

Oxidative Damage and Schizophrenia

An Overview of the Evidence and Its Therapeutic Implications

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Abstract

Free radicals are highly reactive chemical species generated during normal metabolic processes, which in excess can lead to membrane damage. Elaborate antioxidant defence systems exist to protect against oxidative stress.

There is accumulating evidence of altered antioxidant capacity in schizophrenia. Membrane dysfunction can be secondary to free radical-mediated pathology, and may contribute to specific aspects of schizophrenic symptomatology and complications of its treatment. Specifically, free radical-mediated abnormalities may contribute to the development of a number of clinically significant consequences,

including prominent negative symptoms, tardive dyskinesia, neurological 'soft' signs and parkinsonian symptoms. Our previous results showing altered membrane dynamics and antioxidant enzyme activities in schizophrenia, and findings from other investigators, are consistent with the notion of free radical-mediated neurotoxicity in schizophrenia. These findings provide a theoretical basis from which the development of novel therapeutic strategies such as fatty acid and antioxidant supplementation can occur in the future.

Schizophrenia is one of the major mental disorders and is of unknown aetiology. It is associated with a great deal of morbidity and annual economic costs that total billions of dollars. The illness has a variable course, with a substantial proportion of patients having poor outcome in spite of the use of the best currently available treatments. Thus, the development of novel strategies to improve outcome, even for a modest number of patients, will be of great benefit.

Many theories abound conceptualising the pathophysiology of schizophrenia, including neuronal maldevelopment, impaired neurotransmission, viral infections, autoimmune dysfunction and others. Further, there are a broad variety of apparently disparate biological findings in schizophrenia,^[1] possibly due to aetiological heterogeneity; however, it is possible that a final common pathogenetic pathway(s) may mediate the recognisable syndromes of schizophrenia. The scientific challenge is to identify the pathological process(es) that may account for the diverse clinical and biological features of schizophrenia.

The neuronal membrane can serve as a point of convergence for the above-mentioned theoretical models. The neuronal membrane is the structural and functional site of neurotransmitter receptors, ion channels, signal transduction and drug effects. The membrane is also a point where there is a natural intersection between genetic and environmental factors.^[2] Membrane defects, such as those induced by decreased amounts of polyunsaturated fatty acids (PUFAs) in phospholipids,^[3,4] can significantly alter a broad range of membrane functions, and presumably modify behaviour through multiple 'downstream' biological effects.

Previous results from our laboratories^[5-7] and those

of other investigators^[8-11] show decreased levels of PUFAs in both peripheral and central membranes of individuals with schizophrenia. It is well known that oxidative stress can lead to the type of membrane abnormalities that have been observed in schizophrenia.^[12] Thus, free radical-mediated pathological processes may provide an explanatory model for the specific membrane alterations in schizophrenia, indicating that a role for oxidative stress might exist for some domains of schizophrenic pathophysiology as has been proposed by several investigators.^[12-16] This review details the intimate connections between oxidative stress and membrane pathology, with findings specific to schizophrenia.

1. Free Radicals and the Antioxidant Defence System (AODS)

1.1 Free Radicals and Membrane Perturbations

Free radicals are generated *in vivo* during many normal biochemical reactions involving oxygen and nitric oxide (fig. 1), including the mitochondrial electron transfer chain, NADPH-dependent oxidation, and auto-oxidation of PUFAs and catecholamines.^[17-19] These free radicals (primarily the reactive oxygen species, superoxide and hydroxy radicals) play an important role in membrane lipid peroxidation.

PUFAs are major components of membrane phospholipids. They are highly susceptible to free radical insult and auto-oxidation, which result in the formation of peroxyradicals and lipid peroxide intermediates. The existence of these products within cell membranes results in an unstable membrane structure, altered membrane fluidity and permeability, and impaired signal transduction.^[20]

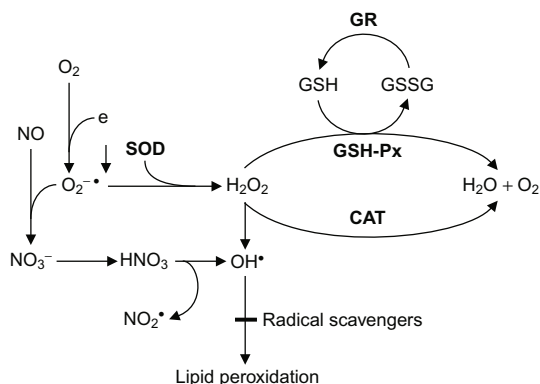


Fig. 1. Production of reactive oxygen species and the defence mechanism against damage by reactive oxygen. Superoxide dismutase (SOD) catalyses the conversion of superoxide radicals ($O_2^{\bullet -}$) to hydrogen peroxide. Catalase (CAT) and glutathione peroxidase (GSH-Px) convert hydrogen peroxide to water. Glutathione (GSH) is utilised by GSH-Px to yield the oxidised form of glutathione (GSSG), which is converted back to GSH by glutathione reductase (GR). Hydrogen peroxide is susceptible to auto-oxidation to form hydroxyl radicals (OH^{\bullet}), particularly in the presence of metal catalysts such as iron. In addition, nitric oxide, which is the product of a 5-electron oxidation of the amino acid L-arginine, can also produce hydroxyl radicals as well as nitron dioxide radical (NO_2^{\bullet}).

Hydroperoxides can further decompose to other toxic species (aldehydes, including malonyldialdehyde), which can damage adjacent cells, membrane-bound enzymes and receptors, cause cross-linking between various types of molecules, and result in membrane breakdown, cytotoxicity, mutagenicity and enzyme modification.^[20-22] Aldehydes can react with lipids and proteins forming lipofuscin, which accumulates in neuronal cells, particularly in regions of active free radical metabolism.

Thus, the unchecked effects of free radicals can result in cellular dysfunction, loss of membrane integrity and even cell death. The brain, which is rich in PUFAs, is particularly vulnerable to free radical-mediated damage.

1.2 The AODS and the Pathological Significance of Its Disruption

Under physiological conditions the potential for free radical-mediated damage is kept in check by

the antioxidant defence system (AODS), which is comprised of a series of enzymatic and non-enzymatic components.

The critical antioxidant enzymes include superoxide dismutase (SOD; E.C.1.15.1.6), catalase (CAT; E.C.1.11.1.6) and glutathione (GSH) peroxidase (GSH-Px; E.C.1.11.1.9). These enzymes act cooperatively at different sites in the metabolic pathway of free radicals (see fig. 1). Hydrogen peroxide produced by SOD is decomposed to water and oxygen by the haeme protein CAT, thereby preventing the formation of hydroxyl radicals. Failure of this first line of antioxidant defence may lead to an initiation of lipid peroxidation. Selenium-dependent GSH-Px protects against lipid peroxidation by converting hydrogen peroxide to water, or more critically by converting toxic hydroperoxides to less toxic alcohols. Since SOD, CAT and GSH-Px are critical to different stages of free radical metabolism, altered activity of one enzyme without compensatory changes in the other enzymes may leave the membranes vulnerable to damage. Thus, the differential patterning of the antioxidant enzyme activities may provide important clues to the pathogenetic mechanisms of abnormal free radical metabolism.^[23]

The non-enzymatic antioxidant components that may be equally important in the overall AODS consist of molecules that react with activated oxygen species and thereby prevent the propagation of free radical chain reactions. The most common non-enzymatic antioxidant molecules are albumin, uric acid, bilirubin, GSH, α -tocopherol (vitamin E), ascorbic acid (vitamin C) and β -carotene.

Oxidative stress is a state when there is a disequilibrium between pro-oxidant processes and the AODS in favour of the former. There are potentially multiple pathological consequences of increased oxidative stress, due either to increased free radical production and/or inefficient antioxidant systems (e.g. increased SOD and/or decreased CAT activity), which lead to lipid peroxidation.

Such changes in free radical metabolism have implications for the pathophysiology of schizophrenia:

- lipid peroxidation can alter the PUFA content of cell membranes, and such changes in PUFA have been reported in patients with schizophrenia^[5-7,9,10,24]
- high levels of hydrogen peroxide and lipid peroxides lead to decreased synthesis of prostaglandins,^[25] which also has been reported in patients with schizophrenia^[26,27]
- lipid peroxidation is associated with increased dopamine and decreased γ -aminobutyric acid (GABA) uptake by synaptosomes^[28]
- hydroxyl radicals decrease synaptic efficiency and impair action potential generation in hippocampal pyramidal cells^[29]
- hydrogen peroxide can inhibit dopamine β -hydroxylase^[30]
- free radicals may decrease brain GABA receptor function.^[31]

It has been suggested that increased scavenging activity of SOD in the absence of increased superoxide production can depress free radical-dependent reactions, such as those catalysed by oxygenases,^[32] thus resulting in decreased catecholamine production.

2. Evidence for Alterations in the AODS in Schizophrenia

Free radicals are involved in brain membrane pathology,^[33] and may play an important role in neuropsychiatric disorders.

Hoffer et al.^[34] first proposed a role for toxic radicals in the aetiology of schizophrenia in 1954. The first study evaluating any indices of the AODS, however, was reported over 20 years later.^[35] Subsequent studies have generally examined indirect measures of free radical activity, since direct measures of free radicals *in vivo* are difficult and cumbersome. The majority of studies have examined the levels of nonenzymatic antioxidants as well as activities of key antioxidant enzymes in plasma and red blood cells (RBCs), based on the notion that changes in the levels of antioxidant molecules and enzyme activities may lead to oxidative stress. A few studies, however, have provided more direct evidence of oxidative membrane

damage by examining levels of lipid peroxidation products. A brief review of recent findings in schizophrenia is presented in sections 2.1. to 2.3.

2.1 Non-Enzymatic Antioxidant Molecules

2.1.1 Plasma Antioxidant Capacity

A major contribution to the total antioxidant capacity comes from antioxidant molecules in plasma. The relative contribution of each plasma antioxidant *in vivo* depends not only on its efficacy but also on its level in biological fluid. It is known that albumin, uric acid and ascorbic acid are the major contributors (>85%) to the total antioxidant capacity in human plasma.^[36,37] This predominance is largely due to their high levels relative to those of other antioxidants in blood, e.g. bilirubin, α -tocopherol and β -carotene. Although individual antioxidants play a specific role in the AODS, these antioxidants may act cooperatively *in vivo* to provide synergistic protection to the organs against oxidative damage. Therefore, it is more meaningful to evaluate the AODS by measuring both the individual levels of antioxidants and the overall antioxidant status.

We have recently established procedures to monitor plasma total antioxidant status (TAS). TAS and levels of individual antioxidants (i.e. albumin, bilirubin and uric acid) were measured in plasma of male patients with schizophrenia, using a within-patient, repeated measures, on-off haloperidol treatment design. Plasma TAS was significantly lower in drug-free and haloperidol-treated patients with schizophrenia compared with that in healthy controls (table I).^[38] Plasma TAS in patients was not significantly correlated with smoking status, as assessed by plasma levels of cotinine (the major metabolite of nicotine). Individual plasma levels of antioxidants (albumin, bilirubin^[39] and uric acid^[40]) were also significantly lower in patients with schizophrenia than in healthy controls (table I). In summary, the observed decreases in plasma TAS, as well as levels of individual antioxidants, in patients with schizophrenia suggest an increased risk for oxidative damage, and lend additional support to our hypothesis that oxidative

Table I. Comparison of plasma total and individual antioxidant status in patients with schizophrenia and healthy volunteers

Plasma antioxidant	Healthy volunteers	Patients with schizophrenia		P value (2-tailed, unpaired <i>t</i> test)		P value (paired <i>t</i> test)
		HD-treated	DF	HD-treated vs healthy volunteers	DF vs healthy volunteers	HD-treated vs DF
Total (TAS) [mmol/L] ^[38]	1.13 ± 0.15	1.06 ± 0.11	1.06 ± 0.10	0.020	0.024	0.576
Individual						
Albumin (g/dL) ^[39]	4.06 ± 0.31	3.83 ± 0.42	3.89 ± 0.36	0.017	0.054	0.299
Bilirubin (mg/dL) ^[39]	0.44 ± 0.25	0.32 ± 0.15	0.33 ± 0.15	0.020	0.026	0.849
Uric acid (mg/dL) ^[40]	5.09 ± 0.97	4.62 ± 0.99	4.29 ± 0.95	0.050	0.008	0.018

DF = drug-free; HD = haloperidol; TAS = total antioxidant status.

stress may have a pathophysiological role in schizophrenia.

2.1.2 Water- and Fat-Soluble Vitamins

Ascorbic acid is a biological antioxidant that acts as a chain-breaking scavenger for peroxy radicals and acts synergistically with α -tocopherol. In the brain, the basal ganglia, which is rich in dopamine and glutamate, contains high levels of ascorbic acid, ranking among the tissues with the highest levels of this vitamin.^[41] Therefore, the antioxidant function of ascorbic acid may serve as an essential defence line against dopamine- or glutamate-induced neurodegenerative processes.^[42]

Previous studies of the levels of ascorbic acid in patients with schizophrenia have reported variable results. More recently, in a carefully controlled study, Suboticanec et al.^[43] demonstrated that both plasma and urinary ascorbic acid levels were decreased in patients with chronic schizophrenia¹ relative to healthy individuals, even after controlling for diet. After ascorbic acid supplementation for 1 month, group differences were no longer significant. It is thus suggested that ascorbic acid requirements for patients with schizophrenia may be higher than for healthy individuals.

It is well known that the fat-soluble vitamin α -tocopherol is involved in oxidative metabolism and associated with diseases linked to oxidative stress. McCreadie et al.^[44] found lower ratios of α -tocopherol to cholesterol in patients with schizophre-

nia compared with that in healthy controls. Specifically, inpatients who were most seriously ill had the lowest ratios. More recently, Brown et al.^[45] also reported decreased lipid-corrected α -tocopherol levels in patients with schizophrenia who had antipsychotic-induced tardive dyskinesia (TD), relative to healthy controls, but not in antipsychotic-treated patients without dyskinesia.

2.2 Scavenging Antioxidant Enzymes

Among the 3 key scavenging antioxidant enzymes, SOD has been the most frequently studied. Increased SOD activity has been reported in the RBCs of patients with schizophrenia by some investigators^[35,46-50] but not by others.^[51] Antipsychotic-naïve patients with first-episode schizophreniform disorder and schizophrenia show both increased^[52] and decreased^[53] SOD activity. In the latter study,^[53] the mean duration of illness was 4.46 days, much shorter than in other studies, which may account for the discordant findings. It is possible that with progression of the illness, the SOD levels rise as a compensatory response to oxidative stress.^[53]

In contrast, blood GSH-Px activity was found to be lower, relative to healthy control individuals, in antipsychotic-treated patients with chronic schizophrenia,^[54] in drug-free female patients with schizophrenia^[46] and in antipsychotic-naïve psychotic children.^[55] Zhang et al.^[56] have recently reported increased plasma GSH-Px activity in long term antipsychotic-free as well as antipsychotic-naïve patients with schizophrenia. However, our data demonstrated a significant and positive correlation

¹ In the studies referred to throughout the rest of this article, patients with chronic schizophrenia were being treated with antipsychotics, unless stated otherwise.

between plasma GSH-Px activity and psychosis severity.^[57] Furthermore, no differences were found in GSH-Px levels of skin fibroblasts from patients with schizophrenia and healthy control individuals,^[56] suggesting that plasma GSH-Px elevation in patients may be a state-dependent change and not a consequence of the course of illness or treatment with antipsychotics. In platelets, Buckman et al.^[58] found that GSH-Px activity was inversely correlated with computed tomographic (CT) scan measures of brain atrophy in patients with chronic schizophrenia, specifically in those with nonparanoid schizophrenia with a predominance of negative symptoms.

Although the above studies show abnormalities in individual antioxidant enzymes, the physiology of the AODS suggests that examining a single enzyme may have limited value for elucidating the role of abnormal free radical metabolism in disease processes.^[49] Since SOD, CAT and GSH-Px are critical to different stages of free radical metabolism, as mentioned in section 1.2, alteration in the activity of one enzyme without compensatory changes in other enzymes may leave membranes vulnerable to damage. Thus, the differential patterning of the antioxidant enzyme activities may provide important clues to the pathogenetic mechanisms of abnormal free radical metabolism.^[49]

Recently, we evaluated the 3 critical enzymes of the AODS (SOD, CAT and GSH-Px) in patients with schizophrenia using a within-subject, repeated measures, on-off haloperidol treatment design.^[50,57]

Among these enzymes in erythrocytes and plasma, only RBC SOD was found to be significantly higher in drug-free patients with schizophrenia than in age- and sex-matched healthy volunteers (table II). Our finding is consistent with most previous reports of increased SOD activity in erythrocytes of drug-free and drug-treated patients with schizophrenia.^[12] On the other hand, the AODS enzyme activities were not significantly correlated with age, or the age at onset or the duration of illness. In addition, none of the major AODS enzymes showed significant differences between relapsed and clinically stable patients.

2.3 Factors Influencing Antioxidant Status

2.3.1 Age

Free radical production and the AODS are thought to play important roles in the mechanism of aging.^[59] For example, SOD activity in the brain increases significantly with aging,^[60-62] reflecting a self-protection mechanism against an increased production of superoxide radicals in the brain. On the other hand, a decreased level of GSH is found in the brain with aging, suggesting an increased susceptibility to oxidative damage with accelerated aging.^[63] Therefore, the rate of membrane oxidative damage by free radicals may provide an index to determine life span.^[64-67] Antioxidant supplementation has been used to prolong life span in experimental animals.^[68-70] It is thus surmised that the aging process may be simply the sum of the

Table II. Comparison of AODS enzymes in patients with schizophrenia and healthy volunteers

AODS enzymes	Healthy volunteers (n = 40)	Patients with schizophrenia (n = 38)		P value (2-tailed, unpaired <i>t</i> test)		P value (paired <i>t</i> test) HD-treated vs DF
		HD-treated	DF	HD-treated vs healthy volunteers	DF vs healthy volunteers	
RBC ^[50]						
SOD (U/mg Hb)	1.85 ± 0.19	1.91 ± 0.18	1.97 ± 0.18	0.158	0.009	0.059
CAT (U/mg Hb)	156 ± 36	159 ± 59	149 ± 36	0.784	0.446	0.240
GSH-Px (U/g Hb)	50 ± 15	47 ± 12	50 ± 13	0.469	0.954	0.037
Plasma ^[57]						
GSH-Px (µg/mg protein)	1.08 ± 0.43	1.07 ± 0.35	1.11 ± 0.41	0.740	0.770	0.454

AODS = antioxidant defense system; **CAT** catalase; **DF** drug-free; **GSH-Px** = glutathione peroxidase; **HD** haloperidol; **RBC** = red blood cell; **SOD** = superoxide dismutase.

deleterious radical reactions going on continuously throughout life in cells and tissue.^[71]

We have previously examined the relationship between plasma antioxidant protein levels and clinically relevant demographic features, e.g. age, age at onset of illness, duration of illness, days on drug treatment, duration of drug-free period, and body mass index (BMI), in individuals with schizophrenia.^[39] The reduction of plasma antioxidant protein levels in schizophrenia appears to be age-related. In healthy volunteers, plasma albumin levels were not correlated with age. By contrast, there was an inverse correlation between this parameter and age in patients with schizophrenia, although there was no significant difference in mean age between the two groups. A similar inverse correlation with age is seen in plasma uric acid levels in patients with schizophrenia.^[40] Plasma total bilirubin levels were positively correlated with age in healthy volunteers, whereas an age-related decrease was observed in patients with schizophrenia. Furthermore, these reductions in plasma albumin and bilirubin level were not related to other clinically relevant demographic factors, particularly duration of illness.

Taken together, age appears to be an important factor modifying the AODS in schizophrenia.

2.3.2 Antipsychotic Treatment

It is not known whether antipsychotics have a similar effect on antioxidant enzyme activities in patients with first-episode and in those with chronic schizophrenia. It is interesting that low erythrocyte SOD activity has been found in never-treated patients experiencing first-episode psychosis and was associated with impaired premorbid school functioning.^[72] Follow-up studies in this patient population could provide more robust evidence of antipsychotic effects. Further, the effects of conventional and atypical antipsychotic drugs, such as clozapine, olanzapine and risperidone, should be assessed as they may differ.

The controlled discontinuation of haloperidol in patients allows the examination of the effects of the drug on biochemical indices involved in the AODS. Recently, we have compared levels of an-

tioxidants and AODS enzymes in patients during haloperidol treatment and during withdrawal from the drug.^[38-40,50,57] In plasma, neither TAS nor individual antioxidant levels were significantly affected by haloperidol withdrawal or treatment. Moreover, the duration of the drug free period had no effect. Although a clearer picture of treatment effects can be best provided by prospective treatment studies of drug-naïve patients, the decreased plasma antioxidant capacity seen in chronic schizophrenia does not appear to be due to the effects of antipsychotics.

In erythrocytes, among the 3 major AODS enzymes, GSH-Px activities were shown to be significantly higher in patients undergoing haloperidol withdrawal compared with haloperidol-treated patients (table II). In addition, SOD activities showed a trend towards being higher. Abdalla et al.^[46] found that SOD and GSH-Px activities were not significantly different between patients with schizophrenia on and off antipsychotic treatment. Buckman et al.^[58] also found that platelet GSH-Px activity was not correlated with antipsychotic treatment. The above studies, however, did not utilise a within-patient repeated measures design, which is a more conservative way to address this issue.

It has been shown that haloperidol increases SOD activity in certain rat brain regions and is associated with decreases in lipid peroxidation.^[73] Our findings are the opposite of that found in rat brains, suggesting that haloperidol may not have a direct regulatory effect on AODS enzymes in patients. Discontinuation of antipsychotics in patients with chronic schizophrenia is often associated with worsening of symptoms, probably a consequence of alterations in the dopaminergic system due to lifting the chronic antipsychotic-induced blockade of dopamine D₂ receptors. Worsening symptoms and accompanying stress that may be due to a hyperdopaminergic state may lead to increased superoxide radical production and consequently increased SOD activity as observed in our study. The observed increase in GSH-Px activity could be compensatory, due to increased SOD-induced production of hydrogen peroxide. It is well recognised that

nonspecific stress can induce oxidative stress.^[74,75] Thus, alterations in SOD activity in our study may be a consequence of a changed behavioural state rather than direct effects of haloperidol, with secondary increases in GSH-Px activity.

2.3.3 Cigarette Smoking

The prevalence of cigarette smoking in patients with schizophrenia is between 70 to 90%, compared with that of 35 to 54% for all psychiatric patients and 30 to 35% for the general population.^[76-78]

Cigarette smoke contains, in addition to nicotine, thousands of substances in the gas and tar phases, many of which are free radicals such as peroxy radicals, oxides of nitrogen, hydroquinones and other radical species.^[79-82] Chronic cigarette smoking is associated with oxidative stress due to the increased free radical burden, reflected in alterations of various oxidative stress indices,^[83] and may contribute directly to decreases in the levels of antioxidants. In light of this, it is important to know whether cigarette smoking accounts for the AODS abnormalities seen in schizophrenia.

We examined plasma cotinine levels as an index of smoking. In our studies,^[38-40] it appears that cigarette smoking does not significantly reduce the levels of plasma TAS and individual antioxidants, with the exception of bilirubin. Although bilirubin is a more efficient antioxidant than albumin, the overall contribution to plasma TAS from bilirubin is <5%, as compared with >40% from albumin.^[37] Therefore, cigarette smoking plays an insignificant role in altering levels of plasma antioxidant proteins.

We further examined whether tobacco smoking may affect AODS enzyme activities via smoking-induced oxidative stress. In healthy control individuals, there was an inverse relationship between erythrocyte SOD activity and plasma cotinine levels.^[50] In patients, there was no such relationship when patients were medicated with haloperidol. Thus, smoking cannot account for an increased erythrocyte SOD activity in patients with schizophrenia after haloperidol discontinuation, although we are unable to account for the absence of the

relationship between cotinine level and SOD activity in patients.

2.3.4 Diet

Diet and alcohol are the major factors affecting both the antioxidant system and the production of free radicals and reactive oxygen-containing species.^[84] Plasma levels of antioxidants of dietary origin (e.g. tocopherols, ascorbic acid, carotenoids, etc.) are influenced directly by nutritional supplements as well as food and alcohol consumption.^[85-87]

Among the major plasma antioxidants, only uric acid levels were correlated significantly and positively with BMI in patients with schizophrenia (both on and off haloperidol treatment); no such correlation was seen in healthy volunteers.^[40] No patients were receiving any uricosuric agents that potentially could decrease uric acid levels. If anything, they were at greater risk for increased uric acid levels because of their higher BMI. In our studies,^[38-40,50,57] all patients were hospitalised and maintained on a control balanced diet without alcohol consumption. Therefore, it is unlikely that their decreased plasma antioxidant status resulted from a dietary deficiency or alcohol consumption as compared with their healthy volunteer counterparts.

3. Free Radical Pathology in Schizophrenia

3.1 Lipid Peroxidation

Changes in the AODS do not necessarily reflect increased oxidative stress and subsequent membrane lipid damage. While evidence of peroxidative damage is limited in patients with schizophrenia, the findings have been consistent. Increased blood levels of malondialdehyde^[44,88,88,89,90,91] and a similar increase in the levels of lipid peroxides in CSF samples^[92,93] have been reported. More recently, Mahadik et al.^[94] reported elevated plasma lipid peroxides levels at the onset of psychosis in never-medicated, first-episode patients. Their findings, if replicated, may indicate the presence of oxidative stress very early in the course of illness, and independent of treatment.

Increased levels of pentane, another marker of lipid peroxidation, have also been reported in patients with schizophrenia relative to control individuals.^[95,96] We are currently examining the issue of whether such a reduction of antioxidant level is associated with increased levels of lipid peroxides in plasma and postmortem brain tissues of patients who had had schizophrenia.

3.2 Mitochondria

Mitochondria process most of the cellular oxygen to provide energy that drives almost all metabolic processes, and also are the site of significant free radical production. About 3% of all oxygen consumed is converted to superoxide, and subsequently to hydrogen peroxide.^[97] Thus, there is an enormous and continuous free radical burden associated with mitochondria. Antioxidant systems keep this in check. When the equilibrium between prooxidant and antioxidant systems are disturbed in favour of the former, mitochondrial damage can occur. Mitochondrial membranes, similar to neuronal membranes, are vulnerable to lipid peroxidation. Any impairment in mitochondrial oxidative phosphorylation can lead to a broad range of cellular disturbances, including decreased neurotransmission, decreased DNA repair and finally cell death.

Cytochrome-c oxidase (COX, Complex IV) is a key enzyme in the mitochondrial electron transport chain. Decreased activity of this enzyme has been reported in the frontal cortex and caudate nucleus of patients with schizophrenia.^[98] Interestingly, COX activity is maintained by cardiolipin, a key lipid in the mitochondrial membrane, which is highly susceptible to peroxidation during oxidative stress.^[99] Several lines of evidence suggest decreased oxidative metabolism in some brain areas in schizophrenia,^[100] and may be explained in part by mitochondrial dysfunction. We propose that this is secondary to oxidative stress due either to decreased antioxidant capacity and/or increased free radical burden.

3.3 Findings in the Brain

There have been only 2 reports of postmortem studies involving investigation of SOD activity in schizophrenia. An early study by Wise et al.^[101] found no difference in the diencephalons (thalamus, and epi-, sub- and hypothalamus) between patients with schizophrenia and control individuals. The recent study by Loven et al.,^[102] however, demonstrated an increased activity of manganese-SOD, a key antioxidant enzyme in mitochondria, in the temporal and frontal cortices of antipsychotic-treated patients who had had schizophrenia.

Several electron microscopic studies of brains from patients with schizophrenia have found large amounts of lipofuscin-like material in oligodendrocytes,^[103] abnormal pigment-laden neurons,^[104] and axonal deposits of lipofuscin-like bodies.^[105] As noted in section 1.1, lipofuscin is a by-product of lipid peroxidation. While some studies have reported gliosis, a potential response to neuronal loss, in patients with schizophrenia, others have not.^[106] A general assumption but possible misconception is that cell death is a necessary consequence of oxidative stress. Nevertheless, these microscopic findings may provide indirect evidence of oxidative stress in schizophrenia.

3.4 Tardive Dyskinesia

There is general consensus that antipsychotics are a necessary, but not sufficient, factor for the development of TD,^[107] although some have argued that TD is integral to the schizophrenic disease processes.^[108] Free radical-mediated pathology has been implicated in the development of TD.^[12,109,110]

Lohr et al.^[93] found a significantly higher level of lipid peroxidation products (diene conjugates) in the CSF of patients with schizophrenia who had TD compared with those who did not have TD. They also demonstrated that the severity of TD was positively correlated with levels of diene conjugates, and, more critically, high levels of diene conjugates were associated with subsequent development of TD. Recently, Peet et al.^[90] found a

highly significant correlation between plasma levels of lipid peroxidation products and dyskinesia severity. Zubenko and Cohen^[111] have shown that platelet membrane fluidity is altered in patients with TD, but not in similarly antipsychotic-treated patients without TD. In addition, treatment with α -tocopherol reduces the severity of TD in patients with schizophrenia.^[112,113] If indeed free radicals play a role in the development of TD, patients with an inadequate AODS would be more likely to develop TD.

4. Evidence for Altered Membrane Dynamics in Schizophrenia

4.1 Phospholipids and Polyunsaturated Fatty Acids in Peripheral Tissues

Early findings, comprehensively reviewed by Rotrosen and Wolkin,^[26] demonstrated variable alterations in the levels of phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylinositol (PI), and consistent decreases of phosphatidylethanolamine (PE) levels in RBC membranes, in psychotic patients. These results are diverse and inconsistent due primarily to differences in patient groups and methodology. Such discrepancies, however, may be explained in part by the more recent finding of a bimodal distribution of RBC PUFAs in patients, in contrast to the unimodal distribution seen in healthy controls.^[10] Phospholipid abnormalities have also been found in medication-free patients with schizophrenia,^[114] and decreases in all 4 key membrane phospholipids were found in fibroblasts from antipsychotic-naïve patients with schizophrenia.^[115] These latter findings suggest that phospholipid and fatty acid abnormalities may be disease-related. At the very least, they are present early in the course of illness, prior to the initiation of treatment.

Significant decreases in the levels of both arachidonic acid [AA; 20:4(n-6)] and linoleic acid [18:2(n-6); a precursor of AA], but an increase in total n-3 fatty acid levels, in the plasma of patients with schizophrenia who were from England, Scotland and Ireland has been reported.^[116] Such de-

creases in the levels of PUFAs were also demonstrated in RBC membranes of patients with schizophrenia.^[5,8,10,117] Moreover, the decreases of RBC PUFA levels were independent of haloperidol treatment.^[6] There are initial data from fibroblasts of drug-naïve patients with first-episode schizophrenia of decreased AA levels.^[118]

Studies to date, however, have focused on patients with chronic illness and poor outcome. Since outcome is often determined early, critical questions are whether low PUFA levels are seen *early* in the course of illness and are associated with later poor outcome. To address this issue, a longitudinal study of patients with first-episode schizophrenia was initiated. Preliminary findings from 17 patients and 15 control individuals showed a significant reduction in RBC AA levels at the antipsychotic-naïve baseline.^[119] Moreover, a significant correlation was demonstrated between RBC phospholipid PUFA levels and ³¹P magnetic resonance spectroscopic (MRS) measures (for details see section 4.2.1) of phospholipid metabolites in the combined right and left frontal lobe, but not other brain regions including caudate, occipital, parietal and temporal areas.^[120] Specifically, both total and individual PUFA (20:4, 22:5 and 22:6) levels were significantly ($p < 0.02$) and positively correlated with phosphomonoesters levels. These findings further support the notion that decreased membrane fatty acid levels in peripheral tissue may be associated with similar changes in the brain.

In light of findings of membrane defects in a variety of peripheral cell types (platelets, RBC and fibroblasts), it has been proposed that in schizophrenia, membrane compositional defects may occur in all cell membranes in the body, and are thus detectable in both extra-neural tissues and the brain.^[121]

4.2 Membrane Lipids in the Brain

4.2.1 In Vivo Magnetic Resonance Spectroscopic Measurements

Using ³¹P-MRS, Pettegrew et al.^[122,123] demonstrated significantly reduced levels of phosphomonoesters (phospholipid precursors) and significantly

Table III. Comparison of phospholipid subclass levels in the caudate region of postmortem brain tissue in patients who had had schizophrenia and control groups with and without other psychiatric disorders (reproduced from Yao et al.,^[7] with permission)

Phospholipids	Patients with schizophrenia ^a	Controls without PD ^a	Controls with other PD ^a	ANCOVA ^b		
				F	Df	P
PE	66.2 ± 6.3	83.2 ± 14.4	79.8 ± 4.2	3.86	2,25	0.0345
PI	4.8 ± 0.4	4.4 ± 0.4	5.0 ± 0.8	3.18	2,25	0.0588
PS	20.2 ± 2.1	20.1 ± 3.2	19.3 ± 1.8	0.95	2,25	0.4018
PC	36.0 ± 6.2	43.5 ± 5.3	40.3 ± 4.0	5.19	2,25	0.0130
SM	15.0 ± 2.9	13.5 ± 1.9	13.6 ± 1.0	2.98	2,25	0.0691
Total PL	142.2 ± 12.1	164.8 ± 21.7	158.0 ± 8.2	2.46	2,25	0.1055

a Each value (µg/mg dry weight) represents unadjusted cell mean and standard deviation.

b 1-way analysis of variance with covariates (age, PMI, brain weight and storage time).

PC = phosphatidylcholine; PD = psychiatric disorders; PE = phosphatidylethanolamine; PI = phosphatidylinositol; PL = phospholipids; PMI = postmortem interval; PS = phosphatidylserine; SM = sphingomyelin.

increased levels of phosphodiester (phospholipid breakdown products) in the frontal cortices of anti-psychotic-naïve first-episode patients as compared with controls. In addition, increased ATP and decreased inorganic orthophosphate levels were also found in the frontal cortex. The authors^[123] suggested that changes in membrane phospholipids might be related to molecular changes that precede the onset of clinical symptoms and brain structural changes in schizophrenia, while changes in high energy phosphate metabolism may be state dependent.

Other groups^[124-128] also reported similar findings of membrane phospholipid perturbations in both acutely and chronically ill patients. Also based on ³¹P-MRS findings, Keshavan et al.^[129] suggested a possible familial basis for membrane phospholipid changes in schizophrenia.

Direct evidence of decreased fatty acid levels comes from postmortem studies (see section 4.2.2) of patients with schizophrenia relative to healthy control individuals, findings that may underlie the phospholipid abnormalities observed using ³¹P-MRS.

4.2.2 High Pressure Liquid Chromatographic Measurements in Postmortem Samples

We have recently developed a procedure to separate and quantify various major membrane phospholipids by high pressure liquid chromatography using an evaporative light scattering detector. Analysis of covariance was conducted to control for significant potential effects of the brain collection and

storage variables on the group differences for the levels of membrane phospholipids in the caudate region of postmortem brain tissue.^[7] The membrane phospholipid levels were significantly different between the schizophrenia and control groups (table III). Both PE and PC levels were significantly reduced in the schizophrenia group. Such changes were not associated with any of the brain collection and storage parameters, and were unlikely to be caused by the effects of antipsychotics. In addition, a small but marginally significant increase in PI level was also found in the schizophrenia group. There are no significant differences between the control groups with and without other mental disorders. The present findings lend further support to the concept of membrane phospholipid deficits in schizophrenia.

Furthermore, a robust reduction of total levels of PUFAs was found in the brains of those individuals who had had schizophrenia (table IV), relative to healthy controls and to those with other psychiatric disorders. This is consistent with the observed reduction of membrane levels of PE and PC. Specifically, the decrease of PUFA levels was largely attributable to reductions in AA and, to a lesser extent, its precursors linoleic acid and 20:2(n-6) in the caudate region. A similar decrease of AA was also found in the frontal cortex of patients who had had schizophrenia.^[9] These data are in accordance with findings of plasma and RBC membrane fatty acid levels.

Thus, there is ample evidence for the existence of membrane phospholipid and fatty acid defects in early and chronic schizophrenia. As is known from membrane physiology, changes in the composition of membranes leads to a number of functional disturbances, and such pathological changes of relevance to schizophrenia are reviewed in section 5.

5. Oxidative Stress, Membrane Dysfunction and Neurotransmission

Much of the research focus in schizophrenia has been on neurotransmitter systems. Although the role of dopamine in the pathophysiology of schizophrenia remains preeminent, recent findings suggest that multiple neurotransmitter systems may be faulty. In many ways, schizophrenia can be conceptualised as being associated with 'multi-neurotransmitter' pathology. Whether these are primary or secondary to other pathological processes, such as oxidative stress and membrane dysfunction, will need to be determined in future studies. It is important to recognise, however, that alterations in the metabolism of several neurotransmitter systems can both contribute to, and be modified by, oxidative stress (or membrane dysfunction).

5.1 Neurotransmitters as Contributors to the Free Radical Burden

While schizophrenia is not necessarily viewed as a neurodegenerative disorder, findings from known neurodegenerative syndromes such as Parkinson's disease can provide heuristic models applicable to schizophrenia. For example, in Parkinson's disease the dopamine turnover rate may be a determinant in the severity of dopamine neuronal degeneration, because neurons with higher metabolic rates are subjected to greater oxidative stress from dopamine-derived free radicals, such as dopamine semiquinones. It is well known that the metabolism of dopamine yields free radicals under normal physiological conditions.^[130] A number of dopamine metabolic pathways exist that lead to the generation of hydroxyl radicals (fig. 2). Dopamine is susceptible to auto-oxidation when the AODS is weak.^[131] Moreover, the progressive loss of dopamine triggers an increase in turnover of the neurotransmitter in the remaining neurons and facilitates the accumulation of toxic byproducts of dopamine metabolism.

Interestingly, it has been recognised that dopamine-mediated toxicity is also mediated through the action of dopamine at *N*-methyl-D-aspartate (NMDA) glutamate receptors.^[132-134] There is accumulating evidence that NMDA receptor-mediated

Table IV. Quantitative determination of polyunsaturated fatty acids (PUFAs) in postmortem brain tissues of healthy individuals and patients who had had schizophrenia

Brain region	PUFAs	Healthy controls	Patients with schizophrenia	Psychiatric controls ^a
Frontal cortex ^{b,c}	18:2(n-6)	0.8 ± 0.5	0.4 ± 0.2*	Not available
	20:4(n-6)	15.2 ± 1.1	13.8 ± 1.7*	Not available
	22:4(n-6)	7.1 ± 0.4	7.5 ± 1.1	Not available
	22:6(n-3)	34.0 ± 3.5	34.2 ± 4.4	Not available
Caudate ^{d,e}	18:2(n-6)	3.2 ± 1.3	2.1 ± 0.8	2.7 ± 0.7
	20:4(n-6)	17.6 ± 1.6	15.4 ± 3.0*	19.5 ± 1.8
	22:4(n-6)	5.3 ± 0.6	4.8 ± 1.1	5.0 ± 0.5
	22:6(n-3)	9.8 ± 1.7	8.6 ± 2.8	10.9 ± 1.7

a Psychiatric control group consisted of individuals with major depression (n = 4) and bipolar disorders (n = 1).

b From Horrobin et al.,^[9] PUFAs were isolated from phosphatidylethanolamine.

c Values from frontal cortex are expressed as mean and standard deviation of mg/100mg total lipids.

d From Yao et al.,^[7] PUFAs were isolated from total phospholipids.

e Values from caudate are expressed as mean and standard deviation of nmol/mg dry weight.

* significant difference (p < 0.05) versus healthy or psychiatric controls.

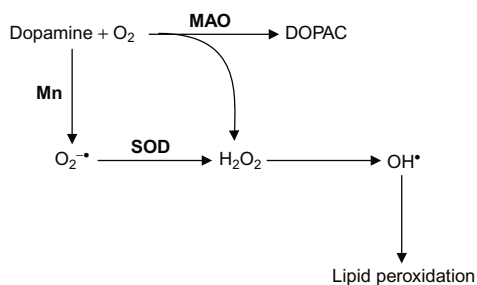


Fig. 2. Auto-oxidation and oxidative deamination of dopamine pathways. **DOPAC** = 3,4-dihydroxy-phenylacetic acid; **MAO** = monoamine oxidase; **Mn** = manganese; **OH[•]** = hydroxyl radicals; **SOD** = superoxide dismutase; **O₂^{•-}** = superoxide radicals.

excitotoxicity involves free radicals such as superoxide and nitric oxide.^[135,136] In fact, antioxidants (e.g. ascorbate and α -tocopherol) protect neurons against glutamate neurotoxicity.^[137,138]

Neurotransmitters, particularly glutamate, can induce other metabolic processes that increase free radical production. Activation of NMDA receptors by glutamate stimulates phospholipase A₂ (PLA₂) activity and results in the release of AA to act as a second messenger, which in turn can lead to the formation of free radicals.^[139] Decreased availability of AA, due either to increased PLA₂ activity or lipid peroxidation, can lead to impaired glutamatergic neurotransmission, which has been proposed as a pathogenetic mechanism in schizophrenia.^[140] A dopamine-glutamate imbalance has also been implicated in schizophrenia.^[141] Antipsychotics that block dopamine receptors may also enhance glutamatergic neurotransmission.

5.2 Membrane Dynamics and Neurotransmission

Changes in membrane dynamics can affect transmembrane processes.^[142] Inhibition of transmembrane dopamine uptake by increasing the synaptic plasma membrane cholesterol to phospholipid ratio (C/PL) has previously been shown.^[143] Thus, the function of the dopamine transporter receptor (DATR) is highly influenced by the lipid composition of membrane environment. Decreased DATR density has been found in cortical areas

with high metabolic activity in the brains of individuals with schizophrenia.^[144] Further, both n-6 and n-3 series of PUFAs may be involved in the presynaptic receptor control of dopamine release.^[145]

Serotonin 5-HT₂ receptors in the brain are thought to play a regulatory role in behaviour.^[146] However, conflicting findings from brain imaging and autopsy studies had left unresolved the issue of whether serotonergic function is abnormal in schizophrenia. The recent development of serotonin-dopamine antagonists as antipsychotic drugs (i.e. atypical antipsychotics) that potently block 5-HT₂ receptors has renewed interest in this area. Modification of 5-HT_{2A} receptors in particular, and probably 5-HT_{1A} and 5-HT_{2C} and possibly 5-HT₆, 5-HT₇ and 5-HT₃ receptors, may have important modulatory roles to play in schizophrenia, as the research with the next generation of antipsychotics suggests.

5-HT₂ receptors stimulate the release of AA in hippocampal neurons through the activation of PLA₂ that is independent of inositolphospholipid hydrolysis.^[147] Thus, serotonin may potentially mediate some pathophysiological processes through receptor-stimulated AA or eicosanoids. We have recently demonstrated that drug-free patients with schizophrenia exhibited reduced physiological responsivity mediated through the platelet 5-HT₂ receptor complex. This could be normalised by haloperidol treatment,^[148] even though haloperidol is a less potent blocker of 5-HT_{2A} receptors than clozapine, risperidone or olanzapine. Serotonin is also believed to have a role in excitotoxic neurotoxicity.^[149] Future studies should explore further the interaction between serotonergic transmission and phospholipid metabolism.

Oxidative stress can both be caused by and lead to abnormal neurotransmitter metabolism. The subsequent membrane dysfunction can have important adverse effects on central neurotransmitter systems that contribute to development of some clinical features of schizophrenia.

6. Clinical Implications

Since the pathophysiology in schizophrenia still remains unclear, understanding of the clinical implications of oxidative stress and membrane deficits is also limited. Several intriguing associations have been demonstrated between biochemical indices and clinical assessments in schizophrenia. For example, there is evidence for both phospholipid abnormality^[123] and impaired free radical metabolism^[102] in the prefrontal cortex of individuals with schizophrenia, which is a key brain area implicated in the disorder. Since PUFAs are preferentially vulnerable to free radical insult, it is conceivable that membrane essential fatty acid (EFA) levels in these areas may also be decreased. Prominent negative symptoms have been associated with low levels of AA in RBCs (table V)^[10] and low levels of platelet GSH-Px.^[58] Positive symptoms, however, have been positively correlated with SOD activity.^[52] Decreased SOD activity is associated with deteriorating premorbid school functioning^[53] and TD.^[150]

To test whether defects in the AODS are related to the severity of psychopathology, we have systematically examined relations between the 3 key scavenging antioxidant enzymes (SOD, CAT and GSH-Px) and various psychosis rating scales in drug-free groups of patients with schizophrenia.^[50,57,151] To reduce the likelihood of Type I errors, we adjusted the standard significance level ($p = 0.05$) using

Table V. Comparison of red blood cell fatty acid levels in patients with schizophrenia who had predominantly negative and positive symptoms (reproduced from Glen et al.,^[10] with permission)

Fatty acids	Patients with:		p (Student's <i>t</i> -test)
	negative symptoms (n = 13)	positive symptoms (n = 12)	
16:0	31.7 ± 3.6	24.6 ± 4.5	<0.001
18:0	13.3 ± 2.6	9.8 ± 1.9	0.001
18:1(n-9)	28.8 ± 2.0	20.6 ± 2.1	<0.001
18:2(n-6)	10.6 ± 1.6	12.2 ± 2.0	0.043
20:3(n-6)	1.0 ± 0.3	1.5 ± 0.4	0.002
20:4(n-6)	7.4 ± 2.3	15.5 ± 3.5	<0.001
20:5(n-3)	0.5 ± 0.3	1.3 ± 0.4	<0.001
22:6(n-6)	2.2 ± 1.8	5.9 ± 1.8	<0.001

Table VI. Correlation between the antioxidant defense system (AODS) and symptom severity in drug-free patients with schizophrenia

Biochemical indices of AODS	r (correlation coefficient)			
	BHPR	BPRS (total)	SANS (5 items)	AIMS
RBC				
SOD ^[151]	-0.3831**	-0.2236	-0.3212*	0.0828
CAT ^[151]	-0.0707	0.0591	0.0655	0.0902
GSH-Px ^[151]	0.4069**	0.2895	0.2969	-0.0889
Plasma				
GSH-Px ^[57]	0.4005**	0.1882	0.2111	0.1394
Plasma				
TAS ^[38]	-0.3807*	-0.4295**	-0.3887*	-0.0010
Albumin ^[39]	-0.0420	0.0542	0.2390	-0.2542
Bilirubin ^[39]	0.1505	0.3510*	0.2689	-0.3039
Uric acid ^[40]	-0.4072**	-0.2149	-0.0194	-0.0558

AIMS = Abnormal Involuntary Movement Scale; **BHPR** = Bunney-Hamburg Psychosis Ratings; **BPRS** = Brief Psychiatric Rating Scales; **CAT** = catalase; **GSH-Px** = glutathione peroxidase; **RBC** = red blood cell; **SANS** = Scale for the Assessment of Negative Symptoms; **SOD** = superoxide dismutase; **TAS** = total antioxidant status; * $p < 0.05$, ** $p < 0.02$.

a Bonferroni correction for each biochemical index (adjusted $\alpha = 0.05/4$; clinical measures = 0.013). Among the 3 major AODS enzymes, both RBC and plasma GSH-Px were found to be significantly and positively correlated with the 3-day mean Bunney-Hamburg Psychosis Rating (BHPR) scores (table VI). Such a correlation was present in patients both on and off haloperidol treatment. In addition, SOD activities were inversely and significantly correlated with the BHPR and SANS scores. We also showed a significant inverse correlation of plasma TAS to various psychopathology ratings in the patients (fig. 3). A similar inverse correlation between plasma level of uric acid (a major antioxidant in plasma) and BHPR scale was also demonstrated in patients both on and off haloperidol treatment.^[40]

On the other hand, a positive correlation has been demonstrated between plasma bilirubin and the BPRS scores in drug-free patients with schizophrenia (table VI). As mentioned earlier (section 2.3.1), there is an increase in plasma total bilirubin level with aging.^[39] Since bilirubin is one of the

most efficient antioxidants in plasma,^[37] such an increased production of bilirubin may reflect an increased antioxidant protection against aging-related oxidative stress. Likewise, an association between plasma total bilirubin and the psychosis severity also suggests a need for increased production of antioxidants to combat oxidative stress.

Taken together, these findings suggest that alterations in AODS enzymes and antioxidant status may be a consequence of changed symptom severity rather than a direct effect of antipsychotic drug treatment, and further suggest the possibility of therapeutic approaches using currently available treatments.^[15,152,153]

7. Therapeutic Treatment

It is abundantly evident that there are abnormalities in AODSs in schizophrenia, predisposing to oxidative stress, which in turn is likely to contribute to the membrane deficits that have also been observed in individuals with the disorder. These observations offer an opportunity to develop novel adjunctive therapeutic strategies that take advantage of known and existing methods of reducing oxidative stress^[154] and repairing membrane deficits.^[155] The use of antioxidants in treating and preventing oxidative stress is a very active area of research, particularly for a variety of brain disorders.^[156]

Oxidative stress, however, is an outcome of complex and multidetermined interconnected systems of free radical generation and antioxidant systems. There are likely to be many risk factors for oxidative stress in schizophrenia.^[12,15] Exogenous risk factors, such as increased calorie intake, smoking and alcohol use are frequently seen in individuals with schizophrenia, and may offer targets for reducing oxidative stress.

7.1 Decreasing Free Radical Production

Oxidative stress can be reduced by lowering the substrates for free radical formation and by inhibiting enzyme activities that produce free radicals. There are several exogenous factors including diet, smoking, pollution, and possibly a sedentary life-

style, which can be modified to reduce free radical formation and increase overall antioxidant capacity. Therefore, the regimens outlines in sections 7.1 to 7.3 may assist patients with schizophrenia to reduce their oxidative stress.

7.1.1 Reducing Caloric Intake

It has been shown that caloric intake is correlated with oxidative stress, particularly in the brain,^[64] and high caloric intake is associated with decreased maximal longevity, presumably due to oxidative stress. By contrast, decreased caloric intake in animals is associated with increased maximal

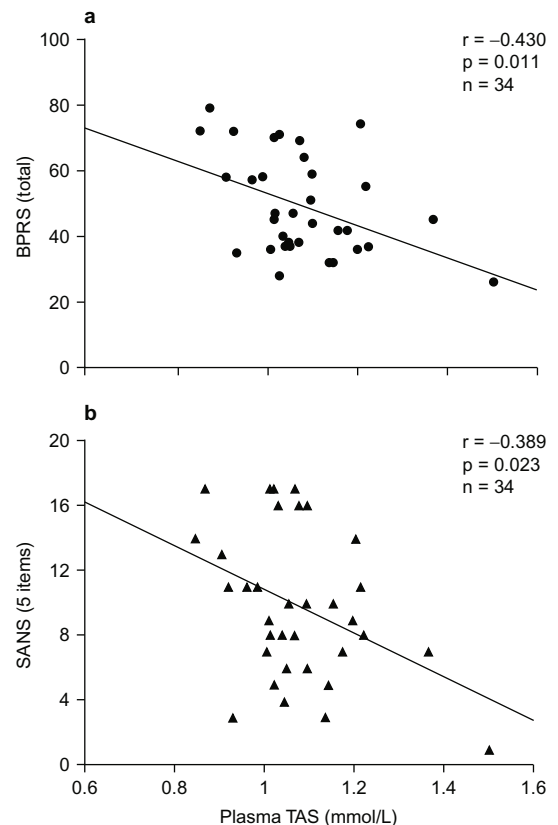


Fig. 3. Relationships of plasma total antioxidant status (TAS) to (a) Brief Psychiatric Rating Scale (BPRS) score and (b) the scale for the Assessment of Negative Symptoms (SANS) score in drug-free patients with schizophrenia (reproduced from Yao et al.,^[38] with permission).

longevity, and maintenance of cognitive and motor skills for longer duration.^[157] It is unclear whether total calorie intake or the origin of the calories makes a difference in the generation of free radicals. Diets high in fats and iron (e.g. from red meat) can increase free radical production. There are, however, no studies in schizophrenia that have examined caloric restriction as a means of reducing oxidative stress.

7.1.2 Cessation of Cigarette Smoking

It is well known that cigarette smoking is associated with reduced levels of plasma antioxidants of dietary origin.^[80-82] One of the major compounds in the gas phase of tobacco smoke is nitric oxide. It has been suggested that nitric oxide reacts with smoke olefins to form carbon-centred radicals.^[81] In addition, the tar phase contains a semi-quinone radical that promotes hydrogen peroxide formation.^[79] Moreover, tobacco smoke may increase free radical formation by activating neutrophils.^[158]

We have demonstrated that higher levels of plasma cotinine did not account for the lower levels of plasma TAS seen in our group of patients with schizophrenia.^[38] It is not clear, however, whether plasma TAS is affected by chronic tobacco consumption. Future investigations will need to determine whether plasma TAS can be improved by cessation of smoking.

7.1.3 Decreasing Alcohol Consumption

Peroxidation of membrane lipids has long been considered as a possible cause for ethanol-induced hepatic lesions.^[159,160] In humans, ethanol is mainly derived from consumption of alcoholic beverages. Most of the absorbed ethanol is removed by oxidation to first acetaldehyde and then to acetate. Chronic alcohol consumption is likely to diminish the oxidising capacity of the liver. The resulting accumulation of acetaldehydes may stimulate lipid peroxidation, and subsequently cause mitochondrial damage. Thus, excessive and prolonged ethanol ingestion is associated with an increase in oxidative stress. Therefore, reducing alcohol intake could be of benefit in reducing oxidative stress in patients with schizophrenia.

7.2 Antioxidant Treatment

The use of antioxidants in schizophrenia has been almost exclusively limited to the use of α -tocopherol in the treatment of TD. α -Tocopherol is a highly efficient lipid-soluble 'chain-breaking' antioxidant that acts to stabilise plasma membranes. Membrane α -tocopherols are susceptible to oxidation, which converts them to tocopherol-quinone. It is conceivable that such a metabolite may be increased under pathological conditions.^[161] Numerous studies in animals and humans have demonstrated protective effects of α -tocopherol against a large variety of free radical-mediated pathological insults.^[162] Supra-normal doses of α -tocopherol have been safely and effectively used to reduce the severity of TD. Several studies,^[90,112,113,163,164] albeit with relatively small sample sizes, have reported decreases in the severity of dyskinesia with α -tocopherol treatment; however, others have not.^[165-167] A shorter duration of TD was associated with better therapeutic response.

Most studies utilised a dosage range of α -tocopherol 1200 to 1600 IU/day. Peet et al.^[90] found a clinically significant response with 1200 IU/day, with the therapeutic effect maintained for 7 to 13 months after discontinuation of α -tocopherol.

While most studies have been relatively brief (<12 weeks), Adler et al.^[167] conducted a 36-week study that showed robust effects of α -tocopherol in reducing dyskinesia severity. By contrast, the largest (n = 158) and longest (12 months) study to date^[168] has shown reductions in dyskinesia severity in both treatment and placebo groups, but there was not a significant difference between the 2 groups.

In another study, significant decreases in psychiatric symptoms were also found in patients with schizophrenia following α -tocopherol treatment.^[169]

The above findings, albeit indirectly, provide further evidence for a role of free radical-mediated pathology in some dimensions of schizophrenic illness and its complications. Furthermore, patients with an increased oxidative stress are more likely to develop TD, possibly antipsychotic-induced.

7.3 Essential Fatty Acid Supplementation

There is evidence of an improvement in clinical state, albeit modest, with EFA supplementation in schizophrenia.^[152] This strategy is based on the fairly consistent evidence that there are a variety of membrane deficits in schizophrenia.^[3,4,121]

It is not known whether patients with schizophrenia in Western countries have a low dietary intake of EFAs. There is an indication, however, that outcome is better in so-called underdeveloped countries (where the diet is characterised by a high intake of EFAs) than in Western countries.^[170] Moreover, a highly significant correlation was found between low dietary fat intake and good schizophrenia outcome.^[171] Thus, there is a close correlation between a good lifetime outcome for schizophrenia and a high ratio of unsaturated to saturated fat intake.^[172]

Early studies of EFA supplementation were aimed at dyskinesia, using an animal model.^[173,174] Subsequently, in a double-blind, placebo-controlled study of evening primrose oil supplementation in a group of patients with psychiatric disorders (primarily schizophrenia), no significant effects on TD were seen, although a significant improvement was noted on the Weschler memory scale and psychopathology scores.^[8] Similarly negative results on the antidyskinetic effect of EFA supplementation were also obtained by other investigators.^[175-177] Such a failure to improve the movement disorder may have resulted from one or a combination of factors such as low dosage, inadequate duration of dietary trial, as well as chronicity and irreversibility of TD.^[178]

However, a recent open-labelled study by Mellor et al.^[179] has shown that dietary intake of eicosapentaenoic acid (EPA) led to significant improvements of scores on rating scales for both schizophrenic symptomatology and TD. In a separate study conducted in India,^[180] an improvement of total scores on the Positive and Negative Syndrome Scales (PANSS) was also noted in patients with schizophrenia following antipsychotic treatment in conjunction with EPA supplementation.

More recently, in a small double-blind trial of EFA supplementation, Peet^[181] observed the greatest improvements in total PANSS, positive symptoms and general psychopathology in antipsychotic-treated patients who received EPA supplementation as compared with those given antipsychotics and a docosahexaenoic acid-enriched oil or a corn oil placebo. No significant differences in the improvement in negative symptoms were demonstrated among the 3 treatment groups. When EPA alone was used, Puri et al.^[182] reported sustained remission of positive and negative symptoms in one drug-naïve patient with schizophrenia over a 1-year period. The improvement of schizophrenic symptomatology was further accompanied by a correction in RBC membrane phospholipid abnormalities.

Taken together, the above findings suggest further support for a potential therapeutic effect of EPA in the treatment of schizophrenia. Careful double-blind studies may now be indicated.

8. Conclusions

Extensive, albeit fragmentary, findings from neurochemical and neuroendocrine studies of schizophrenia have not provided conclusive evidence for any specific aetiological theory of the disorder,^[1] perhaps due to aetiopathogenetic heterogeneity.^[183] However, there exists a point of convergence for many of these theoretical models, one that occurs at the level of the neuronal membrane, which is the site of neurotransmitter receptors, ion channels, signal transduction and drug effects. Membrane deficits, specifically free radical-mediated, can significantly alter a broad range of membrane functions. Previous results showing altered membrane dynamics and antioxidant status in schizophrenia, and findings from other investigators (see section 2), are consistent with the notion of free radical-mediated neurotoxicity in schizophrenia.

The neurotoxicity can be a consequence of increased free radical production and/or an inadequate AODS. It is clear that there are multiple pathways that lead to excess free radical generation and subsequent oxidative stress (fig. 4). The

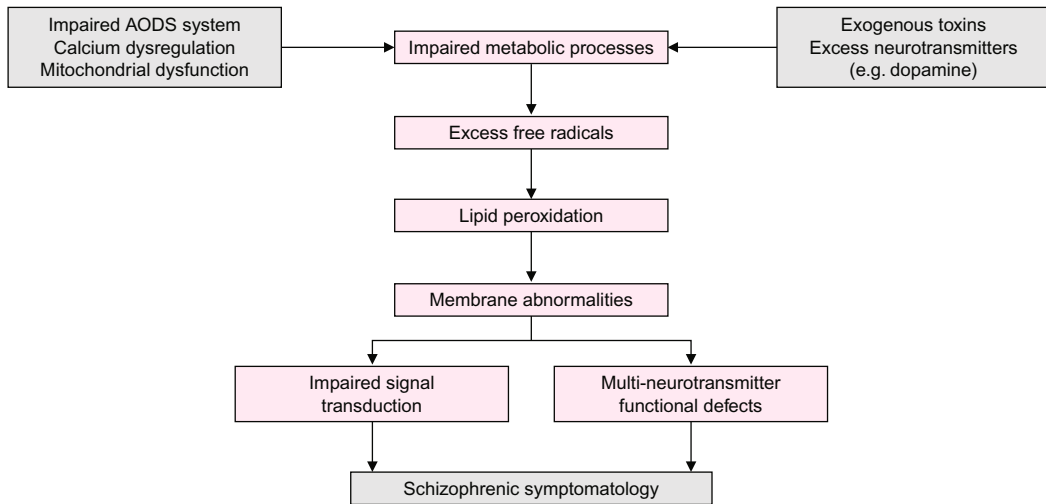


Fig. 4. Schematic model of multiple interactions between free radical production and membrane pathology in schizophrenia. **AODS** = antioxidant defence system.

pathological effects of oxidative stress are likely to be mediated through the well recognised effects of lipid peroxidation on neuronal and mitochondrial membrane components.^[97,184]

If oxidative stress can be proven to have a key role in the pathophysiology of schizophrenia, then the next step is the development of novel, adjunctive antioxidant and membrane protective treatments. It is possible that adjunctive interventions can be ‘tailored’ to respond to specific pathological mechanisms that lead to excess free radical generation, whether it is mitochondrial dysfunction or calcium dysregulation. A major question remains about the timing and duration of such interventions. There is evidence, at least for TD, that treatment with α -tocopherol is more effective in patients with a shorter TD duration.^[185] Encouragingly, the development of treatment trials with adjunctive antioxidants should be extremely short because there already exist many antioxidant compounds with relatively low known toxicity, and essential fatty acid supplementations.^[12,153]

Caution, however, is indicated in utilising antioxidants in the treatment of schizophrenia in the absence of evidence for their efficacy in controlled studies, with the exception of α -tocopherol for

treating TD. More importantly, antioxidants should not be utilised as a primary treatment for schizophrenia. Although there is a general assumption that administration of antioxidants is benign, this may not be necessarily true. Findings that the administration of the antioxidant β -carotene to smokers actually increased cancer rates, an effect opposite to what was expected, highlights this point.^[186] Quenching free radicals in excess may have unintended consequences, since free radicals are also important in many biological reactions.^[187] Therefore, future studies will need to carefully determine which antioxidants, at what dosages and in what combinations will have the greatest therapeutic benefit with the least risk.

Recently, dietary supplementation with EFAs has shown promise in ameliorating some of the clinical symptoms of schizophrenia^[117,180,182] as well as cognitive impairments associated with dyslexia and attention deficit hyperactivity disorder.^[188,189] Thus, investigating EFA metabolism has proved fruitful for generating and testing novel pathophysiological hypotheses and new therapeutic agents for schizophrenia.^[190] A tremendous advantage to the clinical use of EPAs includes their

low cost, lack of significant adverse effects and ease of use.

If indeed oxidative stress leads to membrane deficits, then there may be merit in combining membrane protective approaches, such as antioxidant and EFA supplementation. The efficacy of a combined antioxidant and EFA supplementation strategy may be greater than either approach alone. EFA supplementation alone may be sufficient to repair the loss of membrane fatty acids, but not in reducing or preventing ongoing oxidation of fatty acids by free radicals. Testing such a proposal is eminently feasible, utilising a double-blind, random-assignment approach. Further, the findings may have important ramifications in terms of preventive and early intervention approaches in populations at risk for schizophrenia, and may even shed light on the aetiological processes involved.

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