Increased 8-hydroxy-deoxyguanosine, a marker of oxidative damage to DNA, in major depression and myalgic encephalomyelitis / chronic fatigue syndrome

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Abstract

BACKGROUND: There is now evidence that major depression and myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS) are accompanied by partially overlapping pathophysiological mechanisms, i.e. activation of various inflammatory and oxidative & nitrosative (IO&NS) pathways.
OBJECTIVE: The aim of the present study was to examine the urinary excretion of 8-hydroxy-deoxyguanosine (8-OhdG), a marker of oxidative damage to DNA, in depression; ME/CFS; and depression and ME/CFS.
METHODS: Toward this end, morning urine was sampled for the assays of 8-OHdG and creatinine, in 44 patients with ME/CFS; 25 with major depression; 23 with depression and ME/CFS; and 17 normal controls. Severity of fatigue and somatic symptoms was measured by means of the Fibromyalgia and CFS Rating (FF) scale.
RESULTS: We found that 49.0% of the variance in the urinary excretion of 8-OHdG was predicted by the regression on creatinine. Consequently, the urinary 8-OHdG excretion should be expressed as the residualized 8-OHdG values after partialling out the effects of creatinine and not by computing the 8-OHdG / creatinine ratio. We found that the residualized urinary excretion of 8-OHdG (adjusted for creatinine) was significantly higher in patients with depression and ME/CFS than in normal controls and all other patients. In the patient group, there were significant correlations between the urinary 8-OHdG and the total score on the FF scale and sadness and flu-like malaise.
CONCLUSIONS: The findings show increased oxidatively generated DNA damage in patients with major depression and ME/CFS and, therefore, further extent the role played by IO&NS pathways in the pathophysiology of both disorders. Since oxidatively damage to DNA is a risk factor for atherosclerosis and neurodegeneration, our results also explain previous findings on increased cardiovascular morbidity in depression and ME/CFS, and neurodegenerative processes in depression.

ORIGINAL ARTICLE

INTRODUCTION

There is a significant clinical overlap between major depression and myalgic encephalomyelitis / chronic fatigue syndrome (ME/CFS). Depression frequently occurs during the course of ME/CFS (Skapinakis *et al.* 2003; 2004). Some authors go so far to suggest that chronic fatigue is a "form fruste" of depression because many patients with fatigue syndromes have "comorbid" affective disorders (Roy-Byrne *et al.* 2002). On the other hand, "psychosomatic symptoms", reminiscent of ME/CFS, are prominent in major depression. Thus, somatic factors have been found to be an important part of depressive phenomenolgy (Hamilton, 1959; Beneke, 1987; Mountjoy and Roth, 1982a; 1982b). Other authors employ the label "somatic depression" to describe a type of depression characterized by "somatoform" symptoms, such as chronic fatigue, pain, paraesthesiae, irritable bowel, sexual inhibition, and vertigo (Alonso Fernandez, 2001; Silverstein, 2002). Rating scales which are used to measure severity of depression, e.g. the Hamilton Depression Rating Scale (HDRS) and the Beck Depression Inventory (BDI) (Hamilton, 1960; Beck *et al.* 1961) consider somatic items, such as "somatic symptoms general", that is muscle aches, fatigue and loss of energy; hypochondriasis; somatic anxiety, that is sweating, autonomic symptoms, headache; and fatigue and loss of energy. Recently, we have shown that fatigue and somatic (F&S) symptoms, such as aches and pain, muscular tension, fatigue, concentration difficulties, failing memory, irritability, irritable bowel, headache, and a subjective experience of infection, are a major feature of major and in particular melancholic depression and largely predict the severity and chronicity of depression (Maes, 2009b).

The symptomatic similarities between ME/CFS and depression may be explained by recent findings that partially overlapping pathophysiological mechanisms, i.e. aberrations in inflammatory and oxidative and nitrosative stress (IO&NS) pathways, underpin both disorders (Maes, 2009a; Maes *et al.* 2009e). Thus, inflammatory reactions characterize both disorders: increased pro-inflammatory cytokines, increased intracellular inflammation and gut-derived inflammation (Maes, 2009a; Maes and Leunis, 2008; Maes and Twisk, 2009; Maes *et al.* 2007a; 2007b; 2008a; 2009e). Moreover, both depression and ME/CFS are accompanied by increased O&NS, including increased levels of radical oxygen species (ROS), lipid peroxidation and nitrosative damage to proteins (Maes *et al.* 2006b; 2007c; 2008b; 2009d). Finally, lowered levels of different antioxidants are observed in depression and ME/CFS as well (Maes *et al.* 2005; 2006a; 2009a; 2009b; 2009c).

Importantly, the F&S symptom profiles that characterize ME/CFS and that are a key feature of major and melancholic depression are the expression of disorders in the abovementioned IO&NS pathways. In ME/CFS, there is some evidence that the abovementioned F&S symptoms, e.g. aches and pain, muscular tension, fatigue and malaise are the expression of intracellular inflammation, damage to membrane fatty acids by O&NS and lowered levels of antioxidants, such as coenzyme Q10 (CoQ10), zinc and dehydroepiandrosterone (DHEA) (Maes, 2009b; Maes *et al.* 2006a; 2006b; 2007a; 2007b; 2007c; 2009a). In depression, the F&S symptom cluster corresponds to inflammatory markers, O&NS and decreased levels of antioxidants, such as zinc, CoQ10 and glutathion peroxidase (Maes *et al.* 1993; 2007c; 2008a; 2008b; 2009b; 2009c; 2009d; 2009e). It has been shown that the abovementioned F&S symptoms and specific depressive symptoms, such as anhedonia, anorexia, weight loss and psychomotor retardation may be induced by peripheral and central (neuro)inflammation (Goshen *et al.* 2008; Qin *et al.* 2007). In humans, immunotherapy with cytokines has been shown to induce F&S symptoms appearing early after treatment, while the cognitive symptoms of depression appear some weeks later (Martin *et al.* 2007; Wichers *et al.* 2005).

ROS formed during normal metabolism constantly attack nuclear and mitochondrial DNA causing oxidized nucleosides and thus DNA lesions with high frequency. One of those mutagenic lesions is 8-hydroxy-2'-deoxyguanosine (8-OHdG), the product of hydroxylation of guanine at carbon 8. The nuclear and mitochondrial DNA lesions are excised through the base excision repair pathway so that the DNA lesions cannot be replicated. Consequently, the oxidative damage to DNA is removed and the formed 8-OHdG is eliminated and eventually excreted in the urine (Wu *et al.* 2004; Bohr *et al.* 2002). Inflammatory reactions not only induce nitric oxide (NO) generation which causes IO&NS-induced damage of DNA, but also inhibit the 8-OHdG base excision repair pathways through NO-mediated processes and consequently potentiate DNA damage (Jaiswal *et al.* 2001).

The serum levels of 8-OHdG are significantly increased in depressed patients and are higher in patients with recurrent depressive episodes (Forlenza and Miller, 2006). 8-OHdG is significantly higher in the peripheral leukocytes of depressed patients (Irie *et al.* 2005). In ME/CFS increased indicants of DNA damage were detected in muscle specimens, such as the vastus lateralis muscle (Fulle *et al.* 2000). 8-OHdG can easily be measured in the urine and urinary 8-OHdG is considered to be a biomarker of generalized oxidative stress and oxidative DNA damage (Wu *et al.* 2004; Valavanidis *et al.* 2009).

The present study has been carried out in order to examine whether depression and ME/CFS are accompanied by increased urinary excretion of 8-OHdG and whether the latter is related to specific F&S symptoms.

SUBJECTS AND METHODS

Subjects

One-hundred and nine subjects participated in this study, i.e. 17 normal volunteers; 44 patients with ME/CFS; 25 patients with major depression and 23 patients with both major depression and ME/CFS. All patients were admitted to the Maes Clinics, Antwerp, Belgium. The diagnosis major depression was made using the DSM-IV-R criteria (APA, 2000) by means of a semi-structured interview. The presence of ME/CFS was made using the Center for Disease Control and Prevention (CDC) criteria (Fukuda *et al.* 1994). Of course, the CDC criteria rule out that the diagnosis ME/CFS can be made when melancholia is present. Nevertheless, we employed the CDC criteria to conclude that ME/CFS is present: 1) the patient suffers from severe chronic fatigue for at least six months; and 2) the patient suffers from at least four of the following symptom: substantial impairment in short – term memory or concentration; sore throat; muscle pain; multi – joint pain without selling or redness; headache of new type; unrefreshing sleep; and post exertion malaise lasting more than 24 hours. The severity of the F&S symptoms was measured by means of the Fibromyalgia and Chronic Fatigue Syndrome (CFS) Rating (FF) Scale (Zachrisson *et al.* 2002). This scale rates the following F&S symptoms: pain, muscular tension, fatigue, concentration difficulties, failing memory, irritability, sadness, sleep disturbances, autonomic disturbances, irritable bowel, headache, and subjective experience of infection. The total sum on the FF scale was computed as a index for the severity of F&S symptoms.

We excluded subjects with a life-time diagnosis of psychiatric DSM IV-R disorders, e.g. psychotic, substance use and organic mental disorders. We excluded controls or patients with medical illnesses, such as inflammatory bowel disorders, diabetes type 1 or type 2, hypertension, and atherosclerosis. Patients who had been treated with anti-psychotic drugs, anticonvulsants or mood stabilizers the year prior to this study were omitted. Subjects with clinical signs of infections the last two months prior to the study and heavy smokers (> 10 cigarettes/day) were excluded. Patients and controls gave written informed consent after the study protocol was fully explained; the study has been approved by the local ethical committee.

Methods

8-OHdG has been determined with a competitive in vitro enzyme linked immunosorbent assay (ELISA), catalog no KOG-200S/E from the Japan Institute for Control of Aging (Fukuroi, Shizuoka, 437-012, Japan). This ELISA is based on 8-OHdG linked to a carrier protein and coated to a microtitre plate solid phase to which samples, standards and monoclonal antibody are added in a first reaction step. 8-OHdG in the sample and standards (range 0.5 to 200 ng/ml) reacts competitively with the solid phase 8-OHdG for the monoclonal antibody. After a washing step to remove unbound monoclonal antibody, a second incubation step is perfomed with a HRP-conjugated anti mouse antibody followed again by a washing step. Finally the reaction is quantified by addition of the HRP substrate hydrogen peroxide and the chromogen 3,3',5,5'-tetramethylbenzidine and the substrate conversion is measured at 450 nm. The standard curve from the assay is generated by plotting absorbance at 450 nm versus the concentration of the standards. The absorbance values of the test samples are used to determine the 8-OHdG concentrations in the samples.

Creatinine concentrations in urine samples have been determined with the kinetic colorimetric assay by a modified version of a Jaffe method on a fully automated instrument, Modular P of Roche. In alkaline solution, creatinine forms a yellow-orange complex with picrate. The measuring range in urine for the methods is 4.0 to 650 mg/dL.

STATISTICS

Differences between group means were checked with the analyses of variance (ANOVAs) or covariance (ANCOVAs). The least significant difference (LSD, at $p<0.05$) or the Dunn test were used to assess post-hoc differences amongst multiple treatment means. Correlations between different variables were measured by means of Pearson's product-moment correlation coefficients, semipartial correlation calculations, and simple regression and automatic stepwise multiple regression (with an p–to-enter of p=0.05) analyses. The independence of classification systems was checked by means of analysis of contingence Tables (χ^2-test). In order to normalize the data distribution of the 8-OHdG data we used a Box-Cox transformation. The significance was set at $\alpha=0.05$ (two tailed).

RESULTS

In the total study group we found that 49.0% of the variance in urinary 8-OHdG was explained by the regression on the urinary excretion of creatinine ($r=0.71$, $F=106.2$, $df=1/107$, $p<10^{-5}$). We found that there was only a moderate semipartial correlation between the 8-OHdG values adjusted by means of regression analysis for creatinine and the 8-OHdG / creatinine ratio ($r=0.70$, $F=101.5$, $df=1/107$, $p<10^{-5}$). In the normal controls, we found that 68.0% of the variance in urinary 8-OHdG was explained by the regression on the urinary excretion of creatinine ($r=0.82$, $F=31.8$, $df=1/15$, $p=0.0001$), while the semipartial correlation between the residualized 8-OHdG values and the 8-OHdG / creatinine ratio was only $r=0.52$ ($R^2=27.5\%$, $F=7.1$, $df=1/15$, $p=0.02$). This shows that the urinary 8-OHdG values should be expressed as the residualized values after partialling out the effects of creatinine by means of

regression analysis and not as the 8-OHdG / creatinine ratio. Therefore, in this paper we report on the residualized 8-OHdG values only and not on the 8-OHdG / creatinine ratio. When examining the differences between diagnostic groups in 8-OHdG, creatinine should be used as a covariate in an ANCOVA. When examining correlations between urinary 8-OHdG and other variables, semipartial correlations should be used with the effects of creatinine partialled out.

In our study sample, the regression equation we computed was: predicted 8-OHdG (in ng/mL) = 2.735 + 0.107 x creatinine (in mg/dL). For example, when the creatinine flow equals 50 mg/dL, the predicted 8-OHdG is 8.07 ng/mL. When urinary creatinine equals 200 mg/dL, the predicted 8-OhdG is 24.09 ng/mL. The residual 8-OHdG values are then the difference between the observed 8-OhdG values and the predicted values.

A factorial ANCOVA with age and creatinine as covariates and diagnostic groups (normal controls; ME/CFS; depression; and depression + ME/CFS) and gender as groups showed: a significant difference between the diagnostic groups (F=2.7, df=3/99, p=0.04); no significant differences between men and women (F=0.1, df=1/99, p=0.7); no significant interaction between the diagnostic groups X gender (F=0.2, df=3/99, p=0.9); no significant effect of age (F=1.7, df=1/99, p=0.2) and a highly significant effect of creatinine (F=91.8, df=1/99, $p<10^{-5}$). LSD showed significantly higher ($p<0.05$) residualized 8-OHdG values in patients with simultaneous major depression + ME/CFS (median value=0.28 ng/mL with q25 and q75 values of 0.00 and 0.46 ng/mL, respectively) than in normal controls (median=0.08 ng/mL, -0.43 and 0.26 ng/mL, respectively), patients with major depression (median=-0.03 ng/mL, -0.23 and 0.20 ng/mL respectively) and patients with ME/CFS (median=-0.15 ng/mL, -0.35 and 0.12 ng/mL, respectively). Dunn's test showed significantly higher (t=3.13, p=0.003) residualized 8-OHdG values in patients with simultaneous major depression + ME/CFS than in all other subjects combined. A factorial ANCOVA performed on the creatinine data with age as covariate and gender as second groups showed: no significant differences in creatinine between the diagnostic groups (F=1.5, df=3/100, p=0.2); significantly higher creatinine urinary excretion in men than in women (F=4.6, df=1/100, p=0.03); no significant interaction pattern between diagnosis X gender (F=0.8, df=3/100, p=0.5); and no significant effect of age (F=0.00, df=1/100, p=0.97). There were minor differences in age between the study groups (F=3.5, df=3/105, p=0.02), i.e. normal controls (mean age ±SD=45.1 ±9.2 years); ME/CFS (mean=36.6 ±14.2 years); major depression (mean=41.4 ±9.8 years) and depression + ME/CFS (mean=46.7 ±10.6 years). There were significant differences (χ^2=18.1, df=3, p=0.0004) in the male/female ratio between normal controls (male/female ratio=5/12); ME/CFS (male/female ratio=2/42); major depression (male/female ratio=13/12) and simultaneous depression + ME/CFS (male/female ratio=9/14). In any case, possible effects of age and gender were ruled out using the appropriate statistical methods.

In the combined patient group, we found significant semipartial correlations (after partialling out the effects of creatinine on the 8-OHdG values) between the residualized 8-OHdG values and the total score on the FF scale (r=0.27, p=0.008). Stepwise multiple regression analysis with a p-to-enter=0.05 showed that 6.5% of the variance in the residualized 8-OHdG values was predicted (F=4.2, df=2/91, p=0.01) by two FF symptoms, i.e. sadness (F=4.6, p=0.03) and a subjective experience of infection (F=4.6, p=0.03). In depressed patients, we found that the residualized 8-OHdG values were best predicted by a subjective experience of infection (F=4.2, p=0.04). In patients with ME/CFS, we found that the residualized 8-OHdG values were best predicted by muscle tension (F=5.6, p=0.02).

DISCUSSION

The first major finding of this study is that up to 49.0% of the variance in the urinary excretion of 8-OHdG could be explained by the regression on the excretion of creatinine. Until now, it is common practice to express the urinary 8-OHdG excretion as the 8-OHdG / urinary creatinine ratio. However, in the present study we found that the shared variances between the residualized 8-OHdG values and the 8-OHdG / urinary creatinine ratio were 27.5% and 49.0% in controls and all subjects combined, respectively. These correlations are insufficient for the 8-OHdG / urinary creatinine ratio to be regarded as a valid index for 8-OHdG urinary excretion. Thus, the correction for the possible effects of creatinine should be carried out by partialling out the effects of urinary creatinine on the urinary 8-OHdG excretion (by means of regression analysis). Thus, when examining differences in the urinary 8-OHdG excretion among groups, ANCOVA should be employed with urinary creatinine as a covariate. When examining correlations between the urinary 8-OHdG excretion and other variables, semipartial correlation coefficients should be calculated controlling for the effects of the urinary creatinine excretion.

The second major finding of this study is that depressed patients with ME/CFS have a significantly higher 8-OHdG excretion as compared to normal volunteers and all other patients. Neither depressed patients without ME/CFS, nor ME/CFS patients showed significant increases in 8-OHdG excretion. As explained in the Introduction, 8-OHdG in urine is a biomarker of oxidative DNA damage and more generally of cellular oxidative stress (Wu *et al.* 2004; Valavanidis *et al.* 2009). Thus, it appears that oxidative DNA damage is a hallmark for simultaneous major depression and ME/CFS and not for any of these disorders alone. These results are difficult to compare with those of previous studies in depression (Forlenza and Miller, 2006; Irie

et al. 2005) because they did not consider the effects of the presence of F&S symptoms and because they meausured 8-OHdG levels in serum or in peripheral leukocytes. To the best of our knowledge, no studies examined urinary 8-OHdG in ME/CFS, although there is one study reporting increased oxidative damage to DNA in the vastus lateralis muscle of ME/CFS patients (Fulle *et al.* 2000).

Since increased urinary 8-OHdG is also a general indicator for increased oxidative stress, the results of the present study confirm previous reports that some patients with depression and ME/CFS suffer from induction of the O&NS pathways. As such, our results corroborate the findings of previous studies in depression (Edwards *et al.* 1998; Maes *et al.* 1999; 2007c; 2008b; 2009d; Forlenza and Miller, 2006; Sarandol *et al.* 2007) and ME/CFS (Vecchiet *et al.* 2003; Kennedy *et al.* 2005; Jammes *et al.* 2005; Smirnova and Pall, 2003; Maes *et al.* 2006b; 2007b; 2007c; 2008b). Also, translational research shows increased O&NS in depression and ME/CFS. For example, in post-morten brain of bipolar patients and patients with recurrent depressive episodes, increased 4-hydroxynonenal (4-HNE), a major product of lipid peroxidation (Wang et al. 2009) or increased xanthine oxidase activity (Michel *et al.* 2008) have been found. In the rodent, chronic mild stress (CMS)-induced depression, is characterized by increased lipid peroxidation in the striatum and cerebellum (Lucca *et al.* 2009a; 2009b). In a mice model of ME/CFS, i.e. mice subjected to forced swimming for one 6-minute session on each day for 7-15 days, increased brain lipid peroxidation and increased nitrite and myeloperoxidase levels were detected (Singh *et al.* 2002; Singal *et al.* 2005; Dhir and Kulkarni, 2008).

Another major finding of this study is that increased urinary 8-OHdG is significantly related to F&S symptoms, such as a subjective feeling of infection and muscle tension, and to sadness. Thus, it appears that increased 8-OHdG - and thus oxidative damage to DNA - is related to F&S and depression symptoms as well. This shows that disorders in the O&NS pathways may be more related to specific symptom dimensions than to diagnostic classifications. As described in the Introduction, F&S symptoms are a key component of major and melancholic depression (Maes, 2009b), while depression and sadness often occur in ME/CFS (Skapinakis *et al.* 2003; 2004). The hypothesis of Roy-Byrne et al. (2002) that CF is a "form fruste" of depression because many of those patients have "comorbid" affective disorders in not in accordance with established symptomatic and other epidemiological differences between ME/CFS and depression (Griffith and Zarrouf, 2008; Hawk *et al.* 2006). As explained previously, it is our view that activation of the IO&NS pathways are the pathophysiological underpinnings of the above depression and F&S symptom profiles (Maes, 2009b). The label "comorbidity" between depression and ME/CFS is therefore misleading. In fact, the label "comorbidity" indicates that a medical condition exists independently from another medical condition or indicates that a specific medical condition in a patient causes or is caused by another medical condition (Valderas *et al.* 2009; First, 2005). However, neither of these definitions can be applied here as both the depression and F&S dimension may be the consequence of activated IO&NS pathways and thus reflect different symptomatic expressions of similar pathophysiological underpinnings. Therefore, we consider major depression and ME/CFS not to be mutually exclusive nor co-occurring diagnostic categories but rather two symptomatic dimensions, i.e. the depression dimension (sadness, anorexia, weight loss, anhedonia, psychomotor retardation, feelings of guilt, etc) and the F&S dimension (pain, muscle tension, a subjective feeling of infection, etc), which both are induced by activated IO&NS pathways.

There is now also evidence for a strong "comorbidity" between depression and ME/CFS, on the one hand, and cardiovascular disorders, on the other. In fact, both depression and ME/CFS have been shown to increase the mortality due to cardiovascular disorders (Jason *et al.* 2006; Somberg and Arora, 2008; Dickens *et al.* 2008). These findings suggest that ME/CFS and major depression are risk factors to cardio-vascular disorders and due mortality and should be added to the Charlson "comorbidity" index (Charlson *et al.* 1987). We have discussed that the increased risk to cardiovascular disorder in ME/CFS may be explained by various IO&NS disorders in that illness, i.e. inflammation, increased oxidation and peroxidation, gut-derived inflammation, a lowered omega-3 PUFA, zinc and co-enzyme Q10 status, and viral and bacterial infections (Maes, 2009a). We have also discussed that the increased risk to atherosclerosis and heart disorders in major depression may be explained by activated IO&NS pathways, such as lipid peroxidation, inflammation, a lowered omega-3 PUFA status, and lowered levels of antioxidants, e.g. co-Q10, zinc and glutathion peroxidase (Maes *et al.* 2009b; 2009c; 2009d). The results of the present study further extent the number of possible IO&NS-related factors that create an environment which promotes cardiovascular disorders. Indeed, increased urinary 8-OHdG is considered a risk factor for artherosclerosis. One of the arguments is that increased amounts of 8-OHdG and oxidatively modified DNA may be detected in atherosclerotic plaques (Wu *et al.* 2004). The levels of 8-OHdG in DNA isolated from lymphocytes are significantly higher in atherosclerotic patients than in normal controls (Gackowski *et al.* 2001).

There is also evidence that central neurodegeneration is another characteristics of major depression (Goshen *et al.* 2008; Campbell and MacQueen, 2006; Stockmeier *et al.* 2004; Duman, 2002). We have argued that activated IO&NS pathways may cause neurodegeneration through neuroinflammation, the neurotoxic effects of by-products of induced IO&NS pathways, such as kynurenine and malondialdehyde,

and damage by O&NS (Maes *et al.* 2009d; 2009e). This hypothesis was labelled the inflammatory and neurodegenerative (I&ND) hypothesis of depression (Maes *et al.* 2009e). The results of the present study that 8-OHdG is increased in a subgroup of depressed patients further underscores that the consequences of activated IO&NS pathways, such as O&NS-induced DNA damage, may underpin the neurodegeneration in major depression. There are now many reports showing that O&NS-induced DNA damage plays an important role in neurodegeneration. For example, in subacute sclerosing panencephalitis, it was shown that not only lipid peroxidation and glutamate transport disturbances, but also oxidative stress to DNA contribute to neuronal damage (Hayashi *et al.* 2002). The progressive neurodegeneration of ataxia telangiectasia is characterized by oxidative damage to DNA as measured by 8-OHdG (Reichenbach *et al.* 2002). In the R6/2 transgenic mouse model of Huntington's disease, increased 8-OHdG was found in striatal microdialysates, while 8-OHdG staining was even higher in the late stages of that illness (Bogdanov *et al.* 2001). Accumulations of deleted mitochondrial DNA are considered to be the primary factors in (neuro)degenerative disorders causing defects in the mitochondrial respiratory chain and thus increased ROS leakage, which in turn cause secondary mitochondrial DNA mutations in postmitotic cells (Tanaka *et al.* 1996). Alzheimer's disease (AD) is characterized by an accumulation of ROS-induced mitochondrial DNA mutations causing mitochondrial dysfunctions and genomic DNA damage (de la Monte *et al.* 2000). The presence of 8-OHdG immunoreactivity in cortical neurons of AD but not control postmortem brains, and increased 8-OHdG levels in the cerebrovascular fluid of patients with AD suggests that DNA damage influences the course of AD (de la Monte *et al.* 2000; Markesbery and Carney, 1999). Taken together, these results suggest that oxidative DNA damage is involved in the pathogenesis of neuronal degeneration and, thus, could play a role in the I&ND hypothesis of depression.

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