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Review Article

Oxidative Damage to RNA in Neurodegenerative Diseases

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Since 1999, oxidative damage to RNA molecules has been described in several neurological diseases including Alzheimer's disease, Parkinson's disease, Down syndrome, dementia with Lewy bodies, prion disease, subacute sclerosing panencephalitis, and xeroderma pigmentosum. An early involvement of RNA oxidation of vulnerable neuronal population in the neurodegenerative diseases has been demonstrated, which is strongly supported by a recent observation of increased RNA oxidation in brains of subjects with mild cognitive impairment. Until recently, little is known about consequences and cellular handling of the RNA damage. However, increasing body of evidence suggests detrimental effects of the RNA damage in protein synthesis and the existence of several coping mechanisms including direct repair and avoiding the incorporation of the damaged ribonucleotides into translational machinery. Further investigations toward understanding of the consequences and cellular handling mechanisms of the oxidative RNA damage may provide significant insights into the pathogenesis and therapeutic strategies of the neurodegenerative diseases.

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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 (8 OHdG) and 8-hydroxyguanosine (8 OHG), 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, 8 OHdG and 8 OHG are two of the best characterized and studied forms of DNA and RNA oxidation, respectively [4, 5]. The availability of highly specific antibodies with 8 OHdG and 8 OHG 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 8 OHdG/8 OHG 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 8 OHdG/8 OHG immunoreactions showed cytoplasmic predominance, which indicated

TABLE 1: Summary of studies on RNA oxidation in the central nervous system. (ELISA: enzyme-linked immunosorbent assay; HPLC: high-performance liquid chromatography; IB: immunoblot; ICC: immunocytochemistry; IEM: immunoelectronmicroscopy; RT-PCR: reverse transcription and polymerase chain reaction.)

Year	Human neurological diseases	Materials/Procedures	Authors
1999	Alzheimer's disease	Brain (hippocampus/cerebral cortex)/ICC	Nunomura et al [8]
	Parkinson's disease	Brain (substantia nigra)/ICC	Zhang et al [9]
2000	Down syndrome	Brain (cerebral cortex)/ICC	Nunomura et al [10]
2001	Alzheimer's disease	Brain (hippocampus/cerebral cortex)/IEM	Nunomura et al [11]
	Dementia with Lewy bodies	Brain (hippocampus/cerebral cortex)/ICC	Nunomura et al [12, 13]
	Familial/sporadic Creutzfeldt-Jakob disease	Brain (cerebral cortex)/ICC	Guentchev et al [14]
	Subacute sclerosing panencephalitis	Brain (cerebral cortex)/ICC	Hayashi et al [15]
	Alzheimer's disease	Cerebrospinal fluid/HPLC	Abe et al [16]
2002	Parkinson's disease/multiple-system atrophy	Cerebrospinal fluid/ELISA	Kikuchi et al [17]
	Alzheimer's disease	Brain (hippocampus/cerebral cortex)/IB and RT-PCR (mRNA)	Shan et al [18, 19]
	Parkinson's disease	Cerebrospinal fluid/HPLC	Abe et al [20]
2004	Familial Alzheimer's disease	Brain (cerebral cortex)/ICC	Nunomura et al [21]
2005	Alzheimer's disease	Brain (hippocampus)/IB and RT-PCR (rRNA)	Honda et al [22]
	Xeroderma pigmentosum (group A)	Brain (globus pallidus)/ICC	Hayashi et al [23]
	Gerstmann-Straussler-Scheinker disease	Brain (hippocampus/cerebral cortex)/ICC	Petersen et al [24]
	Alzheimer's disease/mild cognitive impairment	Brain (cerebral cortex)/IB (rRNA)	Ding et al [25]
Year	Experimental conditions	Materials/Procedures	Authors
2002	Old rat	Brain (hippocampus)/ICC	Liu et al [26]
2003	Adult rat with intermittent hypoxia	Brain (hippocampus)/ICC	Row et al [27]
2004	Culture neuron under proteasome inhibition	Mixed astrocyte and neuron cultures/ICC and IB	Ding et al [28]

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-Straussler-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_2O_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_2O_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

TABLE 2: Summary of evidence suggesting temporal primacy of RNA oxidation in the process of neurodegeneration.

Materials/subjects	Findings
Postmortem brains of patients with Alzheimer's disease	RNA oxidation is more prominent in cases with lesser amounts of A β plaque deposition or shorter disease duration [11].
	RNA oxidation is more prominent in hippocampal neurons free of neurofibrillary tangles compared to neurons with neurofibrillary tangles [11].
	RNA oxidation is increased in a presymptomatic case with presenilin-1 gene mutation [21].
Postmortem brains of subjects with mild cognitive impairment	RNA oxidation is increased in brains of subjects with mild cognitive impairment, who, at least in part, represent a prodromal stage of dementia [25].
Postmortem brains of patients with Down syndrome	RNA oxidation precedes A β plaque deposition in a series of Down syndrome brains, a model of Alzheimer's-type neuropathology [10].
Postmortem brains of patients with subacute sclerosing panencephalitis	RNA oxidation is observed in cases with shorter disease duration, while lipid peroxidation is observed in cases with longer disease duration [15].
Cerebrospinal fluid of patients with Alzheimer's disease	RNA oxidation is more prominent in cases with shorter disease duration or higher scores in mini-mental state examination [16].
Cerebrospinal fluid of patients with Parkinson's disease	RNA oxidation is more prominent in cases with shorter disease duration [20].

into RNA through the normal action of RNA polymerase [40, 41]. Not only 8 OHG, but also 8-hydroxyadenosine, 5-hydroxycytidine, and 5-hydroxyuridine have been identified in oxidized RNA [41], which may have altered pairing capacity and thus may be at the origin of erroneous protein production. Indeed, the 8 OHG can pair with both adenine and cytosine, and thus the oxidized RNA compromises the accuracy of translation [40].

The biological consequence of oxidatively damaged mRNA species has been investigated in vitro by expressing them in cell lines. Oxidized mRNAs lead to loss of normal protein level and protein function, and potentially produce defective proteins leading to protein aggregation, a common feature of neurodegenerative diseases [18]. Also the biological consequence of ribosomal oxidation has been investigated in vitro by translation assay with oxidized ribosomes from rabbit reticulocyte, which shows a significant reduction of protein synthesis [22]. Recently, a study on brains of subjects with AD and MCI has demonstrated ribosomal dysfunction associated with oxidative RNA damage [25]. Isolated polyribosome complexes from AD and MCI brains show decreased rate and capability for protein synthesis without alteration in the polyribosome content. Decreased rRNA and tRNA levels and increased 8 OHG in total RNA pool, especially in rRNA, are accompanied with the ribosomal dysfunction, while there is no alteration in the level of initiation factors.

These findings have indicated that RNA oxidation has detrimental effects on cellular function whether the damaged RNA species are coding for proteins (mRNA) or performing translation (rRNA and tRNA). It is noteworthy that studies on some anticancer agents have shown that RNA damage can lead to cell-cycle arrest and cell death, as much as DNA damage does [42]. RNA damage may cause cell death via pathway

involving either p53-dependent mechanism associated with inhibition of protein synthesis or p53-independent mechanism different from inhibition of protein synthesis.

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, alkylation damage in RNA is repaired by the same mechanism as a DNA-repair, catalyzed in the bacterium *Escherichia coli* 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 alkylation 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 cellular investment in counteracting 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.

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