagnoses were schizophrenic disorder in 17 patients and schizophreniform disorder in nine patients. Normal subjects were screened using the SCID-Non-Patient Version. All subjects were medically healthy and those with a history of substance use or dependence or head injury with loss of consciousness were excluded. Also, since diet and smoking affects lipid peroxidation, this information was collected on each subject. All laboratory values were within normal limits. Normal control subjects were additionally required to have a negative family history of psychosis, major mood disorder requiring treatment, dementia, mental retardation, and Huntington's disease in first degree relatives. The research was approved by the Institutional Review Boards of all institutions involved, and all patients gave written informed consent for participation in research.

Clinical Evaluations

Patients were evaluated for their clinical state at baseline and findings recorded using an anchored Brief Psychiatric Rating Scale (BPRS). For the purpose of this study, only BPRS scores for negative symptoms and positive symptoms were examined. The former comprised BPRS items of emotional withdrawal, motor retardation, and blunted affect; the latter comprised BPRS items of suspiciousness, hallucinatory behavior, and unusual thought content. The item of conceptual disorganization was excluded from the positive symptom construct in light of the recent evidence that disorganization syndrome may represent a symptom dimension that is discrete from negative and positive symptoms. The BPRS was completed by two of the authors (RS and EEC), and interrater reliability was acceptable for both negative and positive symptom scores (intraclass correlation $>$ 0.85 for both).

At the time of blood drawing, 23 patients had received no neuroleptic treatment, and three patients had received a single dose of a neuroleptic. All neuroleptic treated patients underwent a drug-free period of at least 96 hours before blood drawing. Venous blood was drawn in the early morning after an overnight fast, and centrifuged at 1000 $\times$ g for 20 minutes to remove RBCs. The platelet-rich plasma was then centrifuged at 10,000 $\times$ g for 15 minutes to obtain the clear plasma fraction that was stored at -80°C until the time of assay, which was typically done within 3–4 months of blood sampling.

Determination of Plasma Levels of Lipid Peroxidation Products [Malondialdehyde (MDA)]

Plasma MDA, a commonly used index of peroxidation of membrane polyunsaturated fatty acids (PUFAs), was determined as thiobarbituric acid reactive substances (TBARS) with a widely used procedure (Ohkawa et al 1979) as well as with a high performance liquid chromatographic (HPLC) procedure for a PUFA specific TBARS (Young and Trimble 1991). All assays were performed blind to clinical information on the subjects. Plasma samples were treated with butylated hydroxytoluene to protect against oxidation. An aliquot (0.1 ml) in the standard range (2–25 nmoles) was treated with 1 μM $FeCl_3$ 25 μm vitamin C, 0.3% TBA, 0.405% SDS, and 7.5% acetic acid in a final volume of 3.6 ml at pH 3.5 in a water bath at 95°C for 1 hour. Samples were cooled and 1 ml of water was added. The red fluorescent 1:2 MDA:TBA adduct formed was extracted with 3 ml of n-butanol and determined by a double beam spectrophotometer reading at 532 and 600 nm. The extinction coefficient was determined simultaneously for adduct with standard (1,1,3,3,-tetramethoxy propane, MDA bis-dimethyl acetal) at 532 nm $= 1.56 \times 10^5$ $cm^{-1}M^{-1}$. All assays were done in quadruplicate and the average of the three closest values used for the estimation of TBARS, which was expressed as nmoles per ml plasma. Coefficient of variance for intraassay was $<$ 5% and for interassay was $<$ 7%. The levels of RBC superoxide dismutase, glutathione peroxidase, and catalase were determined as we have described earlier (Reddy et al 1991; Mukherjee et al 1996).

Statistical Methods

Data were analyzed using nonparametric statistics. Specifically, between group comparisons were examined using Mann-Whitney tests, and bivariate comparisons were examined using Spearman rank correlation coefficients *(r)*. Values were corrected for ties, and two-tailed significance values were used throughout.

Results

Although all subjects were younger than 40 years of age, patients were significantly younger than normal controls (Table 1; mean $\pm$ SD of age $= 23.2 \pm 4.6$ and 27.9 ± 8.3 years, respectively; Mann-Whitney test, $P = 0.01$). The groups did not differ on their respective distributions of gender or race. The average duration of psychosis in the patients was 4.5 ± 2.8 days (range: 2–10 days).

On the average, plasma TBARS levels were significantly higher in the patients than in the normal controls [Table 1; Mean $\pm$ SD, 8.01 ± 5.5 versus 4.5 ± 1.8 and 5.16 ± 1.85 versus 2.35 ± 0.78 nmoles per ml plasma by procedures of Ohkawa et al (1979) and Young and Trimble (1991), respectively; Mann-Whitney test, $P <$ 0.002 for both values]. We analyzed plasma TBARS by two different procedures since inspite of some contribution of TBARS from nonlipid source (Janero 1990), both methods showed significant differences between patients and normal controls. As shown in the scatterplot in Figure 1, 16 patients had TBARS levels that were above the range found in normal controls (1.6–7.5 nmoles/ml plasma). Since there was no difference in TBARS levels between

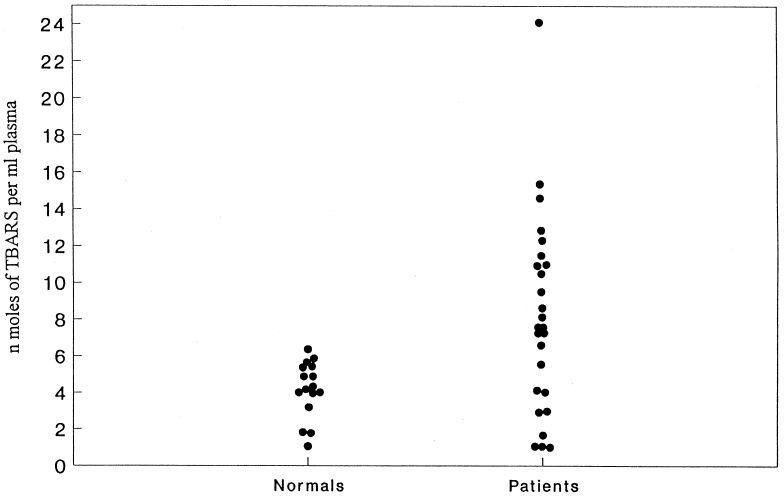

Figure 1. Scattergram of levels of plasma lipid peroxidation products in patients at the onset of nonaffective psychosis and normal controls. The demographic characteristics of patients (N = 26) and normal controls (N = 16), and the laboratory procedures for analysis of plasma lipid peroxides as TBARS are described in Methods Section. All values are in nmoles of TBARS per ml of plasma.

schizophrenic and schizophreniform patients, the two groups were combined in all subsequent data analyses. There was no effect of age, gender (20 men, 8.2 ± 5.9 versus 6 women 7.4 ± 2.7 nmoles/ml plasma), or age at onset of psychosis on plasma TBARS levels. There was no difference in TBARS levels between patients who had never received neuroleptics and those who had received a single neuroleptic dose.

In patients, plasma TBARS level was significantly correlated with the BPRS negative symptom score (r = 0.54, $P < 0.01$), but not with the BPRS positive symptom score ($P > 0.30$) at initial presentation. Data on RBC activities of antioxidant enzymes were available in 16 of 26 patients from our published study (Mukherjee et al 1996). Plasma TBARS levels were significantly and inversely correlated with RBC activity of glutathione peroxidase ($R = 0.62$, $P < 0.01$), but not correlated with RBC activities of superoxide dismutase or catalase ($P > 0.10$ for both comparisons).

Discussion

The most salient finding of this study is that plasma levels of membrane lipid peroxidation products were found to be elevated in never-medicated patients experiencing a first episode of nonaffective psychosis that met DSM-IIIR criteria for schizophrenia or schizophreniform disorder. Given that the duration of psychosis in the patients was less than 5 days on average, the data indicate ongoing oxidative injury at the very onset of psychosis. These findings are consonant with our previous observation that the enz~ymatic antioxidant defense is impaired in these first-episode patients (Mukherjee et al 1996).

The patients in this study were significantly younger than the normal controls. Although the membrane lipid peroxidation is known to increase with aging, such increase is found in a population > 55 years of age. Also, if age had an effect on plasma levels of TBARS in this study, it would have attenuated the difference between the patients and normal controls. Previous studies that examined large representative populations of normal subjects (age range: 35–60 years) using similar procedures used in this study, have reported TBARS levels (Tatbishi et al 1987) that are lower than more than one-half of the patients in this study. We did not find the gender effect but that may be a function of limited power due to small number of women subjects.

The very short duration of psychosis (< 5 days on

Table 1. Demographic Characteristics and Plasma Levels of TBARS of Patients and Normal Controls

	First-episode psychosis patients	Normal controls
Number	26	10
Men:women	20:6	6:4
Age (years)	23.2 ± 4.6	27.9 ± 8.3[a]
Duration of psychosis (days)	4.5 ± 2.8 (range: 2–10)	
Schizophrenic:schizophreniform	17:9	
TBARS (nmoles/ml plasma)	8.01 ± 5.5[b]; 5.16 ± 1.85[c]	4.5 ± 1.8[b]; 2.35 ± 0.78[c]

[a] $P = 0.01$.
[b] Analyzed by the procedure of Ohkawa et al., 1979.
[c] Analyzed by the procedure of Young and Trimble, 1991. $P = < 0.002$ for both.

average) and the drug-naive status of most of the patients at the time of study suggest that elevated lipid peroxidation may be integral to the schizophrenic disease process, and not simply a consequence of prolonged disease state or its treatment. Levels of plasma lipid peroxides can increase owing to increased production of reactive oxygen species (ROS), an impaired antioxidant defense even if ROS production is normal, or both. We found that lower RBC activity of glutathione peroxidase was associated with higher plasma levels of TBARS. Glutathione peroxidase is an antioxidant enzyme that plays a critical role in removing hydrogen peroxide as well as lipid peroxides and thereby preventing peroxidation of cell membrane lipids (Cohen and Hochstein 1963). Levels of plasma lipid peroxides were not related to RBC-SOD (superoxide dismutase), primarily Cu-Zn-SOD). It needs to be investigated whether it is related to a specific isoform of SOD (e.g., mitochondrial Mn-SOD).

Evidence is increasing to support the impaired antioxidant defense and increased oxidative injury in schizophrenia (Mahadik and Mukherjee 1996). However, whether or not the plasma TBARS as well as the other peripheral indices of oxidative cell injury reflect some of the brain pathophysiologic abnormalities in schizophrenic patients remains to be investigated directly (i.e., studies on CSF and possible association of peripheral indices to the brain morphometric changes). Unfortunately, it has been very difficult to get consents of these young patients for CSF. Low peripheral glutathione peroxidase activity has previously been found to be associated with greater brain morphologic abnormalities (larger ventricles and greater cortical sulcal prominence) in chronic schizophrenic patients (Buckman et al 1987; 1990). There is substantial evidence that oxidative stress is a global phenomenon and peripheral measures reflect the brain injury (Kramer et al 1987; Mahadik and Mukherjee 1996; Simonian and Coyle 1996).

It is unlikely that the elevated levels of lipid peroxides are the result of prodromal symptoms of psychosis a long time before becoming floridly psychotic and requiring hospitalization because such conditions cannot remain undetected during army recruitment procedures as well as during every day rigorous training. The body mass index (BMI) is known to be associated with the lipid peroxidation. However, all the patients were physically fit and none of them had obesity indicating very little variability in BMI. Furthermore, all the patients were receiving an identical diet during both before and 6 months after the admission. In addition, although smoking can increase the lipid peroxidation (Morrow et al 1995) and heavy smoking is common in psychotic patients, none of our patients were smoking at the time of admission and at least 1–2 weeks after the admission. All blood samples were collected during this smoke-free period. However, it is likely that the acute episode of psychosis, and associated excitement and agitation have triggered the oxidative stress resulting into increased lipid peroxidation. These states may increase the "stress" that can increase the global oxidative stress. The prolonged stress may preferentially cause the permanent brain damage (O'Brien 1997). This is difficult to resolve since it will require to determine the prehospitalization (i.e., almost prepsychotic) levels of TBARS. It is also difficult to have additional nonpsychotic hospitalized controls since each illness condition may have its own effects on lipid peroxidation.

Most of the published studies to date have reported elevated levels of lipid peroxidation products in plasma as well as CSF from chronic schizophrenic patients receiving neuroleptic treatment, especially in those with movement disorders (Pall et al 1987; Lohr et al 1990; Peet et al 1993). The possibility that neuroleptic treatment exacerbates the oxidative injury and increase lipid peroxidation needs to be more systematically examined in future follow-up studies. Chronic neuroleptic treatment in animals causes impaired antioxidant defense and increased lipid peroxidation (Murthy et al 1989; Cadet and Perumal 1990) as well as leads to irreversible neuropathologic changes in the brain (Mahadik et al 1988; Mahadik and Mukherjee 1995; Jeste et al 1992; Miller and Chouinard, 1993). Similar neuropathologic changes have been reported in schizophrenia (e.g., Jeste and Lohr 1989; Bloom 1993; Freeman and Karson 1993). McCreadie et al (1995) have suggested that the increased oxidative injury in schizophrenia is irrespective of the neuroleptic status. These studies and our findings suggest that oxidative injury might play a role in the development of the deficit syndrome of schizophrenia. This possibility also needs to be examined in longitudinal prospective studies.

Increased oxidative injury has been implicated in a variety of neurodegenerative brain disorders, including Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis, to name but a few (Simonian and Coyle, 1996). It is likely that oxidative cell injury might also exist to some extent in other psychiatric disorders. This nonspecificity makes it unlikely that oxidative injury is a primary event in the pathogenesis of schizophrenia or other psychoses. Rather, we suggest that ongoing oxidative injury modifies the course of schizophrenia and contributes to a deteriorating course and development of the deficit syndrome. At what stage of pathogenesis such effects are exerted is yet to be investigated. For example, whether or not peroxidative injury to membrane lipids contributes to adverse neurodevelopmental events involved in the pathogenic cascade of some cases of schizophrenia is not known. Our earlier finding that impaired RBC activity of the antioxidant enzyme superoxide dis-

mutase was associated with deterioration of school performance from childhood to early adolescence and impaired school performance during adolescence suggests such a possibility (Mukherjee et al 1996).

The question has been raised whether the deleterious effects of increased peroxidative injury are owing to accumulation of toxic materials or to loss of phospholipids and essential polyunsaturated fatty acids (EPUFAs) (Horrobin 1991; Horrobin et al 1994; Mahadik et al 1996), or damage to cellular proteins and nucleic acids that are not yet investigated. Regardless, the findings that there is increased oxidative injury at the very onset of psychosis, and that this is probably associated with more severe negative symptoms at initial presentation, suggests a possible role of adjunctive antioxidant treatment from the beginning of the illness (Mahadik and Scheffer 1996; Mahadik and Gowda 1996). Further research is required to determine whether such a strategy will suffice, or also require the supplemental use of essential fatty acids to restore membrane EPUFA contents (Mahadik and Evans 1997; Peet et al 1996).

While neuroleptics may remain necessary for controlling the productive psychotic symptoms of schizophrenia, it might be necessary to consider other strategies, such as antioxidant treatment and EPUFA supplementation, to prevent a deteriorating course of illness and development of the deficit syndrome. Albeit some of the interpretations of our study are speculative at this stage, this approach offers an optimistic view of the prospects of treating schizophrenia rather than assume that poor outcome is predetermined by adverse neurodevelopmental events.

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