Research Paper

Oxidative damage to RNA but not DNA in the hippocampus of patients with major mental illness

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Background: Oxidative damage in the central nervous system is increasingly recognized as an important pathological process in many diseases. Previously, our laboratory found that oxidative damage to lipids and proteins was increased in postmortem brain tissue from patients with bipolar disorder and schizophrenia. In the current study, we analyzed oxidative damage to nucleic acids in the CA1, CA3 and dentate gyrus regions of postmortem hippocampus tissue from patients with bipolar disorder, schizophrenia and major depression. **Methods:** We examined oxidative damage to nucleic acids by performing immunohistochemistry with a monoclonal antibody that recognizes both 8-hydroxy-guanosine in RNA and 8-hydroxy-2'-deoxyguanosine in DNA. **Results:** We found that the amount of oxidative damage to nucleic acids was elevated in the CA1, CA3 and dentate gyrus regions of the hippocampus among patients with bipolar disorder, schizophrenia and major depressive disorder. This damage was predominantly in the cytoplasm, suggesting that the damage was primarily to RNA. Compared with oxidative damage in control samples, the magnitude of damage was high in patients with schizophrenia, modest in patients with bipolar disorder and lower in patients with major depression. **Limitations:** The interpretation of our results is limited by a number of factors, including the retrospective review of patient history, the relatively small sample size and the inclusion of patients who had substance abuse and were undergoing various drug treatments at the time of death. **Conclusion:** Our results suggest that oxidative damage to RNA, rather than to DNA, occurs in vulnerable neurons of the brain in patients with major mental illness and may contribute to the pathology of these disorders. The magnitude of RNA oxidative damage may be associated with the severity of mental illness.

Introduction

Oxidative damage results from an overproduction of reactive oxygen species (ROS) that overwhelms the cellular antioxidant capacity. Brain cells are more vulnerable than other cells to oxidative damage because the brain consumes about 20% of the body's total oxygen, although it constitutes less than 2% of total body weight. The significance of oxidative damage as a component of many disease processes in the central nervous system is being increasingly recognized. Oxidative damage has been found in neurologic disorders such as Parkinson disease and Alzheimer disease,[1,2] and it was recently identified in mental illnesses such as bipolar disorder and schizophrenia. For example, increased lipid peroxidation and decreased activity of the antioxidant defence enzymes superoxide dismutase and catalase were found in the plasma of patients with bipolar disorder,[3,4] the expression of the antioxidant enzyme glutathione s-transferase A4 and M3 subtypes was reduced in postmortem brain samples from patients with bipolar disorder,[5] and increased nitric oxide radicals and decreased levels of glutathione and related antioxidant enzymes were found in the postmortem brain tissue of schizophrenia patients.[6,7] These findings suggest that the process of oxidative damage may play an important role in the pathology of bipolar disorder and schizophrenia. Recently, many studies have shown that mood-stabilizing drugs and antipsychotic drugs inhibit oxidative damage and increase various antioxidant enzymes,[8–14] suggesting that the process of oxidative damage may be targeted by these drugs. In a clinical study involving twins, the bipolar twin had increased lipid peroxidation compared with the healthy twin.[15] This elevated lipid peroxidation was normalized after mood-

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stabilizing drug treatment,[15] suggesting that antioxidative action may have a therapeutic indication in this disorder.

Previously, our laboratory found that oxidative damage to lipids and proteins was elevated in the postmortem brain tissue of patients with bipolar disorder and schizophrenia.[16,17] Reactive oxygen species react not only with lipids and proteins but also with nucleic acids, thereby inducing oxidative damage to DNA and RNA. Guanine in DNA and RNA is more sensitive to ROS attacks than are the other bases. Reactive oxygen species oxidize guanine and generate 8-oxo-7,8-dihydroguanosine (8-OHG) in RNA and 8-oxo-7,8-dihydro-2-deoxyguanosine (8-OHdG) in DNA.

The hippocampus is a medial temporal structure involved in the Papez circuitry[18] that is responsible for emotions and is susceptible to damage during chronic stress. Recent studies have indicated the presence of cellular damage and volumetric changes in this brain region of patients with mood disorders and schizophrenia.[19,20] Because one factor that may be important in these changes is oxidative stress, we analyzed the amount of oxidative damage to nucleic acids in the CA1, CA3 and dentate gyrus regions of postmortem hippocampus tissue from patients with bipolar disorder, schizophrenia and major depressive disorder.

Methods

Postmortem brain tissue

We obtained human postmortem sections from the Stanley Medical Research Institute's brain collection. We included sections from the anterior hippocampus of patients with bipolar disorder, schizophrenia and major depressive disorder as well as nonneurologic, nonpsychiatric controls. The tissue sections were 10-μm thick, formaldehyde-fixed, paraffin-embedded, slide-mounted coronal sections. The sections were matched for age, sex, postmortem interval, pH and mRNA quality. Diagnoses were retrospectively established by 2 senior psychiatrists using DSM-IV criteria. The participants' demographic information is summarized in Table 1. Detailed clinical information, diagnostic procedures and other demographic information for these participants are available in previously published studies.[21,22]

Immunohistochemistry

We hydrated the tissue sections through graded ethanol baths, and the paraffin was removed by use of xylene. The tissue sections were then rehydrated, blocked with 5% normal goat serum with 0.3% Triton X-100 for 1 hour at 22°C and incubated with a monoclonal antibody that recognizes both 8-OHG in RNA and 8-OHdG in DNA (1:100; QED Bioscience Inc.) and an antibody for β3-tubulin (1:200; Chemicon International) at 4°C overnight. After washing the sections with phosphate-buffered saline, we incubated them in a 1:1000 dilution of Alexa-568–conjugated antimouse IgG (Invitrogen Canada Inc.) and a 1:1000 dilution of Alexa-488–conjugated anti-chicken IgG (Invitrogen) for 2 hours at room temperature. After washing, we covered the sections with fluorescent antifade mounting media (Invitrogen) and a cover slip.

We examined the fluorescence-labelled sections at 40× magnification using an Eclipse E600 microscope with NIS-Elements Advanced Research version 3.0 software (Nikon Canada). We captured images from 5 fields of each region. In the CA1 and CA3 regions, we examined the intensity of each positively labelled cell. Regions of the field without specific immunoreaction were used as background. We captured all images with uniform threshold and intensity settings. We quantified the fluorescence intensity of each field using image analysis software. The fluorescence intensities of the CA1 and CA3 regions for each slide were calculated as follows:

Fluorescence intensity = (intensity of cell 1 – intensity of the background) + (intensity of cell 2 – intensity of background) + … + (intensity of cell n – intensity of the background).

Because the cells in the dentate gyrus region were so dense, we could not count the individual cells. Thus, we examined the total intensity of 5 fields. This intensity was calculated as follows:

Fluorescence intensity = intensity of 5 fields – intensity of the background.

We processed and evaluated 2 adjacent sections for each patient. Immunohistochemical and imaging analysis were performed in a blinded fashion on 4 groups in parallel.

Statistical analyses

We assessed the differences between groups by use of 1-way analyses of variance followed by post-hoc testing (Tukey honestly significant difference). Because we analyzed data for the CA1, CA3 and dentate gyrus hippocampal subregions, we considered p values less than 0.0167 ($p = 0.05/3$) to be significant because of the Bonferroni inequality adjustment for

Table 1: Demographic characteristics of study participants

Characteristic	Group; mean (SEM) [range]*			
	Control participants	Major depressive disorder	Bipolar disorder	Schizophrenia
Age, yr	48 (2.7) [29–68]	47 (2.3) [30–65]	42 (2.9) [25–61]	45 (3.3) [25–62]
Sex, male:female	9:6	9:6	9:6	9:6
PMI, h	23.7 (2.4) [8–42]	27.5 (2.7) [7–47]	32.6 (4.0) [13–62]	33.7 (3.7) [12–61]
pH	6.3 (0.1) [5.8–6.6]	6.2 (0.1) [5.8–6.5]	6.2 (0.1) [5.8–6.5]	6.2 (0.1) [5.8–6.6]

PMI = postmortem interval; SEM = standard error of the mean.
*Unless otherwise indicated.

multiple comparisons. We used 2-tailed independent-samples t tests to assess the effects of sex. We examined the contributions of age, postmortem interval and pH by Pearson correlation analysis. All statistical analyses were performed with SPSS 16.0 for Windows.

Results

We found that oxidative damage to the nucleic acids was predominantly located in the cytoplasm, not the nucleus (Fig. 1). We found an overall significant effect of diagnosis on oxidative damage across the 4 groups in the neurons of the dentate gyrus ($F_{3,56}$ = 12.138, p < 0.001; Fig. 2), CA3 ($F_{3,56}$ = 7.783, p < 0.001; Fig. 3) and CA1 ($F_{3,56}$ = 11.907, p < 0.001; Fig. 4) in patients with major depressive disorder, bipolar disorder and schizophrenia.

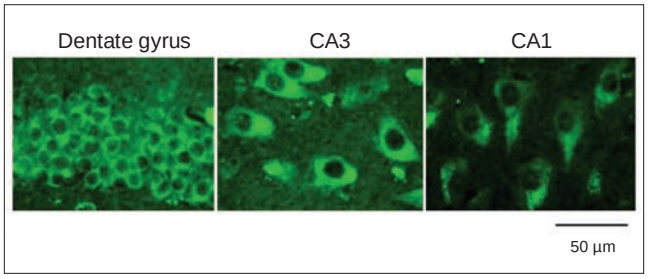

Fig. 1: Immunohistochemical staining of 8-oxo-7,8-dihydroguanosine (8-OHG) and 8-oxo-7,8-dihydro-2-deoxyguanosine (8-OHdG) in the dentate gyrus, CA3 and CA1 regions of postmortem tissue from patients with bipolar disorder. The sections were stained with a monoclonal antibody that recognizes both 8-OHG in RNA and 8-OHdG in DNA.

We assessed the effect of sex, age, postmortem interval and pH on nucleic acid oxidative damage in CA1, CA3 and dentate gyrus regions (Fig. 5). We found no difference between men and women and no correlation between nucleic acid oxidative damage and age or pH in these 3 regions. Although nucleic acid oxidative damage was not correlated with postmortem interval in the CA1 and CA3 regions, the damage was positively correlated with postmortem interval in the dentate gyrus (r = 0.264, p = 0.042). However, when postmortem interval was used as a covariate, the univariate analysis of variance still indicated that oxidative damage was significantly different among the diagnostic groups ($F_{4,55}$ = 9.241, p < 0.001).

Post-hoc comparisons for the dentate gyrus and CA3 regions revealed that the oxidative damage was significantly increased in all 3 mental illness groups. In the dentate gyrus (Fig. 2), nucleic acid oxidative damage was increased by 147% in patients with major depressive disorder (p = 0.004), 159% in patients with bipolar disorder (p < 0.001) and 175% (p < 0.001) in patients with schizophrenia. In the CA3 region (Fig. 3), nucleic acid oxidative damage was increased by 145% in major depressive disorder (p = 0.016), 149% in bipolar disorder (p = 0.007) and 168% (p < 0.001) in schizophrenia.

In the CA1 region (Fig. 4), nucleic acid oxidative damage

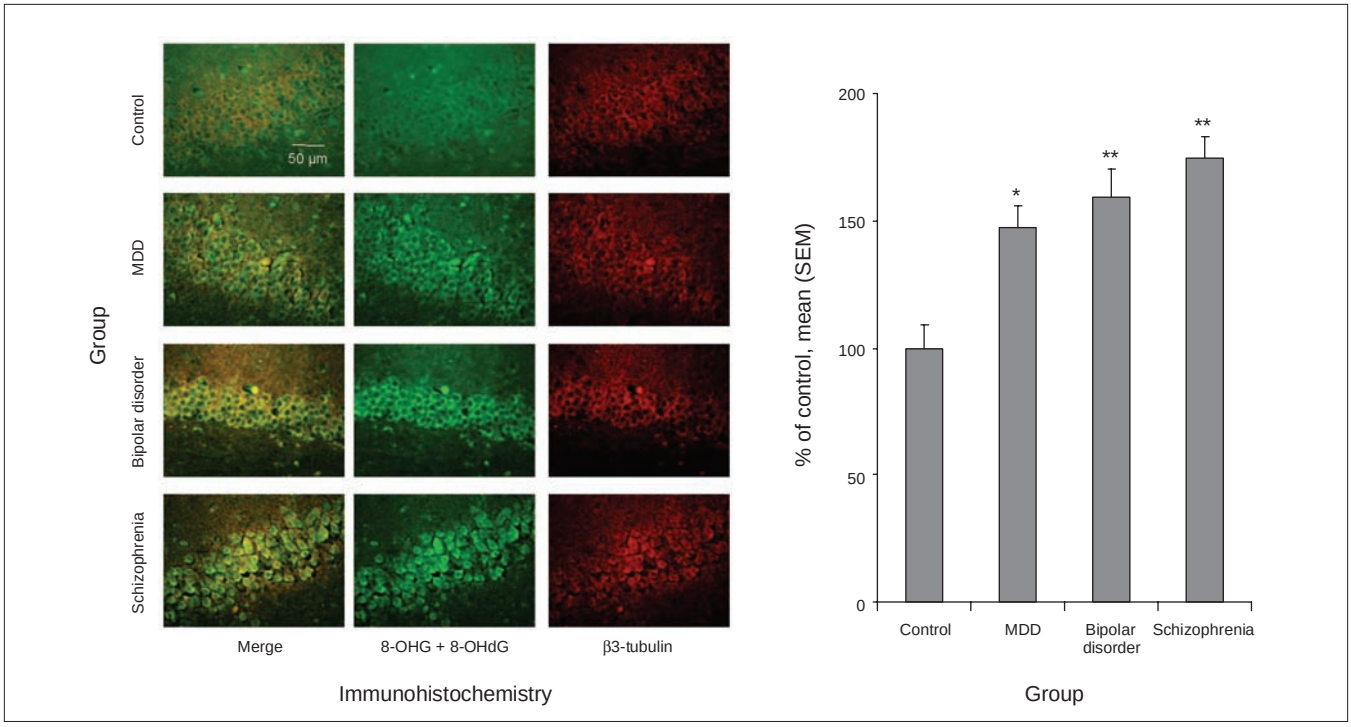

Fig. 2: RNA oxidative damage in the dentate gyrus region of postmortem hippocampus tissue from controls and patients with major depressive disorder (MDD), bipolar disorder or schizophrenia. We assessed the damage to the nucleic acids via immunohistochemical staining with an antibody that recognizes 8-oxo-7,8-dihydroguanosine (8-OHG) in RNA and 8-oxo-7,8-dihydro-2-deoxyguanosine (8-OHdG) in DNA (n = 15 patients per group; original magnification × 40). *p < 0.0167, **p < 0.0033 compared with control. SEM = standard error of the mean.

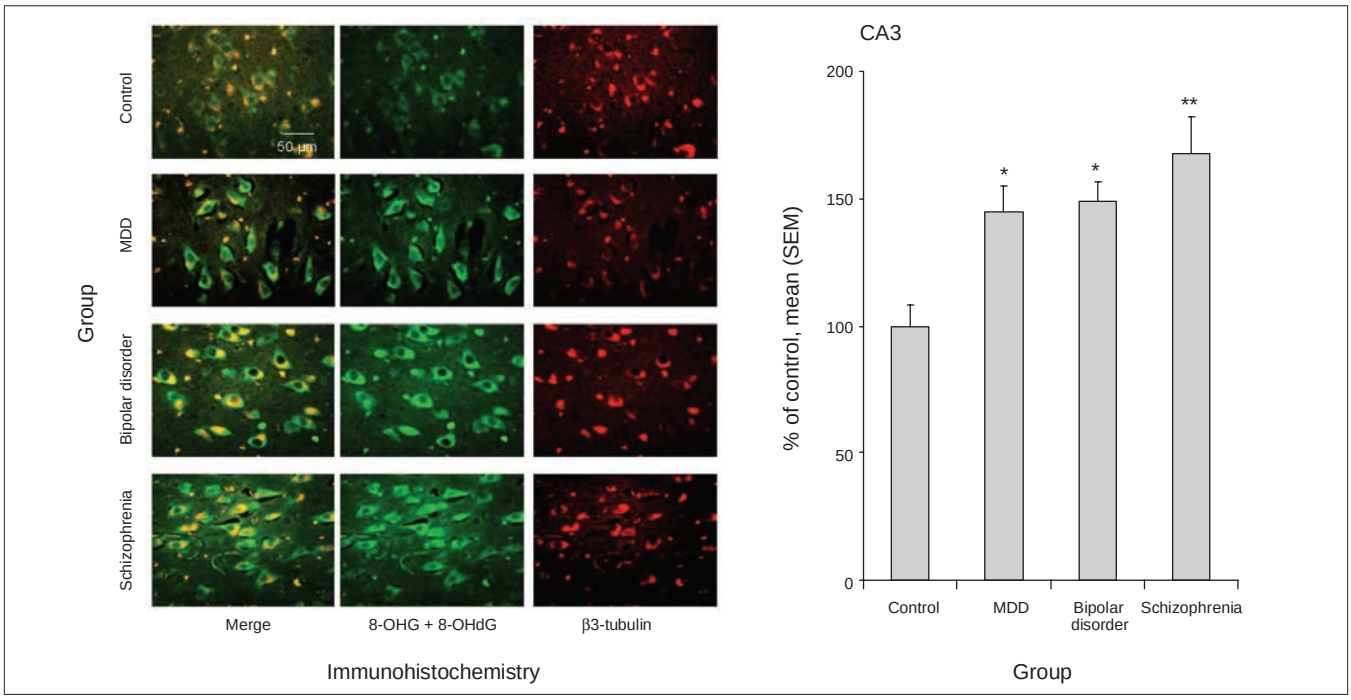

Fig. 3: RNA oxidative damage in the CA3 region of postmortem hippocampus tissue from controls and patients with major depressive disorder (MDD), bipolar disorder or schizophrenia. Damage to nucleic acids was assessed by immunohistochemistry with an antibody for both 8-oxo-7,8-dihydroguanosine (8-OHG) in RNA and 8-oxo-7,8-dihydro-2-deoxyguanosine (8-OHdG) in DNA (n = 15 patients per group; original magnification × 40). An average of 50, 60, 60 and 65 positive cells were counted in the control, MDD, bipolar disorder and schizophrenia groups, respectively. *p < 0.0167, **p < 0.0033 compared with control. SEM = standard error of the mean.

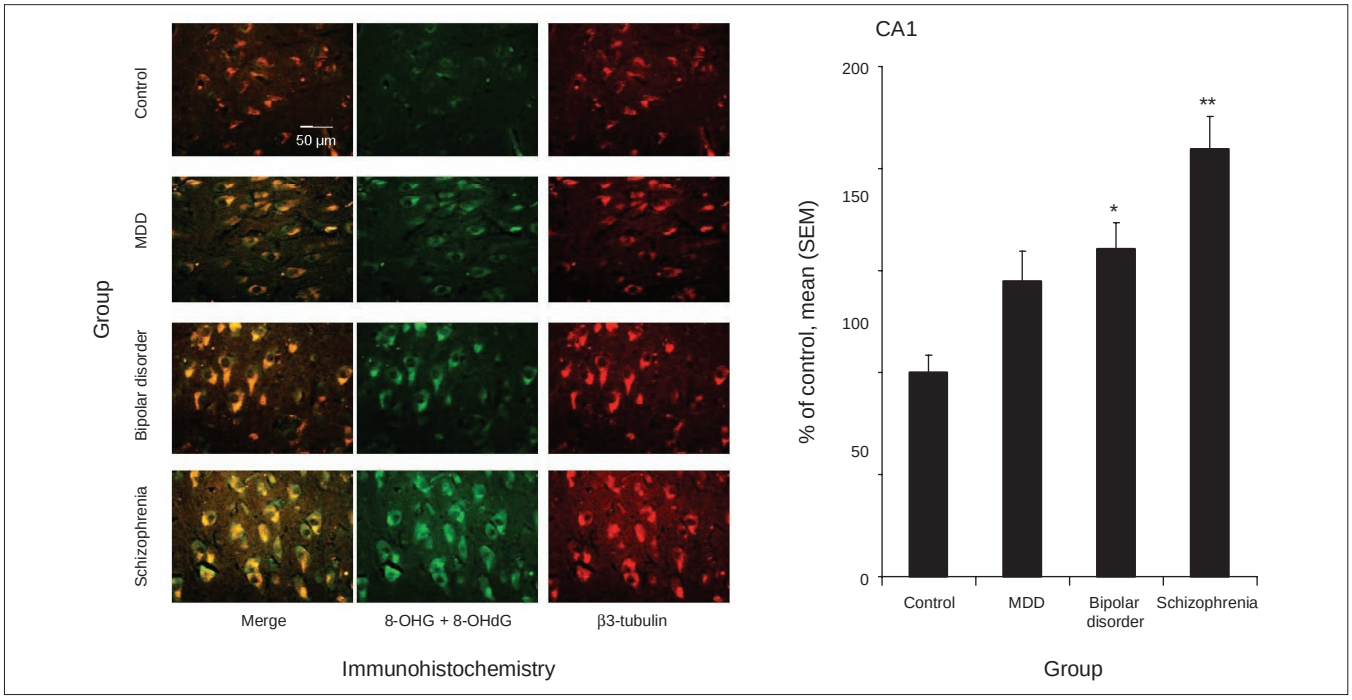

Fig. 4: RNA oxidative damage in the CA1 region of postmortem hippocampus from controls and patients with major depressive disorder (MDD), bipolar disorder and schizophrenia. Damage to nucleic acids was assessed using immunohistochemistry with an antibody for both 8-oxo-7,8-dihydroguanosine (8-OHG) in RNA and 8-oxo-7,8-dihydro-2-deoxyguanosine (8-OHdG) in DNA (n = 15 patients per group; original magnification × 40). An average of 56, 64, 68 and 71 positive cells were counted in controls, MDD, bipolar disorder and schizophrenia groups, respectively. *p < 0.0167, **p < 0.0033 compared with control. SEM = standard error of the mean.

was significantly increased in patients with bipolar disorder and schizophrenia compared with controls. Damage was increased by 161% in bipolar disorder ($p = 0.010$) and 210% in schizophrenia ($p < 0.001$). Although nucleic acid oxidative damage was increased by 144% in patients with major depressive disorder, it was not statistically significant ($p = 0.09$).

Discussion

In this study, we found that oxidative damage to nucleic acids in neurons was significantly increased in the CA1, CA3 and dentate gyrus regions of postmortem hippocampus tissue from patients with major depressive disorder, bipolar disorder and schizophrenia. This suggests that nucleic acid oxidative damage in vulnerable neurons has an important role in the pathological process of mental illness. Our results also indicate that this oxidative damage predominantly occurs in the cytoplasm, not the nucleus, of the cells in the CA1, CA3 and dentate gyrus regions. Because DNA is located in the nucleus and RNA is located in the cytoplasm, our results suggest that RNA but not DNA is damaged by oxidative stress in these diseases. RNA is more vulnerable to oxidative attack than DNA, possibly because the bases in single-strand RNA are not protected by hydrogen bonding as they are in double-strand DNA.[23–25] The pattern of predominant oxidative damage to cytoplasmic RNA rather than nuclear DNA may be reversible after pharmacological intervention. We also found that the magnitude of RNA oxidative damage was highest in patients with schizophrenia, whereas it was more modestly increased in patients with bipolar disorder and less so in patients with major depression. These findings suggest that RNA oxidative damage may be associated with the severity of these mental illnesses.

RNA oxidation is generally associated with reduced protein expression because oxidized bases on RNA transcripts can slow the translation process and induce translation errors.[26,27] Recent proteomic studies have identified many downregulated proteins in the postmortem brain tissue of patients with major depressive disorder, bipolar disorder and schizophrenia.[28–31] It will be interesting to determine whether RNA oxidative damage contributes to the downregulation of these proteins.

Because most of the patients in our study had been taking various medications including antidepressants, mood stabilizers and antipsychotics, increased RNA oxidative damage may be related to drug treatment. Ideally, it would be best to compare RNA oxidative damage in unmedicated and medicated patients. However, in our patients, only 2 patients with major depression, 3 with bipolar disorder and 3 with schizophrenia were not taking any medication at the time of death. In addition, 5 of these 8 patients also had an alcohol or substance abuse problem. Therefore, it would not be practical to perform this comparison. Many recent studies, including ours,[8-11] have shown that antidepressants, mood stabilizers and atypical antipsychotics inhibit oxidative damage in animal models and cells,[8–14] which suggests that RNA oxidative damage in patients with mental illness is not likely a result of treatment with medication.

Increased RNA oxidative damage in patients with bipolar disorder, schizophrenia and major depressive disorder may result from dysfunctional mitochondria because mitochondria are the major source of ROS. A growing body of evidence suggests that bipolar disorder and schizophrenia are illnesses that are particularly associated with mitochondrial dysfunction.[32-37] Studies involving brain imaging have shown that adenosine-5′-triphosphate (ATP) and phosphocreatine are decreased in the frontal lobes and basal ganglia in patients with mood dis-

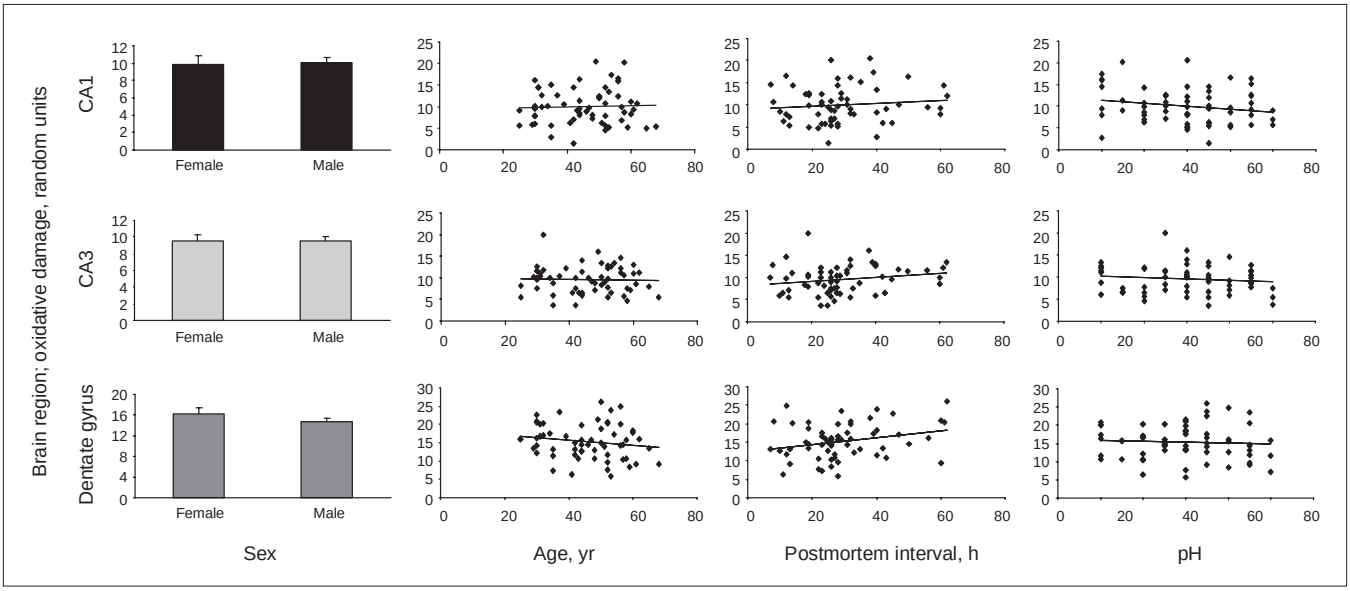

Fig. 5: Difference in the amount of oxidative damage to RNA between men and women and the correlation between RNA oxidative damage and age, postmortem interval and pH in the postmortem hippocampus of control patients, patients with major depressive disorder, bipolar disorder or schizophrenia ($n = 60$; 36 men and 24 women). We used 2-tailed independent *t* tests to assess the effects of sex. The contributions of age, postmortem interval and pH were examined by Pearson correlation analysis.

orders.[32–35] It has also been reported that the density of mitochondria was decreased in the caudate nucleus and putamen of patients with schizophrenia.[36] In addition, mitochondrial ATP production rates have been found to be decreased in the muscle of patients with major depressive disorder.[37] These findings suggest that dysfunctional mitochondria impair energy production in these common mental illnesses.

Recent studies have shown that lactate levels in cerebrospinal fluid are significantly increased in patients with bipolar disorder and schizophrenia compared with controls,[38] which suggests that mitochondrial dysfunction induces a shift in energy production from more efficient oxidative phosphorylation in the mitochondrial electron transport chain to less efficient anaerobic glycolysis. Mitochondrial ATP is produced via oxidative phosphorylation coupled to electron transport chain complexes I–V. Studies have shown deletions and mutations in genes coding for complex I subunits in patients with bipolar disorder.[39] Recent DNA microarray analyses, including ours,[40] in postmortem samples from the frontal cortex and hippocampus found that the expression of many mRNAs coding for subunits of complexes I–V was decreased in patients with bipolar disorder.[40,41] A proteomic study has also shown that subunits of complex I and complex III are downregulated in postmortem samples of the prefrontal cortex of schizophrenia patients.[30] Complexes I and III are the main sites where electrons are leaked to oxygen, resulting in the production of ROS that can cause oxidative damage.

Reactive oxygen species can be also produced by various oxidases.[42] Monoamine oxidases (MAOs) degrade monoamine neurotransmitters and have an important role in the pathology of depression. Monoamine oxidase inhibitors are a pharmacological option for the treatment of depression. It has been reported that the density of MAO-A is increased in the brain of patients with depression.[43] The results of these studies, together with ours,[40] suggest that RNA oxidative damage in bipolar disorder, schizophrenia and major depressive disorder may result from overproduction of ROS caused by mitochondrial dysfunction or abnormal function of MAOs.

Limitations

The interpretation of our results is limited by a number of factors, including the retrospective review of patient history, the relatively small sample size and the inclusion of patients with substance abuse and those undergoing various drug treatments at the time of death. Therefore, confirmation in larger samples will be required if more definitive conclusions are to be made.

Conclusion

We found that RNA oxidative damage was significantly increased in the postmortem hippocampus of patients with major depressive disorder, bipolar disorder and schizophrenia. Together with our previous findings,[16] this suggests that oxidative damage in the brain may contribute to the pathological process of mental illnesses and that antioxidative stress may be an alternative approach to pharmacological treatment of these psychiatric disorders.

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Competing interests: None declared.

Contributors: All authors designed the study. Dr. Che acquired the data, which he and Drs. Wang, and Young analyzed. Drs. Che, Wang and Young wrote the article, which they and Dr. Shao reviewed. All authors approved the final version submitted for publication.

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