INTRODUCTION

Growing body of evidence has indicated that oxidative damage is involved in the pathogenesis of neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease (PD) [1, 2]. Although RNA in a cell should be subject to the same oxidative insults as DNA and other cellular macromolecules, oxidative damage to RNA has not been the major focus in investigating the magnitude and the biological consequence. Because RNA is mostly single-stranded and its bases are not protected by hydrogen bonding and probably less protected by specific proteins, RNA may be more susceptible to oxidative insults than DNA [3]. Indeed, greater oxidation in cellular RNA than that in DNA was demonstrated in several experimental studies on nonneuronal cell lines and tissues, where oxidized nucleosides, 8-hydroxydeoxyguanosine (8OHdG) and 8-hydroxyguanosine (8OHG), were measured as markers for oxidative damage to DNA and RNA, respectively [4–6]. It is now becoming evident that RNA molecules are not only intermediates in the transfer of genetic information from DNA to proteins but also key players in many mechanisms controlling expression of genetic information [7]. Therefore, RNA damage is detrimental to cells and may be involved in the pathogenesis of neurodegenerative diseases [3]. Here we review recent studies demonstrating RNA oxidation in vulnerable neuronal population in several neurological diseases and discuss the biological significance of the damage to RNA.

RNA OXIDATION IN VARIOUS NEUROLOGICAL DISEASES AND EXPERIMENTAL CONDITIONS

Among multiple adducts of nucleoside oxidation, 8OHdG and 8OHG are two of the best characterized and studied forms of DNA and RNA oxidation, respectively [4, 5]. The availability of highly specific antibodies with 8OHdG and 8OHG has enabled us to perform in situ approaches to nucleoside oxidation in postmortem brain samples taken from patients with neurological diseases [29, 30]. In 1999, prominent 8OHdG/8OHG immunoreactions were demonstrated in the vulnerable neuronal populations in postmortem brains of patients with AD and PD [8, 9]. In both AD and PD, the neuronal 8OHdG/8OHG immunoreactions showed cytoplasmic predominance, which indicated
that mitochondrial DNA and cytoplasmic RNA in neurons were major targets of oxidative damage. Because the neuronal 8 OHdG/8 OHG immunoreactions in AD brain were diminished greatly by RNase pretreatment but not by DNase pretreatment, the oxidized nucleoside was predominantly associated with RNA rather than DNA [8]. This notion was further supported by the immunoelectron microscopic observation that most of the oxidized nucleoside was localized to the ribosomal structures [11].

Similar RNA oxidation in neuronal cytoplasm was observed in brain samples of patients with Down syndrome [10], dementia with Lewy bodies [12, 13], Creutzfeldt-Jakob disease [14], and subacute sclerosing panencephalitis [15], as summarized in Table 1. The oxidative damage to RNA was demonstrated not only in sporadic form of the diseases but also in familial form of AD [21] and prion disease, that is, familial Creutzfeldt-Jakob disease and Gerstmann-Strausler-Scheinker disease [14, 24]. Furthermore, nuclear DNA oxidation and cytoplasmic RNA oxidation were observed in brains of patients with a genetic defect of nucleotide excision repair mechanism, xeroderma pigmentosum, showing cutaneous hypersensitivity to sunlight and progressive neurological disturbances [23].

These immunocytochemical studies demonstrating neuronal RNA oxidation in the neurological diseases were followed by biochemical detection of the oxidized nucleoside in AD brain with immunoblot approaches [18, 19, 22, 25]. Shan et al [18, 19] used Northwestern blotting with a monoclonal anti-8 OHG antibody, to isolate and identify oxidized RNA species and showed that significant amount of poly (A)+ mRNA species were oxidized in AD brain. The oxidation to mRNA was further confirmed by cDNA synthesis and Southern blotting of the immunoprecipitated mRNA species. Densitometric analysis of the Southern blot results revealed that 30–70% of the mRNAs from AD frontal cortices were oxidized, while only 2% of the mRNAs were oxidized in age-matched normal controls [19]. Interestingly, reverse transcription-PCR and filter array analyses of the identified oxidized mRNAs revealed that some species were more susceptible to oxidative damage in AD, while no common motifs or structures were found in the oxidatively susceptible mRNA species. Some of the identified known oxidized transcripts were related to AD, which included p21ras, mitogen-activated protein kinase (MAPK) kinase 1, carbonyl reductase, Cu/Zn superoxide dismutase, apolipoprotein D, calpains, but not amyloid β protein precursor or tau [18]. Although these studies by Shan et al [18, 19] focused on mRNA species that account for only a few percent of total cellular RNA, Honda et al [22] and Ding et al [25] reported that rRNA, extremely abundant in neurons, contained 8 OHG in AD brain. rRNA showed higher binding capacity to redox-active iron than tRNA, and consequently oxidation of rRNA
by the Fenton reaction formed 13 times more 8 OHG than tRNA [22].

Of note, both immunocytochemical studies [8, 9, 12, 13] and biochemical studies [18, 25] revealed that the regional distribution of the RNA oxidation in the brain was consistent with the selective neuronal vulnerability in each neurological disease. There were increased levels of 8 OHG in the hippocampus and cerebral neocortex in AD as well as in the substantia nigra in PD, while no alteration in the 8 OHG level was found in the cerebellum in both AD and PD compared with controls [8, 9, 18, 25].

Significantly increased levels of the oxidized RNA nucleoside, 8 OHG, have been identified not only in brain tissue but also in cerebrospinal fluid collected from patients with AD and PD [16, 17, 20] as well as in serum of PD patients [17], which indicates that 8 OHG is a possible biomarker of the diseases. As we describe in the next section, 8 OHG is a potent candidate of an early-stage marker of the diseases or a marker predicting conversion from the prodromal stage into an early stage of the diseases.

Experimental studies with rodent have shown that neuronal RNA oxidation and spatial memory deficit are observed in old animals [26] as well as animals with intermittent hypoxia [27]. In both aging and hypoxia models, antioxidants or mitochondrial metabolites can reduce the oxidative damage and the spatial memory deficit. In another experimental model using mixed astrocyte and neuron cultures [28], DNA oxidation and RNA oxidation have been observed following proteasome inhibition that is associated with several neurodegenerative features such as protein aggregation, activated apoptotic pathways, and induction of mitochondrial disturbances. Interestingly, in this proteasome inhibition model, neuron underwent larger increases in nucleic acid oxidation compared to astrocyte cultures, and RNA appeared to undergo a greater degree of oxidation than DNA, which was exactly identical in AD brain [8].

**RNA Oxidation: An Early Stage Event in the Process of Neurodegeneration**

Because RNA oxidation is involved with a wide variety of neurological diseases (Table 1), it may be considered an event in common neurodegenerative pathway that occurs in a late stage of the diseases. However, that is not the case in AD and PD. There is a considerable amount of evidence supporting an early involvement of RNA oxidation in the pathological cascade of neurodegeneration, especially in AD (Table 2). Namely, RNA oxidation has been observed in postmortem brains of cases with early-stage AD [11], a presymptomatic case with familial AD mutation [21], Down syndrome cases with early-stage AD pathology [10], and subjects with mild cognitive impairment (MCI) who possibly represent prodromal stage of AD [25]. Furthermore, the increased level of RNA oxidation in cerebrospinal fluid is more prominent in cases with shorter duration of AD and PD [16, 20]. Recent studies of MCI subjects have demonstrated also increased oxidation to protein and lipid in postmortem brain [31], increased lipid peroxidation in cerebrospinal fluid, plasma, and urine [32], increased DNA oxidation in peripheral leukocytes [33] as well as decreased plasma antioxidant vitamins and enzymes [34], and decreased plasma total antioxidant capacity [35]. From clinical points of view, the notion of an early involvement of oxidative damage in the pathogenesis of these degenerative diseases should have a great importance to establish a diagnostic tool and a therapeutic target, as we have reviewed recently [36, 37].

Of note, an early stage involvement of neuronal RNA oxidation is identified not only in age-associated neurodegenerative diseases, but also in cases with subacute sclerosing panencephalitis that is caused by persistent measles virus infection in the central nervous system and is pathologically accompanied with brain atrophy and neurofibrillary tangles [15].

**Sources of Reactive Oxygen Species (ROS) Responsible for RNA Oxidation**

The brain is especially vulnerable to oxidative damage because of its high content of easily peroxidizable unsaturated fatty acids, high oxygen consumption rate (accounting for 20–25% of the total body oxygen consumption, but for less than 2% of the total body weight), and relative paucity of antioxidant enzymes compared with other organs (e.g., the content of catalase in brain is only 10–20% of liver and heart) [37]. Therefore, neurons are continuously exposed to ROS such as superoxide, H$_2$O$_2$, and hydroxyl radical that are produced from the mitochondrial electron transport chain through normal cellular metabolism. Hydroxyl radical can diffuse through tissue only in the order of several nanometers and superoxide is hardly permeable through cell membrane. In consideration of widespread damage to cytoplasmic RNA in the neurodegenerative diseases, RNA species are likely attacked by hydroxyl radical, which is formed from the reaction of highly diffusible H$_2$O$_2$ with redox-active metals through Fenton reaction [8, 22]. In AD brain, disrupted mitochondria likely play a central role in producing abundant ROS as well as supplying redox-active iron into the cytosol [38, 39]. Indeed, ribosomes purified from AD hippocampus contain significantly higher levels of redox-active iron compared to controls, and the iron is bound to rRNA [22]. Therefore, mitochondrial abnormality and metal dysregulation are key features closely associated with ROS formation responsible for the RNA oxidation in AD. Interestingly, both of the features are found in the substantia nigra of PD also [36].

**RNA Oxidation and the Biological Consequence**

Although more than 20 different types of oxidatively altered purine and pyrimidine bases have been detected in nucleic acids [40], guanine is the most reactive of the nucleic acid base [41]. Therefore, the oxidized base, 8 OHG, is the most abundant among the oxidized bases [3]. The 8 OHG can be formed in RNA by direct oxidation of the base and also by the incorporation of the oxidized base from the cytosolic pool
into RNA through the normal action of RNA polymerase

...DNA damage can be repaired not only by the mechanism of direct reversal of the modified bases but also by base excision repair mechanism. Specific DNA glycosylases excise the damaged base and DNA polymerases replace the nucleotide with its unmodified form. However, for RNA, this mechanism is less efficient due to the single-stranded nature of RNA.

**COPING WITH RNA DAMAGE**

Until recently, it has been considered that damaged RNA may be only degraded rather than repaired. However, Aas et al [43] has suggested that the cells have at least one specific mechanism to repair RNA damage, indicating that cells may have a greater investment in the protection of RNA than previously suspected [3, 42]. Indeed, alkylating damage in RNA is repaired by the same mechanism as a DNA-repair, catalyzed by the enzyme Alk B and in humans by the related protein [43]. Alk B and its homologue hABH3 cause hydroxylation of the methyl group on damaged DNA and RNA bases, and thus directly reverse alkylating damage. Alk B and hABH3, but not hABH2, repair RNA, since Alk B and hABH3 prefer single-stranded nucleic acids while hABH2 acts more efficiently on double-stranded DNA.

DNA damage can be repaired not only by the mechanism of direct reversal of the modified bases but also by base excision repair mechanism. Specific DNA glycosylases excise the damaged base and DNA polymerases replace the nucleotide [42, 44]. Furthermore, cells have mechanism of dealing with nucleotide damage other than direct repair, which seems to be useful for defense against oxidative damage to both DNA and RNA. Because oxidation of nucleotides can occur in the cellular nucleotide pool and the oxidized nucleotide can be incorporated into DNA and RNA, the mechanism avoiding such incorporation of the oxidized nucleotide is involved in coping with nucleic acid damage [40, 42, 44]. MutT protein
in *E. coli* and its mammalian homologues MTH1 and NUDT5 participate in this error-avoiding mechanism by hydrolyzing the oxidized nucleoside diphosphates and/or triphosphates to the monophosphates [3, 40, 44]. Indeed, the increase in the production of erroneous proteins by oxidative damage is 28-fold over the wild-type cells in *E. coli mutT* deficient cells, which is reduced to 1.2- or 1.4-fold by the expression of MTH1 or NUDT5, respectively [40].

Then, one important question is whether cells have machineries against oxidatively damaged nucleotides that are contained in RNA. Recently, proteins that bind specifically to 8 OHG-containing RNA have been reported, namely, *E. coli* polynucleotide phosphorylase protein (Pnp) and human Y box-binding protein 1 (YB-1) [45, 46]. The binding of the specific protein likely makes the 8 OHG-containing RNA resistant to nuclease degradation [45]. However, it has been proposed that these proteins may recognize and discriminate the oxidized RNA molecule from normal ones, thus contributing to the fidelity of translation in cells by sequestering the damaged RNA from the translational machinery [45, 46].

It is possible that the RNA quality control mechanisms are defective or inefficient in cancer cells as well as cells of neurodegenerative diseases. Further elucidation of the mechanisms of repair or avoidance of RNA damage and their potential role in preventing human diseases might provide new approaches to a number of unresolved issues of life science, while it has not been the major focus in investigation for a long period [3].

**CONCLUSION**

An early involvement of RNA oxidation of vulnerable neuronal population in neurodegenerative diseases such as AD and PD has been demonstrated in immunocytochemical and biochemical studies. Indeed, oxidized RNA is associated with a disturbance in protein synthesis in vitro and in vivo. Although there are only a small number of studies suggesting the existence of coping mechanisms for RNA damage at present, the known mechanisms may be the tip of iceberg of the RNA damage. Understanding of the consequences and cellular handling mechanisms of the oxidative RNA damage may provide clues to both basic research and the treatment of the neurodegenerative diseases.

**REFERENCES**


