Free Radical Research, June 2007; 41(6): 673–680

informa
healthcare

Oxidative inactivation of the proteasome in Alzheimer's disease

VALENTINA CECARINI[1,2], QUNXING DING[3], & JEFFREY N. KELLER[1,3]

[1]*Sanders Brown Center on Aging, University of Kentucky, 205 Sanders-Brown Center on Aging, Lexington, KY 40536-0230, USA,* [2]*Molecular and Cellular Biology, University of Camerino, Camerino, Italy, and* [3]*Anatomy and Neurobiology, University of Kentucky, 205 Sanders-Brown Center on Aging, Lexington, KY 40536-0230, USA*

Accepted by Dr T. Grune

(Received 18 November 2006; in revised form 22 January 2007)

Abstract

In the present study we isolated proteasome complexes from control, mild cognitive impairment (MCI), and Alzheimer's disease (AD) subjects. No significant difference in the amount of proteasomes was detected across the different groups, although impairments in chymotrypsin-like proteasome activity was observed in AD subjects. Large impairments in proteasome- mediated degradation of an oxidized protein substrate was observed in MCI and AD subjects. Incubation with a reducing agent (DTT) had no significant effect on proteasome chymotrypsin-like activity, but fully restored proteasome-mediated protein degradation in MCI and AD subjects. Proteasomes from AD subjects exhibited elevations in protein carbonyls, 4-hydroxynonenal-conjugation, and neuroprostane-conjugation. Together, these data confirm that impairments in the function of purified proteasomes occurs in the earliest stages of AD, and directly support a role for oxidative inactivation contributing to declines in proteasome function in AD.

Keywords: *Aging, brain, 4-hydroxynonenal, neurodegeneration, neuroprostane, oxidative stress*

Introduction

The proteasome is a multicatalytic protease with a molecular weight of ~700 kDa located in the cytosol and nucleus of all eukaryotic cells, representing approximately 1–2 percent of the total cellular proteins [1–3]. The proteasome is responsible for the majority of intracellular protein degradation and plays a pivotal role in several cellular activities including cell cycle regulation, antigen presentation and apoptosis [4,5]. The 20S core proteasome possesses three main catalytic activities: the chymotrypsin-like (ChT-L) activity, the trypsin-like (T-L) activity and the peptidylglutamyl-peptide hydrolase activity (PGPH) that cleave the substrates after hydrophobic residues, basic residues and acidic residues [3], respectively. Cap-like proteins can bind the 20S proteasome and form a new structure, the 26S proteasome, which is responsible for the ATP-dependent degradation of ubiquitinated substrates [2].

Several studies have demonstrated evidence for proteasome impairment in a variety of neurodegenerative conditions including Alzheimer's disease (AD), Parkinson's disease (PD), and ischemia-reperfusion injury [7–9]. Because the proteasome plays an important role in the degradation of oxidized and damaged proteins [10,11], a decrease in proteasome activity could promote an accumulation of potentially toxic proteins and contribute to neuronal degeneration and neuronal death. Despite the progress made in our understanding of proteasome inhibition in the aging and disease, studies have not confirmed if alterations in proteasome homeostasis can be detected in purified proteasome

Correspondence: J. N. Keller, University of Kentucky, 205 Sanders-Brown Center on Aging, 800 South Limestone, Lexington, KY 40536-0230, USA. Tel: 1 859 257 1412. Ext. 333. Fax: 1 859 323 4601. E-mail: jnkell0@uky.edu

ISSN 1071-5762 print/ISSN 1029-2470 online © 2007 Informa UK Ltd.
DOI: 10.1080/10715760701286159

complexes in each of these different conditions. Experimental clarification that alterations in proteasome functionality occurs in purified proteasome complexes is currently a pressing need for the field. Similarly, oxidative modification of the proteasome is believed to play a role in mediating declines of proteasome function in aging and disease [6–11], although it remains unclear whether oxidative modifications to the proteasome complex occurs in AD. Similarly, studies have not identified the specific contribution of oxidative modifications to the proteasome as mediators of proteasome inhibition in age-related diseases of the brain.

The focus of this study was to determine the presence of potential alterations in 20S proteasome complexes purified from subjects with mild cognitive impairment (MCI), the earliest detectible stage of AD, and in subjects with AD. Our data demonstrate that impairments in ChT-L activity, and a robust declines in proteasome-mediated protein degradation, are evident in purified 20S proteasome complexes from subjects with MCI and AD. Additionally, data in this study provide experimental evidence for oxidative damage to the proteasome complex contributing to the impairment of proteasome-mediated protein degradation in AD.

Materials and methods

Human subjects

The diagnosis of MCI, AD, and normal cognitive function was defined by clinical-neuropathological consensus conference [12]. A total of 15 control [9(M)/6(F)], nine MCI (3 M/6 F), and 16 end-stage AD (5 M/11 F) subjects were used for all studies (Table I). The mean ± SEM age was 80 ± 1.30 years for control, 86 ± 4.35 years for MCI and 84 ± 1.83 years for AD subjects. The post mortem interval (PMI) was 2.78 ± 0.23 h for control, 3.08 ± 0.32 h for MCI and 3.20 ± 0.27 h for AD subjects. All patients, including those in the Biologically Resilient Adults in Neurological Studies program, were studied longitudinally as part of the University of Kentucky Alzheimer's Disease Research Center. The clinical criteria used for the diagnosis of amnestic MCI were as described previously [13,14].

Table I. Subject demographic data.

	Age (years)	Sex	PMI (h)
Cont	80 ± 1.30	9 M/6 F	2.78 ± 0.23
MCI	86 ± 4.35	3 M/6 F	3.08 ± 0.32
AD	84 ± 1.83	5 M/11 F	3.20 ± 0.27

Data are presented as the mean and SEM; PMI, post mortem interval.

Proteasome isolation

Proteasome complexes were isolated from the superior and middle temporal gyri (SMTG) and from the cerebellum following the procedure described by Friguet et al. [15] with minor modifications. Briefly, frozen brain specimens were homogenized in Phosphate-buffered saline (pH 7.4), using a Sonic Dismembrator (Fisher Scientific). The resulting homogenate was centrifuged at 10,000*g* for 10 min at 4°C. The supernatant was subjected to a fractionation from 10 to 30% in ammonium sulphate. The 10 and 30% pellets were then combined and resuspended in phosphate-buffered saline (pH 7.4). An ion-exchange chromatography was performed on a HiTrap Q FF column (GE Healthcare) followed by a gel filtration chromatography with a Sepharose 6 Fast Flow column (Amersham Biosciences). The total proteasome content (20S) was calculated as the ratio between the total micrograms of proteasomes present in the sample after the final step of the isolation procedure, in each milligram of brain protein.

Determination of proteasome activity

Assays to test the activities of the proteasome were performed using the fluorogenic substrates Suc-LLVY-AMC (10 mM stock), Boc-LSTR-AMC (10 mM stock) and Boc-LRR-AMC (25 mM stock) for the ChT-L, T-L and PGPH, respectively [16]. The reaction was conducted in 200 μl proteasome activity buffer (10 mmol/l Tris–HCl, pH 7.8, 1 mmol/l EDTA, 0.5 mmol/l dithiotreitol and 5 mmol/l $MgCl_2$) in a mixture containing 10 μl of isolated proteasome and 1 μl of substrate [16]. This gave a final concentration of substrate as being 50 μM for ChT-L and T-L activities, with post-glutamyl peptidase activity being 125 μM. After 1 h of incubation at 37°C the fluorescence emission of the released aminomethylcoumarine product was monitored on a Microplate Fluorescence Reader (Bio-Tech Inc.) using an excitation/emission of 350/440 nm. Fluorescence values obtained in the assay were divided by the total micrograms of proteasomes used in the experiment and graphed as fluorescence/mg proteasome. Values are expressed as the fluorescence units^{-1} × per microgram of proteasome^{-1} × per hour^{-1}. The linearity of the products produced over time was ascertained in initial pilot studies.

The recovery of the ChT-L activity was also tested in the presence of dithiothreitol, DTT (1 M). Samples were treated with DTT for 30 min prior to addition of ChT-L substrate and the enhancement of ChT-L activity determined. The ability of the proteasome to degrade oxidized proteins was determined by incubating proteasomes (1 μg/30 μl final assay volume) with an oxidized protein substrate (1 μg/5 μl) for 30 min, and monitoring the amount of protein degradation.

The substrate used was DQ Green BSA® (Molecular Probes), which was oxidized by 30 min exposure to iron oxide (50 μM), followed by repeated cycles of overnight dialysis and lyophilization to ensure removal of iron oxide. Western blots confirmed the presence of protein carbonyls in BSA exposed to iron oxide as compared to non iron oxide treated BSA (data not shown). The degradation of DQ Green BSA® was quantified by monitoring the increase in fluorescence, generated by degradation of the BSA into peptides which liberates fluorescence, over time as reported previously [17,18]. Briefly, when bound to full length BSA there is a strong quenching of fluorescence due to the fact the protein is heavily labeled with BODIPY dyes. Upon hydrolysis, during proteasome-mediated protein degradation, the individual dye-labeled peptides do not exhibit any quenching and intense fluorescent products. In experiments where proteasomes were treated with DTT and monitored for oxidized BSA degradation the DTT was incubated with purified proteasomes for 30 min, followed by dialysis to remove DTT prior to addition of BSA. The linearity of the products produced over time was ascertained in initial pilot studies.

Analysis of protein oxidation

In order to analyze the level of protein oxidation, the detection of the carbonyl groups was performed using the OxyBlot protein oxidation detection kit (Chemicon International) [7,8]. Briefly, 1 μg of the samples were incubated for 1 h with 2,4-dinitrophenylhydrazine (DNPH) which leads to the derivatisation of the carbonyl groups and the formation of a dinitrophenyl (DNP) hydrazone product. The derivatized carbonyl groups were then detected using a dot blot immunoassay similar to previous studies by our laboratory [16]. In order to detect elevations in 4-hydroxynonenal (HNE) or neuroprostane conjugation, we conducted immunohistochemical studies using antibodies that recognize HNE-conjugated or neuroprostane conjugated proteins. These studies were conducted using dot blots and using commercially available antibodies (Chemicon International).

Results

Proteasome isolation

Proteasome complexes were isolated from the SMTG and from the cerebellum of control, MCI and AD subjects (Table I). The SMTG is a brain region adversely affected in AD, while the cerebellum is known to be spared in AD, thus serving as a control brain region for effects observed in the SMTG. No significant differences in proteasome content were observed between control, MCI and AD brains (Figure 1).

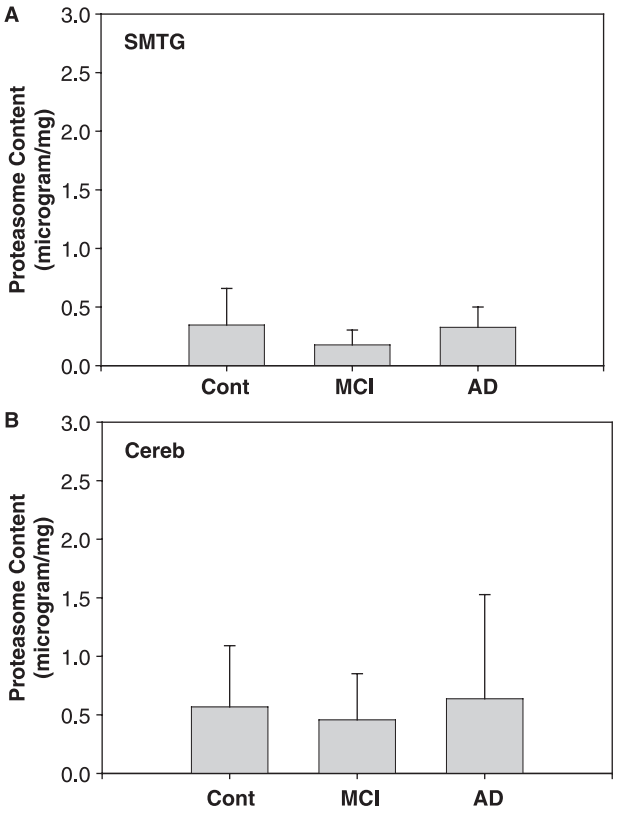

Figure 1. Total amount of proteasomes (μg) isolated from control, MCI and AD brains. (A) Proteasome content isolated from the SMTG of each experimental group. (B) Proteasome content isolated from the cerebellum of each experimental group. Data are presented as the mean and SD.

Proteasome activity

In order to determine the presence of alterations in proteasome activities in MCI and AD brains, we analyzed the ChT-L, T-L and PGPH activities in isolated proteasomes. Figure 2 shows each of the proteasome activities in proteasomes isolated from the SMTG and cerebellum. The Ch-L activity was significantly decreased in the SMTG area of AD brains compared with control subjects (Figure 2A) but was not significantly altered in the cerebellum of AD subjects. The T-L and PGPH activities were not significantly different between the control, MCI and AD subjects (Figure 2). Previous studies have demonstrated that protease activity in AD can be recovered with DTT treatment [19], suggesting that oxidation of proteases can inhibit their activity in AD. Therefore ChT-L activity was also analyzed in the presence of DTT (Figure 3) to determine if ChT-L activity could be enhanced after the treatment with the reducing agent. No significant differences in the recovery of ChT-L activity were found in MCI or AD subjects relative to control subjects (Figure 3).

We then sought to determine if there was a difference in the ability of proteasomes from MCI and AD subjects to degrade an oxidized protein

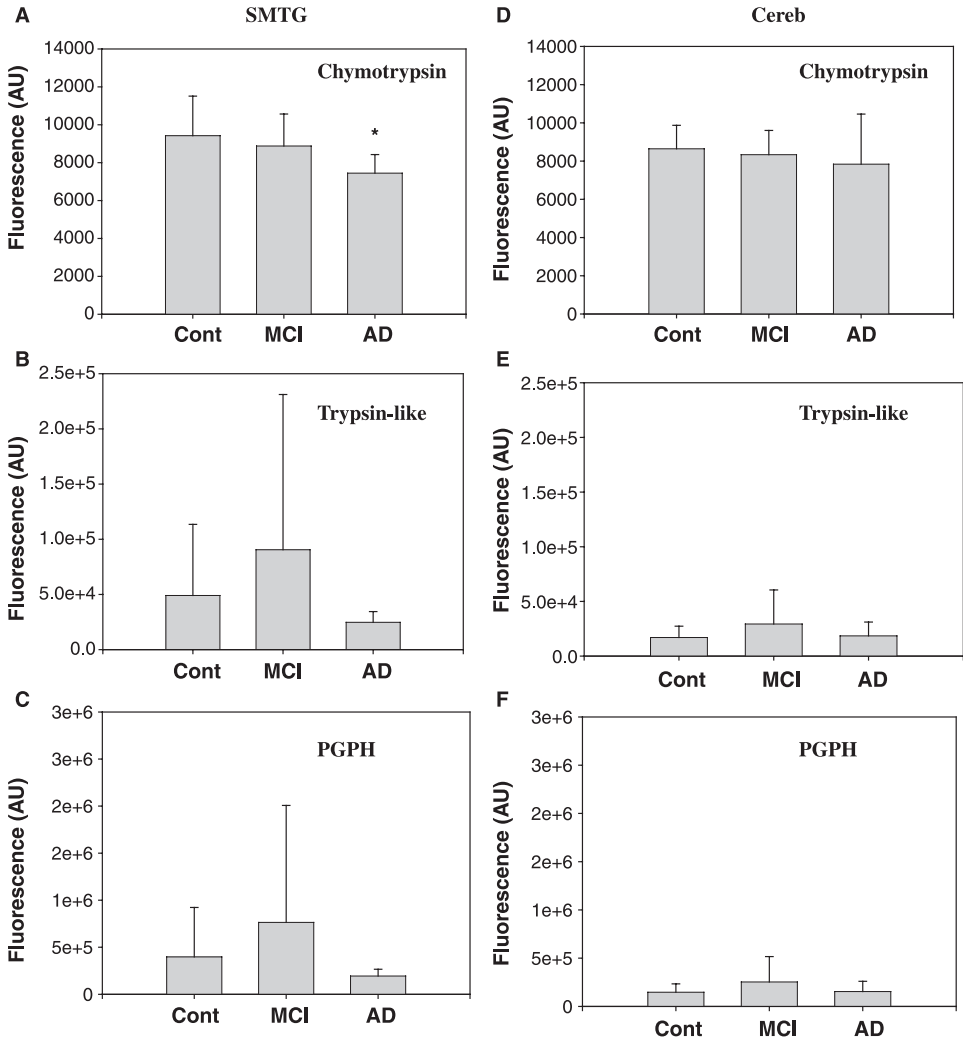

Figure 2. Activities of the proteasome isolated from the control, MCI and AD brains. Chymotrypsin-like (ChT-L) activity from the SMTG (A) and cerebellum (D). Trypsin-like (T-L) activity from the SMTG (B) and cerebellum (E). Post-glutamyl peptidase (PGPH) activity from the SMTG (C) and cerebellum (F). Values are expressed as the fluorescence units^{-1} × per microgram of proteasome^{-1} × per hour^{-1}. Data are presented as the mean and SD. *$p < 0.05$ compared to control subjects.

substrate. Analysis of oxidized bovine serum albumin (oxBSA) degradation revealed that there was a significant impairment in the ability of proteasomes from the SMTG of MCI and AD subjects to degrade oxBSA (Figure 4A), relative to proteasomes from control subjects. This loss of proteasome-mediated protein degradation was selective for the SMTG as no significant difference in oxBSA degradation was observed between proteasomes isolated from the cerebellum of control, MCI and AD subjects (Figure 4B). Interestingly, incubation with the reducing agent DTT was observed to cause a complete recovery of proteasome-mediated protein degradation in proteasomes from the SMTG of MCI and AD subjects (Figure 4A). No effect of DTT on proteasome-mediated protein degradation was observed in the cerebellum of control, MCI and AD subjects (Figure 4B).

A number of oxidative modifications to proteins are elevated in the different neurodegenerative diseases as well as normal aging [20,23]. The most commonly studied forms of protein oxidation are protein carbonyls and HNE-conjugation, due in part to the availability of well characterized antibodies that allow for immunohistochemical detection of these modifications [20–23]. Recent studies have demonstrated a role for neuro-ketals or neuroprostanes, a specialized form of lipid oxidation, as important mediators of oxidative stress [24,25], with recent studies demonstrating the ability of neuroprostanes to inhibit proteasome activity *in vitro* [26]. In order to determine if elevated levels of oxidative modifications were occurring to the proteasome and inhibiting is function during the progression of AD, we conducted studies in which we conducted dot blot immunoassays with the purified proteasomes from the SMTG. In this analysis

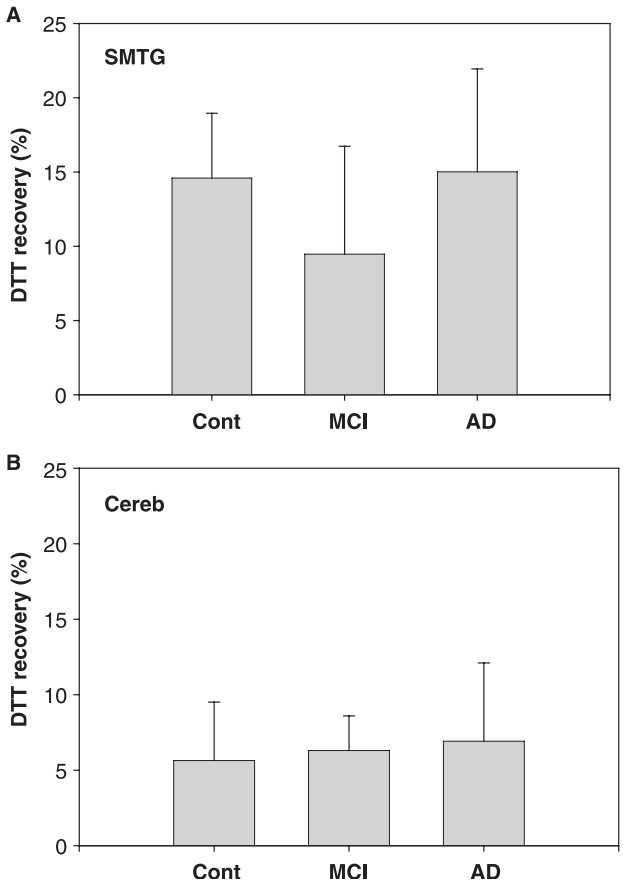

Figure 3. Percentage of DTT recovery for the ChT-L activity in the isolated proteasome. (A) DTT recovery in the SMTG area. (B) DTT recovery in the cerebellum. Values are expressed as the percent increase of activity relative to no DTT treatment. Data are presented as the mean and SD.

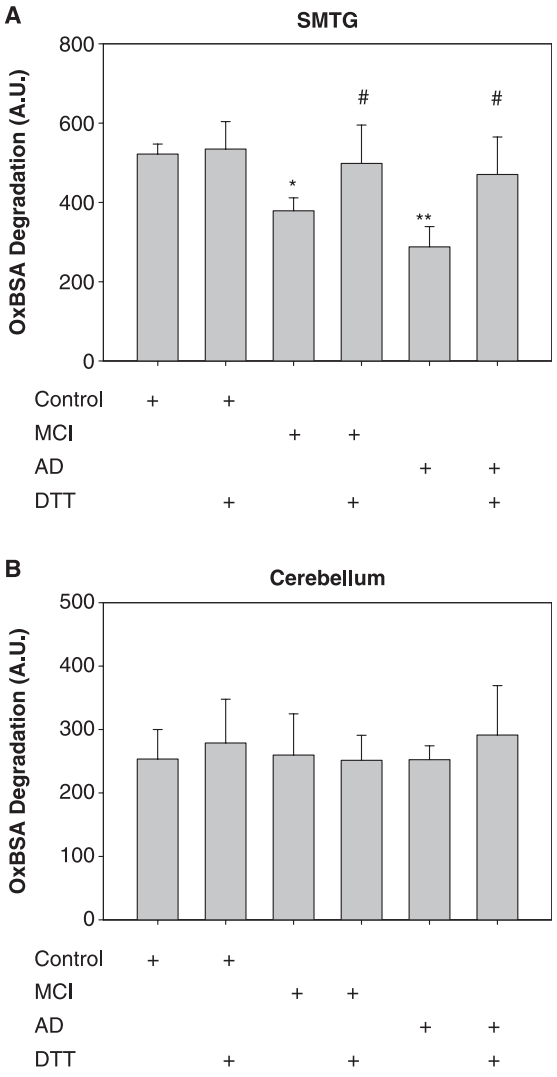

Figure 4. Analysis of proteasome-mediated degradation of oxidized BSA. In this aim the proteasome from SMTG (A) and cerebellum (B) of control, MCI, and AD subjects were analyzed for their ability to degrade oxidized BSA. The degradation of oxidized BSA was quantified by measuring the amount of liberated BODIPY fluorescence (detectible in BODIPY labelled peptides but no BODIPY labelled protein). In this assay system the lower the fluorescence value, the lower the proteasome-mediated degradation of BSA into fluorogenic peptides. Data are presented as arbitrary units (AU) of fluorescence, with the bars representing the mean fluorescence, and the error bars depicting the SD. $\star p < 0.05$ as compared to control subjects; $\#p < 0.05$ compared to non-DTT treated proteasome complexes.

we observed that proteasomes from AD subjects exhibited elevated levels of protein carbonyls, HNE- and neuroprostane-conjugation relative to control or MCI (Figure 5). These data are consistent with oxidative modifications potentially playing a role in mediating declines in proteasome function as reported in other neurodegenerative settings [6–11].

Discussion

The current study shows that isolated proteasome complexes from AD subjects present decreased ChT-L activity and an increased level of oxidative damage. Dramatic decreases in proteasome-mediated protein degradation were observed in purified proteasome complexes from both MCI and AD subjects. Several previous publications have suggested that AD and other neurodegenerative diseases are characterized by alterations in proteasome functionality and that proteasome inhibition often occurs in these disorders [6–11]. However, these studies previous studies were conducted with crude assessment of proteasome activity in tissue lysates, with no current studies demonstrating evidence for altered function in purified proteasomes from each of these conditions. Our results confirm the presence of alterations in the ChT-L activity in the AD subjects, with no significant differences in the other proteasome activities observed in our present studies, and furthermore they demonstrate that there is a robust inhibition of proteasome-mediated protein degradation occurs in both MCI and AD subjects. These findings strongly support evidence for decreased proteasome function in AD, and suggest that the loss of proteasome-

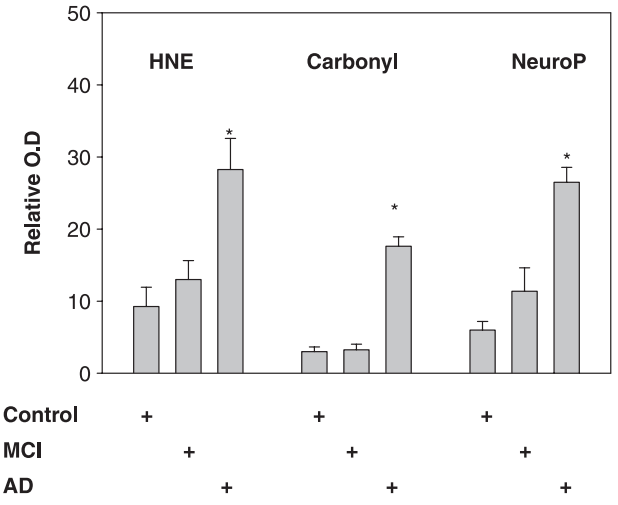

Figure 5. Gross proteasome oxidation. Changes in the levels of protein oxidation in purified proteasomes from the SMTG of control, MCI and AD subjects was determined. All subjects were analyzed for protein oxidation. The amount of protein carbonyls, HNE-conjugation, and neuroprostane-conjugation (NeuroP) in purified 20S proteasomes (1 μg) was measured immunohistochemically using dot blot as outlined in methods. The amount of staining was quantified using image analysis and expressed as the relative optical density (OD). The error bars depict the SD with $*p < 0.05$ as compared to control or MCI subjects.

mediated protein degradation occurs at the earliest stages of AD. Of particular importance is the fact that decreased proteasome-mediated protein degradation occurs prior to the development of dementia, or the development of widespread AD neuropathology, since it is detectible in the SMTG of MCI subjects. It will be important in future studies to characterize the loss of proteasome-mediated protein degradation that occurs in the different cortical and subcortical brain regions, and to correlate the changes in proteasome-mediated protein degradation with specific cognitive and neuropathological alterations.

Previous studies have shown that in AD brains homogenates the treatment with the reducing agent DTT induces a recovery in calpain protease activity [19]. Our results show that purified proteasomes from AD brains do not differ from controls in the amount of ChT-L activity recovery following treatment with DTT. However, a robust recovery of proteasome-mediated protein degradation was observed in MCI and AD proteasome complexes in response to incubation with DTT. These data strongly support a role for elevated levels of oxidative damage, to non-catalytic sites of the proteasome complex, directly contribute to declines in proteasome-mediated protein degradation in MCI and AD subjects. These studies also suggest that oxidative modification to the proteasome complex plays a direct role in mediating a loss of proteasome-mediated protein degradation in the earliest stages of AD, based on the fact that DTT enhanced proteasome-mediated protein degradation in proteasomes from MCI subjects.

In the present study we confirmed the presence of elevated oxidative modifications occurring to the proteasome complexes in AD subjects, relative to the extent of oxidative modification observed in control or MCI subjects. These studies strongly suggest that there is a neurochemical distinction (elevated levels of oxidative damage) between the proteasomes found in AD subjects, relative to those in the earliest stages of AD, or those free of AD pathogenesis. We are interested in understanding if these increases in oxidative modifications in AD are due to an elevation in oxidative stress, or whether they represent a decline in replacement or repair of the proteasome complexes. It is possible that declines in proteasome synthesis potentially contributes to the observed elevation in oxidized proteasomes in AD, due to the fact that generation of new (non-oxidized proteasomes) may be a primary basis for the lower levels of oxidized proteins in control and MCI subjects. It is interesting to note that previous studies have demonstrated that the failure to generate a single proteasome subunit is sufficient to elevate protein oxidation in the brain [27], and that low level exposure to oxidative stressors stimulates proteasome subunit expression in neural cells [28]. The fact that so many oxidative modifications are present in the proteasomes of AD subjects almost certainly mean that oxidative modifications are contributing to the decline in proteasome-mediated protein degradation. Such loss of proteasome function could not only promote the genesis of elevated levels of ubiquitinated and misfolded proteins, but also contribute to declines in protein synthesis [12,29,30]. This is based on recent studies demonstrating a role for proteasome inhibition causing reversible decreases in protein synthesis in neurons [12,29,30]. Together these data indicate a role for declines in proteasome-mediated protein degradation contributing to neuropathology and neurotoxicity via multiple mechanisms in AD.

These data are the first to demonstrate a role for neuroprostanes inhibiting proteasome function during the progression of AD. While many believe that neuroprostanes are relatively inactive in the brain [24,25], the fact that there is a clear segregation of neuroprostane-proteasome conjugation in AD, relative to MCI or control, raises many interesting questions. For example, is the elevation in neuroprostane conjugation due to an increase in neuroprostane attack, or could it potentially be mediated by increased recruitment of neuroprostane modified proteins to the proteasome? Similarly, how selective is the elevation in neuroprostane conjugation to the proteasome in AD, relative to other proteases? Studies are currently underway to address this potentially new area of research, whereby neuroprostanes play a role in inhibiting proteasome function in AD.

The data in this study are consistent with previous studies which have implicated oxidative modification to the proteasome contributing to age-related declines in proteasome function [10,11,31–35]. Additionally, these data support previous reviews which suggested that proteasome inhibition, mediated by oxidative modifications to the proteasome, contribute to oxidative stress and neurodegeneration [10,11,33,36,37]. As such, the rates at which oxidative stressors impair proteasome function may serve as an important trigger for a feed forward cascade for accelerated and exacerbated oxidative stress in AD [10,11,33,36,37], declines in protein synthesis in AD [12], and the activation of pro-apoptotic cascades in AD [6,8,11,34–37]. Our data support a role for oxidative damage contributing to neurochemical deficits observed in the earliest stages of AD, and thereby provide experimental support for oxidative stress playing a role in the onset and progression of AD. As such, our data suggest that therapies which are ably to reduce the levels of oxidative modification to the proteasome (via reducing the levels of oxidative stressors, or enhancing the genesis of proteasomes lacking oxidative modifications), particularly at the earliest stages of AD (pre-MCI), may provide therapeutic benefit by delaying the development of AD.

Acknowledgements

The authors thank the members of the University of Kentucky Alzheimer's Disease Center for their help in obtaining tissues and patient information. The authors thank Dr William Markesbery for his support, and Dr Rodney Guttmann for helpful discussions, and thank all those who donated tissue for the proposed study. This work was supported by grants from the Alzheimer's Association and NIH.

References

[1] Baumeister W, Walz J, Zühl F, Seemüller E. The proteasome: Paradigm of a self-compartimentalizing protease. Cell 1998;92:367–380.
[2] DeMartino GN, Slaughter CA. The proteasome, a novel protease regulated by multiple mechanisms. J Biol Chem 1999;274:22123–22126.
[3] Orlowski M, Wilk S. Catalytic activities of the 20S proteasome, a multicatalytic proteinase complex. Arch Biochem Biophys 2000;383:1–16.
[4] Adams J. The proteasome: Structure, function and role in the cell. Cancer Treat Rev 2003;29:3–9.
[5] Davies KJA. Degradation of oxidized proteins by the 20S proteasome. Biochimie 2001;83:301–310.
[6] Betarbet B, Sherer TB, Greenamyre JT. Ubiquitin-proteasome system and Parkinson's diseases. Exp Neurol 2005;191: 17–27.
[7] Keller JN, Huang FF, Zhu H, Yu J, Ho YS, Kindy MS. Oxidative stress-associated impairment of proteasome activity during ischemia-reperfusion injury. J Cereb Blood Flow Metab 2000;20:1467–1473.
[8] Keller JN, Hanni KB, Markesbery WR. Impaired proteasome function in Alzheimer Disease. J Neurochem 2000;75: 436–439.
[9] Lam YA, Pickart CM, Alban A, Landon M, Jamieson C, Ramage R, Mayer RJ, Layfield R. Inhibition of the ubiquitin-proteasome system in Alzheimer's disease. Proc Natl Acad Sci USA 2000;97:9902–9906.
[10] Grune T. Oxidative stress, aging and the proteasomal system. Biogerontology 2000;1:31–40.
[11] Grune T, Shringarpure R, Sitte N, Davies KJ. Age-related changes in protein oxidation and proteolysis in mammalian cells. J Gerontol A Biol Sci Med Sci 2001;56:459–467.
[12] Ding Q, Markesbery WR, Chen Q, Li F, Keller JN. Ribosome dysfunction is an early event in Alzheimer's disease. J Neurosci 2005;25:9171–9175.
[13] Petersen RC, Smith GE, Waring GC, Ivnik RJ, Kokmen E, Tangelos EG. Aging, memory, and mild cognitive impairment. Int Psychogeriatr 1997;9:65–69.
[14] Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos RG, Kokmen E. Mild cognitive impairment: Clinical characterization and outcome. Arch Neurol 1999;56:303–308.
[15] Friguet B, Szweda LI, Stadtman ER. Susceptibility of glucose-6-phosphate dehydrogenase modified by 4-hydroxy-2-nonenal and metal-catalyzed oxidation to proteolysis by the multicatalytic protease. Arch Biochem Biophys 1994;311: 168–173.
[16] Ding Q, Lewis JJ, Strum KM, Dimayuga E, Bruce-Keller AJ, Dunn JC, Keller JN. Polyglutamine expansion, protein aggregation, proteasome activity, and neural survival. J Biol Chem 2002;277:13935–13942.
[17] Barratt-Boyes SM, Zimmer MI, Harshyne LA, Meyer EM, Watkins SC, Capuano S, Murphey-Corb M, Falo LD, Donnenberg AD. Maturation and trafficking of monocyte-derived dendritic cells in monkeys: Implications for dendritic cell-based vaccines. J Immunol 2000;164:2487–2495.
[18] Jones LJ, Upson RH, Haugland RP, Panchuk-Voloshina N, Zhou M. Quenched BODIPY dye-labeled casein substrates for the assay of protease activity by direct fluorescence measurement. Anal Biochem 1997;251:144.
[19] Marcum JL, Mathenia JK, Chan R, Guttmann RP. Oxidation of thiol-proteases in the hippocampus of Alzheimer's disease. Biochem Biophys Res Commun 2005;334:342–348.
[20] Sayre LM, Moreira PI, Smith MA, Perry G. Metal ions and oxidative protein modification in neurological disease. Ann Ist Super Sanita 2005;41:143–164.
[21] Smith MA, Rottkamp CA, Nunomura A, Raina AK, Perry G. Oxidative stress in Alzheimer's disease. Biochim Biophys Acta 2000;1502:139–144.
[22] Sohal RS. Role of oxidative stress and protein oxidation in the aging process. Free Radic Biol Med 2002;33:37–44.
[23] Cecarini V, Gee J, Fioretti E, Amici M, Angeletti M, Eleuteri AM, Keller JN. Protein oxidation and cellular homeostasis: Emphasis on metabolism. Biochim Biophys Acta 2006 (In press).
[24] Roberts LJ, Fessel JP, Daves SS. The biochemistry of the isoprostane, neuroprostane, and isofuran pathways of lipid peroxidation. Brain Pathol 2005;15:143–148.
[25] Roberts LJ, Fessel JP. The biochemistry of the isoprostane, neuroprostane, and isofuran pathways of lipid peroxidation. Chem Phys Lipids 2004;128:173–186.
[26] Davies SS, Amarnath V, Montine KS, Bernoud-Hubac N, Boutaud O, Montine TJ, Roberts LJ. Effects of reactive γ-ketoaldehydes formed by the isoprostane pathway (isoketals) and cyclooxygenase pathway (levuglandins) on proteasome function. FASEB J 2002;16:715–717.
[27] Ding Q, Martin S, Dimayuga E, Bruce-Keller AJ, Keller JN. LMP2 knock-out mice have reduced proteasome activities and

increased levels of oxidatively damaged proteins. Antioxid Redox Signal 2006;8:130–135.

[28] Ding Q, Reinacker K, Dimayuga E, Nukala V, Drake J, Butterfield DA, Dunn JC, Martin S, Bruce-Keller AJ, Keller JN. Role of the proteasome in protein oxidation and neural viability following low-level oxidative stress. FEBS Lett 2003;546:228–232.

[29] Ding Q, Dimayuga ED, Markesbery WR, Keller JN. Proteasome inhibition induces reversible impairments in protein synthesis. FASEB J 2006;20:1055–1063.

[30] Ding Q, Ceccarini V, Keller JN. Interplay between protein synthesis and degradation in the CNS: Physiological and pathological implications. Trends Neurosci 2007;30:31–36.

[31] Farout L, Mary J, Vinh J, Szweda LI, Friguet B. Inactivation of the proteasome by 4-hydroxy-2-nonenal is site specific and dependant on 20S proteasome subtypes. Arch Biochem Biophys 2006;453:135–142.

[32] Ferrington DA, Kapphahn RJ. Catalytic site-specific inhibition of the 20S proteasome by 4-hydroxynonenal. FEBS Lett 2004;578:217–223.

[33] Hyun DH, Lee MH, Halliwell B, Jenner P. Proteasomal dysfunction induced by 4-hydroxy-2,3-trans-nonenal, an end-product of lipid peroxidation: A mechanism contributing to neurodegeneration? J Neurochem 2002;83:360–370.

[34] Farout L, Friguet B. Proteasome function in aging and oxidative stress: Implications in protein maintenance failure. Antioxid Redox Signal 2006;8:205–216.

[35] Friguet B. Oxidized protein degradation and repair in ageing and oxidative stress. FEBS Lett 2006;580:2910–2916.

[36] Halliwell B. Oxidative stress and neurodegeneration: Where are we now? J Neurochem 2006;97:1634–1658.

[37] Lee MH, Hyun DH, Jenner P, Halliwell B. Effect of proteasome inhibition on cellular oxidative damage, antioxidant defences and nitric oxide production. J Neurochem 2001;78:32–41.