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The Expression of Key Oxidative Stress-Handling Genes in Different Brain Regions in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) has been hypothesized to be associated with oxidative stress. In this study, the expression of key oxidative stress-handling genes was studied in hippocampus, inferior parietal lobule, and cerebellum of 10 AD subjects and 10 control subjects using reverse transcriptase-polymerase chain reaction (RT-PCR). The content of Mn-, Cu,Zn-superoxide dismutases (Mn- and Cu,Zn-SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione reductase (GSSG-R) mRNAs, and the "marker genes" (β-actin and cyclophilin) mRNAs was determined. This study suggests that gene responses to oxidative stress can be significantly modulated by the general decrease of transcription in the AD brain. To determine if the particular oxidative stress handling gene transcription was induced or suppressed in AD, the "oxidative stress-handling gene/β-actin" ratios were quantified and compared with control values in all brain regions studied. The Mn-SOD mRNA/β-actin mRNA ratio was unchanged in all regions of the AD brain studied, but an increase of the Cu,Zn-SOD mRNA/β-actin mRNA ratio was observed in the AD inferior parietal lobule. The levels of peroxidation handling (CAT, GSH-Px, and GSSG-R) mRNAs normalized to β-actin mRNA level were elevated in hippocampus and inferior parietal lobule, but not in cerebellum of AD patients, which may reflect the protective gene response to the increased peroxidation in the brain regions showing severe AD pathology. The results of this study suggest that region-specific differences of the magnitude of ROS-mediated injury rather than primary deficits of oxidative stress handling gene transcription are likely to contribute to the variable intensity of neurodegeneration in different areas of AD brain.

Index Entries: Alzheimer's; neurodegeneration; oxidative stress; antioxidant genes; reverse transcriptase-polymerase chain reaction (RT-PCR); ROS.

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Introduction

Alzheimer's disease (AD) is the major cause of age-related dementia. Evidence that oxidative stress is involved in the pathogenesis of AD is increasing (Benzi and Moretti, 1995; Beal, 1995; Harman, 1995; Sagara et al.,1996; Simonian and Coyle, 1996; Butterfield, 1997; Markesbery, 1997). Hallmarks of oxidative damage, such as the elevated levels of lipid peroxidation products (Sommerville et al., 1991; Lovell et al., 1995; Markesbery and Lovell, 1998), oxidatively modified proteins (Smith et al., 1994; Hensley et al., 1995; Aksenova et al., 1998), and oxidized nucleotides in nuclear and mitochondrial DNA (Mecocci et al., 1994, 1997; Gabbita et al., 1998), have been found in areas of the brain affected by the AD pathology.

Oxidative damage in the CNS of AD patients may result from increased production of free radicals or from the failure of antioxidant defenses. Several reports suggest a significant increase of free radical production in the brain of AD patients (Hoyer, 1993; Zhou et al., 1995). Specific sources of oxidative stress in AD have been suggested to include defective mitochondrial electron transport systems (Nutisya et al., 1994; Parker et al., 1994; Cavelier et al., 1995; Sheehan et al., 1997), amyloid beta peptide (Davis, 1996; Hensley et al., 1996; Butterfield, 1997; Behl and Sagara, 1997), increased concentrations of redox-active metals (Gerlach et al., 1994; Markesbery and Ehmann, 1994), reactive glia (Colton et al., 1994), and protein glycation products (Smith et al., 1996). The degree of oxidative damage in the AD brain was shown to correspond to the regional distribution of AD histopathology (Hensley et al., 1995; Lovell et al., 1995).

Reactive oxygen species (ROS) can directly affect the cellular signaling apparatus and act as potential intracellular and extracellular signaling molecules able to change gene expression in the cell (Palmer and Paulson, 1997). Changes of the expression of oxidative stress handling enzymes play an important role in the mechanism of the cell response to oxidative damage. A set of oxidative stress handling enzymes, which normally prevents an excessive accumulation of ROS in the cell, include Cu/Zn- and Mn-superoxide dismutases (Cu/Zn and Mn-SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-R). Increased antioxidant enzyme activities contribute to the ability of cells to resist the prooxidant attack (Sagara et al., 1996; Yagi et al.,1996) and delay an apoptotic cell death (Greenlund et al., 1995). The function of antioxidant enzyme systems is highly regulated on the transcriptional level. Altered or insufficient induction of antioxidant enzyme-specific mRNA synthesis in response to increased ROS production may cause failure of the cell defense against oxidative stress. It is conceivable that alterations of oxidative stress handling gene response may contribute to the development of oxidative damage in the CNS of AD patients. Assuming that the AD brain is under oxidative stress, one would expect an increase of antioxidant enzyme activities and gene expression in the AD brain. However, the postmortem studies of oxidative stress handling enzymes in AD produce contradictory results (Bowling and Beal,1995; Markesbery, 1997). There is little information about changes of oxidative stress handling gene expression in histopathologically distinct regions of the AD brain. This study was designed to compare the level of expression of five key oxidative stress handling genes (Cu,Zn- and Mn-SOD, CAT, GSH-Px, GSSG-R) in the hippocampus, inferior parietal lobule, and cerebellum of AD and normal control subjects.

Materials and Methods

Tissue Use

Specimens from hippocampus, inferior parietal lobule, and cerebellum were obtained at autopsy from 10 AD patients (mean age 77 ± 1.2 yr) and 10 age-matched control subjects (mean age 86 ± 2.3 yr). Mean postmortem interval was 3.0 ± 0.2 h for AD patients and 3.2 ± 0.3 h for control subjects. All AD patients had clinical diagnoses of probable AD using NINCDS-ADRDA Work Group Criteria (McKhann et al., 1984). For histopathologic diagnoses, hematoxylin and eosin, and the modified Bielschowsky stains were used. All AD patients met accepted standard criteria for the histopathologic diagnosis of AD (Khachaturian, 1985; Mirra et al., 1991; NIA and Reagan Institute Diagnostic Criteria, 1997).

Controls were individuals without a history of dementia, other neurological diseases, or systemic diseases affecting the brain. All control subjects

were from the University of Kentucky normal volunteer control group who underwent annual neuropsychological testing. Neuropathological evaluation of control brains revealed no significant gross alterations and only age associated microscopic changes.

Brain specimens for the study were removed rapidly at autopsy, immediately placed in liquid nitrogen, and stored at –70°C.

RNA Isolation, Reverse Transcription, and PCR Amplification

Total RNA was extracted from frozen brain samples (100–200 mg tissue) following the method of Chromzynski and Sacchi (1987). RNA concentration was determined by 260/280 absorbance measurement. The integrity of the extracted RNAs was monitored using 1% agarose gel electrophoresis (Sambrook et al., 1988).

For reverse transcription, 1 µg of total RNA was mixed with 500 pmol of random hexamers (Boehringer Mannheim) in a volume of 20 µL, incubated at 95°C for 2 min, and then placed on ice. Then the stock solution was added such that the final reaction volume of 30 µL contained 200 U of Superscript, 500 µ*M* dNTPs, 40 U of RNasin, 1X reaction buffer (Life Technologies). The solution was incubated at 20°C for 10 min and at 42°C for 50 min, and the Superscript reverse transcriptase was inactivated by heating to 95°C for 2 min. The Superscript was omitted from the samples, which were then used as a control for possible DNA contamination in PCR amplification ("No RT" controls).

Stock PCR reaction mixtures were prepared on ice and contained 50 µ*M* dCTP, 100 µ*M* each of dGTP, dATP, and dTTP, 10 µCi of dCTP (3000 Ci/mmol), 1.5 µ*M* $MgCl_2$, 1X reaction buffer, 0.03 U/µL of Taq DNA Polymerase (Life Technologies). The stock solutions were separated into 14.5-µL aliquots, and 1/60 of cDNA synthesized in the reverse transcription was added to each aliquot. The reaction mixtures were covered with a drop of mineral oil and subjected to various cycles of PCR.The use of multiple cycles allowed us to determine the minimum number of cycles necessary to detect PCR product and thereby stay in the linear region of PCR. Typical reaction conditions were 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C. After amplification cDNAs were separated by polyacrylamide electrophoresis. The gels were analyzed by PhosphoroImager technology (Molecular Dynamics, Sunnyvale, CA) or stained with SYBR Green I (Molecular Probes, Inc., Eugene, OR), photographed, scanned and digitized by computer-assisted imaging using MCID/M4 software supplied by Imaging Research Inc. (Ontario, Canada). Both procedures of the image analysis were shown to produce equal results. The sequences of the primers, product size, and optimized number of PCR cycles for each particular gene expression analysis were:

For Cu,Zn-SOD:
Sense primer:
5'-GTGATCTCACTCTCAGGAGA-3'
Antisense primer:
5'-TCATTTCCACCTTTGCCCAA-3'
Product size: 88 bp
Number of PCR cycles: 24

For Mn SOD:
Sense primer:
5'-AGATCATGCAGCTGCACCACA-3'
Antisense primer:
5'-GTTCTCCACCACCGTTAGGGC-3'
Product size: 204 bp
Number of PCR cycles: 23

For CAT:
Sense primer:
5'-CTGTTGAAGATGCGGCGAGAC-3'
Antisense primer:
5'-GGCCAAACCTTGGTGAGATCG-3'
Product size: 175 bp
Number of PCR cycles: 25

For GSH-Px:
Sense primer:
5'-TGCTCGGTTTCCCGTGCAA-3'
Antisense primer:
5'-ACCGTTCACCTCGCACTTCT-3'
Product size: 139 bp
Number of PCR cycles: 25

For GSSG-R:

Sense primer:

5'-CCAAGTTGTGAGGGTAAATT-3'

Antisense primer:

5'-TTTTTCCCACTGACCTCTAT-3'

Product size: 175 bp
Number of PCR cycles: 25.

Cyclophilin and β-actin were chosen as marker genes for this study. The sequences of the primers, product sizes, and optimized numbers of PCR cycles for RT-PCR analysis of the expression of marker genes were:

For cyclophilin:

Sense primer:

5'-ATGGTCAACCCCACCGTGTT-3'

Antisense primer:

5'-CGTGTGAAGTCACCACCCT-3'

Product size: 204 bp
Number of PCR cycles: 16.

For β-actin:

Sense primer:

5'-TCCGGAGACGGGGTCACCCA-3'

Antisense primer:

5'-GTCCAGACGCAGCAGGATGGCAT-3'

Product size: 78 bp
Number of PCR cycles: 17.

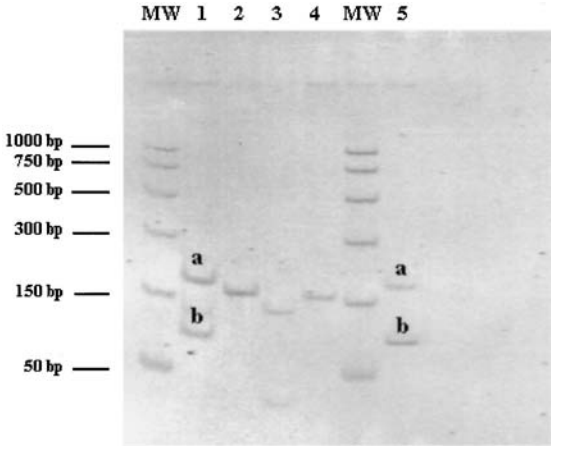

Fig. 1. Antioxidant gene-specific and marker gene-specific RT PCR products. One milligram of total human brain RNA loaded per each reverse transcription reaction mixture. 1/60 of cDNA synthesized in reverse transcription was then amplified with primers specific for antioxidant mRNAs (Cu,Zn-SOD, Mn-SOD, CAT, GSH-Px, GSSG-R), and marker mRNAs (cyclophilin and β-actin). cDNA products were separated by polyacrylamide electrophoresis. The gel was stained with SYBR Green DNA stain (Molecular Probes, Inc., Eugene, OR). MW, molecular weight markers; 1, SOD-specific PCR products; a, Mn-SOD, 204 bp; b, Cu,Zn-SOD, 88 bp; 2, CAT-specific PCR product, 175 bp; 3, GSH-Px-specific PCR product, 139 bp; 4, GSSG-R-specific PCR product, 175 bp; 5, marker genes-specific PCR products; a, cyclophilin, 204 bp; b, β-actin, 78 bp.

Statistical Analysis

Statistical comparisons were made using ANOVA followed by Dunnett's test for multiple comparisons.

Results

The data described below represent the results of imaging analysis of specific PCR products separated by polyacrylamide gel electrophoresis (Fig. 1). Representative gel images which illustrate the results of RT PCR analyses of the expression of oxidative stress handling and marker genes are shown in Fig. 2. To determine if the particular oxidative stress handling gene transcription was induced or suppressed in AD, the "oxidative stress handling gene/β-actin" ratios were quantified, averaged and compared in the hippocampus, inferior parietal lobule, and cerebellum for each AD patient or control subject studied.

Expression of Marker Genes in Three Different Brain Regions in AD

The content of the cyclophilin and β-actin mRNAs was decreased in the hippocampus, inferior parietal lobule, and cerebellum of AD patients. The results of the phosphoroimaging analysis of the yields of marker gene specific PCR products are shown in Table 1. The level of β-actin

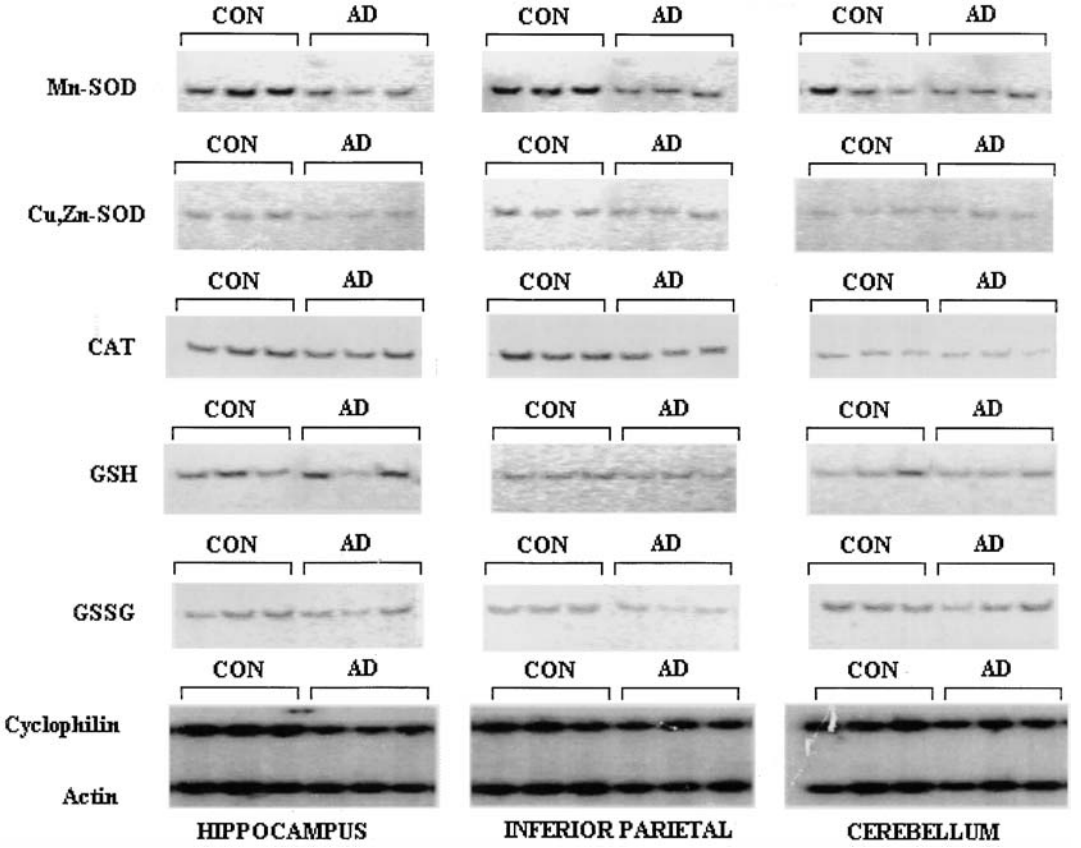

Fig. 2. Representative result of RT PCR analysis of the levels of antioxidant gene-specific and marker gene-specific transcripts in different brain regions of control and AD subjects. Yields of antioxidant enzyme-specific cDNAs and β-actin cDNA, determined by quantification of gel images, were used to quantify the "oxidative stress handling gene/β-actin" ratios in the hippocampus, inferior parietal lobule, and cerebellum for each AD patient or control subject studied.

Table 1
β-Actin and Cyclophilin mRNA Content in Different Regions of the AD and Control Brain[a]

	CON	AD	% of CON	*P* value
β-actin				
HIPP	44,589 ± 5741	26,974 ± 2877	61	0.03
INF	39,434 ± 4957	22,916 ± 2669	58	0.013
CEREB	29,146 ± 2703	20,838 ± 2261	72	0.0009
Cyclophilin				
HIPP	29,287 ± 5124	16,203 ± 1930	55	0.04
INF	35,574 ± 2586	18,548 ± 2586	52	0.026
CEREB	29,834 ± 3562	19,522 ± 3013	65	0.015

[a]The results of imaging analysis of specific RT-PCR product yields are presented as mean ± SEM. Difference between AD and control groups were considered to be significant if *P* value was <0.05.

mRNA was 58–61% of control in the hippocampus and the inferior parietal lobule of AD patients and 72% of control in cerebellum. Similiar results were obtained for another marker gene, cyclo-

Table 2
Mn-SOD Gene Expression in Different Regions of the AD and Control Brain[a]

	CON	AD	% of CON	*P* value
Mn-SOD mRNA				
HIPP	40,010 ± 8563	20,620 ± 3185	52	0.048
INF	46,810 ± 7929	29,260 ± 3696	63	NS
CEREB	31,080 ± 6714	29,890 ± 4552	96	NS
Mn-SOD mRNA/actin RNA				
HIPP	0.89 ± 0.10	0.76 ± 0.07	85	NS
INF	1.18 ± 0.35	1.27 ± 0.06	108	NS
CEREB	1.07 ± 0.18	1.43 ± 0.21	134	NS

[a]The results of imaging analysis of Mn-SOD RT-PCR product yields and Mn-SOD mRNA/β-actin mRNA ratios are presented as mean ± SEM. NS—the difference between AD and controls was not significant at 95% level ($P > 0.05$).

philin. The level of cyclophilin-specific transcript was 52–55% of control value in the AD hippocampus and the inferior parietal lobule and 65% of control value in the AD cerebellum. The decrease of cyclophilin and β-actin mRNAs observed in our experiments is probably the reflection of a reduced yield of total poly(A)+ RNA in AD reported previously by several authors (Sajdel-Sulkowska et al., 1984; Subbarao et al., 1990; Gullimette et al., 1987; Crapper-McLachlan et al., 1988). This general decrease of mRNA production in AD brain should be taken into account when changes of the expression of specific genes are studied. The decrease of β-actin mRNA content observed in this study was consistent with our previous obsevations that showed a decreased content of β-actin protein in the middle temporal gyrus of AD patients (Aksenova, Aksenov, unpublished observation). Because of this we have used the level of β-actin mRNA as a normalization factor for the determination of changes of the oxidative stress handling gene expression in AD brain.

Expression of Oxidative Stress-Handling Genes in Three Brain Regions in AD Cu,Zn- and Mn SOD Gene Expression

An overall decrease of Cu,Zn-SOD mRNA was observed in hippocampus of AD subjects (Table 2). However, the ratio of Cu,Zn-SOD to β-actin mRNA was the same in AD and control hippocampus. A statistically significant ($P < 0.005$) increase in the Cu,Zn-SOD mRNA/β-actin mRNA ratio was found in the inferior parietal lobule in AD. The level of Cu,Zn-SOD mRNA normalized to β-actin mRNA was elevated in the AD cerebellum compared to control, although that difference was marginally significant ($P = 0.05$). If expressed as a percent of control value, the Cu,Zn-SOD mRNA/β-actin mRNA ratio was 46–50% higher in cerebellum and in the inferior parietal cortex of AD patients (Table 2).

The yield of Mn-SOD-specific RT-PCR product was significantly lower in AD hippocampus (Table 3), but the normalization to the β-actin product yield revealed that the 15% difference between Mn-SOD mRNA/β-actin mRNA ratios in AD and control hippocampus was not significant. This ratio also was unchanged in the inferior parietal lobule of AD patients (Table 3). The Mn-SOD mRNA/β-actin mRNA ratio tended to increase in AD cerebellum, but this difference (34% over control value) did not reach statistical significance.

CAT Gene Expression

Without the ajustment for actin mRNA content, levels of CAT mRNA in the hippocampus and inferior parietal lobule of AD and control subjects did not show significant difference (Table 4). However, the level of CAT mRNA normalized to β-actin mRNA level (CAT mRNA/β-actin mRNA ratio) was significantly elevated in hippocampus and in the inferior parietal cortex ($P < 0.005$ and $P < 0.05$, respectively). CAT mRNA/β-actin mRNA ratios were 55 and 40% higher in hippocampus and the inferior parietal lobule of AD patients than

Table 3
Cu,Zn-SOD Gene Expression in Different Regions of the AD and Control Brain[a]

	CON	AD	% of CON	*P* value
Cu,Zn-SOD mRNA				
HIPP	15,067 ± 2247	9130 ± 796	60	0.018
INF	29,720 ± 3108	24,556 ± 2744	83	NS
CEREB	10,550 ± 1891	11,220 ± 1350	106	NS
Cu,Zn-SOD mRNA/actin RNA				
HIPP	0.34 ± 0.04	0.34 ± 0.05	100	NS
INF	0.75 ± 0.08	1.10 ± 0.09	146	0.0018
CEREB	0.36 ± 0.07	0.54 ± 0.09	150	0.05

[a]The results of imaging analysis of Cu,Zn-SOD RT-PCR product yields and Cu,Zn-SOD mRNA/β-actin mRNA ratios are presented as mean ± SEM. NS—the difference between AD and controls was not significant at 95% level ($P > 0.05$).

Table 4
CAT Gene Expression in Different Regions of the AD and Control Brain[a]

	CON	AD	% of CON	*P* value
CAT mRNA				
HIPP	27,080 ± 3219	26,029 ± 2183	96	NS
INF	22,4482 ± 1720	18,350 ± 1448	82	NS
CEREB	20,929 ± 1494	15,591 ± 1249	75	0.013
CAT mRNA/actin mRNA				
HIPP	0.66 ± 0.07	1.02 ± 0.07	155	0.0026
INF	0.57 ± 0.06	0.80 ± 0.08	140	0.014
CEREB	0.76 ± 0.06	0.72 ± 0.07	95	NS

[a]The results of imaging analysis of CAT RT-PCR product yields and CAT mRNA/β-actin mRNA ratios are presented as mean ± SEM. NS–the difference between AD and controls was not significant at 95% level ($P > 0.05$).

in the same brain regions of control subjects (Table 4). The decrease of CAT mRNA level, observed in the AD cerebellum (75% of control, Table 4) was close to the decrease of marker gene mRNAs in this brain region of AD patients (72% of control for β-actin mRNA and 65% of control for cyclophilin, Table 1). Thus, no specific change of CAT gene expression was found in AD cerebellum.

GSH-Px Gene Expression

Yields of GSH-Px -specific RT-PCR product did not show a significant decrease in the AD brain regions studied (Table 5). Thus, changes of overall GSH-Px mRNA level did not correlate with the decrease of the level of marker gene transcription. When normalized to β-actin mRNA content, GSH-Px mRNA level was significantly higher in hippocampus and in the inferior parietal lobule in AD ($P < 0.05$ and $P < 0.01$ subsequently). The GSH-Px mRNA/β-actin mRNA ratio was increased by 47% in hippocampus and by 65% in the inferior parietal lobule in AD. Although the GSH-Px mRNA/β-actin mRNA ratio was 30% higher in AD cerebellum than in controls, this diffrence was not statistically significant (Table 5).

GSSG-R Gene Expression

The results of RT-PCR analysis demonstrated that the decrease of GSSG-R mRNA correlated with the decrease of β-actin and cyclophilin mRNAs in AD cerebellum and the inferior parietal lobule (Table 6). GSSG-R mRNA/β-actin mRNA ratios in AD cerebellum (118%) and the inferior parietal lobule (104%) were close to control (Table 6), which suggests that changes of the level of GSSG-R transcript in these regions of the AD brain are

Table 5
GSH-Px Gene Expression in Different Regions of the AD and Control Brain[a]

	CON	AD	% of CON	*P* value
GSH-Px mRNA				
HIPP	13,367 ± 1100	11,770 ± 1302	88	NS
INF	7474 ± 375	7294 ± 455	98	NS
CEREB	8742 ± 466	7972 ± 536	91	NS
GSH-Px mRNA/actin mRNA				
HIPP	0.30 ± 0.05	0.44 ± 0.07	147	0.047
INF	0.19 ± 0.02	0.32 ± 0.07	167	0.008
CEREB	0.30 ± 0.02	0.38 ± 0.06	127	NS

[a]The results of imaging analysis of GSH-Px RT-PCR product yields and GSH-Px mRNA/β-actin mRNA ratios are presented as mean ± SEM. NS–the difference between AD and controls was not significant at 95% level ($P > 0.05$).

Table 6
GSSG-Red Gene Expression in Different Regions of the AD and Control Brain[a]

	CON	AD	% of CON	*P* value
GSSG-Red mRNA				
HIPP	9715 ± 973	7780 ± 666	80	NS
INF	9722 ± 829	5930 ± 624	61	0.002
CEREB	11,176 ± 524	9450 ± 437	85	0.02
GSSG-Red mRNA/actin mRNA				
HIPP	0.22 ± 0.02	0.29 ± 0.03	132	0.037
INF	0.25 ± 0.03	0.26 ± 0.03	104	NS
CEREB	0.38 ± 0.03	0.45 ± 0.04	118	NS

[a]The results of imaging analysis of GSSG-Red RT-PCR product yields and GSSG-Red mRNA/β-actin mRNA ratios are presented as mean ± SEM. NS–the difference between AD and controls was not significant at 95% level ($P > 0.05$).

likely to reflect the general decrease of mRNA production in the AD brain. The overall level of GSSG-R mRNA was not significantly lower than in control (80% of control value, $P > 0.05$) in AD hippocampus, and the GSSG-R mRNA/β-actin mRNA ratio was elevated (130% of control value, $P > 0.05$) compared to control, which may reflect the induction of GSSG-R mRNA in the AD hippocampus (Table 6).

Discussion

Evidence from several sources indicates that alterations of gene expression occur in the brain of AD patients (Selkoe, 1991). Both neuronal and glial cells in AD-affected neocortex contain an increased proportion of condensed chromatin (Lewis et al., 1981). An increased chromatin condensation is known to be associated with a reduced level of transcription (Knezetic and Luse, 1986). The substantial decrease of polyadenylated RNA in the AD brain was later documented in several studies (Sajdel-Sulkowska et al., 1984; Taylor et al., 1986; Gullimette et al., 1987; Crapper-McLachlan et al., 1988). Our findings of the decreased levels of marker mRNAs, β-actin and cyclophilin mRNA, are in line with the reports that indicated the general decrease of mRNA production in AD-affected brain. The general decrease of polyA+ RNA, documented in the AD brain, complicates the analysis of the expression of the selected genes. Because of the low percentage of

polyA+ RNA in total RNA preparations, a substantial change of mRNA content may not significantly change the total yield of RNA, obtained from the AD brain samples. Thus, if the results of the specific mRNA determination will be normalized per total RNA load, the induction of the particular gene transcription in AD may be underestimated or remain unnoticed (if it is comparable with the general decrease of mRNA level) and the degree of the decrease of the particular mRNA content may be overestimated. We believe that use of the "housekeeping" mRNA content as a normalization factor in our analysis of oxidative stress handling gene expression minimize this problem. Another problem, which may complicate the analysis of gene expression in postmortem autopsy samples is a possible gradual decay of RNA message. Studies of the mRNA integrity in relation to the postmortem interval demonstrated that, both in control and in AD brain autopsy samples, each transcript appears to have its own intrinsic decay rate with half lives from minutes to days (Krowzynska et al., 1985; Lukiw and Crapper-McLachlan, 1990). Although it is a question whether mRNA in AD may be less stable than in control, we minimized the possible unwanted effects of the RNA degradation in our study by using only the short PMI brain specimens for total RNA preparation. The integrity of each preparation of total RNA was also controlled by agarose electrophoresis. Use of RT PCR analysis, which is less sensitive to the total RNA integrity than the other methods of gene expression analysis, also decreased the probability of the wrong estimation of the levels of oxidative stress handling gene transcripts. The RT PCR-based method used in this study was previously validated as a tool for the analysis of the expression of specific mRNAs (Estus, 1997).

Our study demonstrates that quantitative shifts of the antioxidant gene transcription occur in different regions of the AD brain. Consistent with previous studies our results suggest that the gene responses to oxidative stress can be significantly modulated by the general decrease of mRNA production in the AD brain. When statistically significant decreases of oxidative stress handling-specific transcripts per total RNA were observed in our study, normalization to the housekeeping gene mRNA content indicated that this decrease is likely reflected the general decrease of the mRNA level in the AD brain. This fact suggests that specific defects of expression of key oxidative stress handling genes are unlikely to occur in AD-affected brain. Statistically significant quantitative shifts of "oxidative stress handling gene mRNA/β-actin mRNA" ratios, reported in this study, may reflect the induction of the expression of oxidative stress handling genes in response to the increased intensity of ROS production in the CNS of AD patients. Our results demonstrate that the pattern of changes of antioxidant gene expression varies in the different brain regions in AD.

A previously published study of Cu,Zn-SOD expression using *in situ* hybridization technique compared the content of Cu,Zn-SOD mRNA in single cells in hippocampal CA1 and CA2 regions (Smith et al., 1996). Cu,Zn-SOD mRNA was moderately (22%) decreased in CA1 of AD brains with no corresponding reduction in CA2 region. These data coincide with our observations. Without the normalization per the decreased level of the marker gene transcript, which was not done in the study of Sommerville and coauthors, the content of Cu,Zn-SOD mRNA in our study also would be lower in the AD hippocampus than in control. However, the Cu,Zn-SOD mRNA/β-actin mRNA ratio was close in the AD and control hippocampus, which indicates no specific deficit of Cu,Zn-SOD gene expression. While the Cu,Zn-SOD mRNA/β-actin mRNA ratio did not change in the AD hippocampus, it was significantly increased in the inferior parietal lobule of AD patients. This suggests that cellular responses to oxidative stress in different brain regions significantly affected by AD pathology may have their own specific features.

No significant changes of the Mn-SOD mRNA/β-actin mRNA ratio in the hippocampus and inferior parietal lobule of AD patients were found, however, Mn-SOD gene expression tended to increase in the AD cerebellum. The information about changes of Mn-SOD expression in the AD brain is sparse.

The immunocytochemical detection of both SOD isoenzymes in the cerebral cortex and hippocampus of patients with AD (Furuta et al., 1995; Maeda et al., 1997) demonstrated that Cu,Zn- SOD immunoreactivity was enriched in the degenerating neurons, whereas Mn-SOD immunoreactivity occurred in reactive astrocytes, associated with senile plaques. The expression of Mn-SOD is known

to be NF-κB-dependent (Mattson et al., 1997). Kaltshmidt and coauthors recently reported the activation of the transcription factor NF-κB in neurons and astroglia of brain sections from AD patients. However, activated NF-κB was restricted to cells in close vicinity of early plaques (Kaltshmidt et al., 1997). We speculated that the activation of Mn-SOD mRNA transcription may be detected at earlier stages of AD pathogenesis, when early plaques prevail in the areas of the brain affected by AD pathology. An accumulation of mature neuritic plaques in the brain of advanced AD patients, such as been used in our study, abrogates the compensatory increase of Mn-SOD-specific transcription.

Data reported in this study indicate a different shift of the relative levels of Cu,Zn- and Mn-SOD transcripts in two vulnerable regions of the AD brain: hippocampus and inferior parietal lobule. Differences in senile plague density and NFT density, as well as different neurons-to-glia ratios may contribute to the different pattern of gene expression of two SOD isoforms observed in these two brain regions severely affected by AD pathology.

In this study we demonstrated a significant increase of CAT gene expression in the brain regions significantly affected by AD pathology. The brain contains only small amounts of CAT (Coyle and Puttfarcken, 1993). However, this antioxidant enzyme may play a meaningful role in the protection of CNS cells from the oxidative stress in AD. Cell culture studies demonstrated that the increased expression of CAT contributes to the increased resistance of cultured neural cells to Ab toxicity (Sagara et al., 1996; Behl and Sagara, 1997). Recent reports indicate that neurons are particularly sensitive to hydrogen peroxide (Whittemore et al., 1994; Desagher et al., 1996; Hoyt et al., 1997). Astrocytes express much more catalase than neurons and their ability to protect neurons from hydrogen peroxide toxicity and oxidative damage has been documented (Desagher et al.,1996; Wilson, 1997). CAT seems to be the main peroxidase activity in the neuroprotective effect of astrocytes (Desagher et al.,1996). Region-specific differences in the Ab accumulation, activation of microglia, and electron transport chain (ETC) defects may contribute to a differential increase of hydrogen peroxide production in certain areas of the AD brain. The relative increase of CAT mRNA level in vulnerable regions of the AD brain may reflect the cellular response to the increased hydrogen peroxide production in those brain areas.

Although CAT expression is low in neurons, GSH-Px appears to be the main enzymatic activity involved in neuronal defense against H_2O_2 toxicity (Desagher et al., 1996). Changes of GSH-Px mRNA level in different regions of AD brain observed in our study coincided with the changes of CAT gene expression. GSH-Px activity contributes to the removal of toxic lipid peroxides. Thus, it would not be unexpected, that the GSH-Px mRNA transcription would be induced in the areas of AD brain known to exhibit the increased level of lipid peroxidation (Markesbery, 1997).

GSSG-R is one of the enzymatic activities which are expected to provide the primary cellular mechanism for protection and repair of sulfhydryl proteins under oxidative stress. Through the maintenance of GSH/GSSG ratio, this enzyme also contributes to normal functioning of GSH-dependent enzymes like GSH-Px. In this study, statistically significant changes of GSSG-R gene expression were observed only in the AD hippocampus.

Whereas studies of antioxidant enzyme gene expression in AD are sparse, changes of activity of key oxidative stress handling enzymes have been studied by numerous investigators (Markesbery, 1997). The enzyme activity level in the cell results from a complicated interplay between different regulatory mechanisms and events that occur on transcriptional, posttranscriptional and post-trans-lational levels of the process of the transformation of genetic information into the enzyme product. Chan-ges of protein production that do not involve the regulation at the mRNA level (Komori et al., 1997; Rajagopalan and Malter, 1997), and posttranslational modification of proteins by ROS (Butterfield and Stadtman, 1997) may cause an inconsistency between the antioxidant enzyme activity and the level of antioxidant enzyme gene expression.

Several laboratories reported no significant alterations of SOD isoenzymes activity in some regions of the AD brain (Lovell et al., 1995; Balaz and Leon, 1994; Gsell et al., 1995; Kato et al., 1991) whereas others found a moderate increase (Marklund et al., 1985) or decrease of SOD activity (Cu/Zn or Mn-SOD) (Chen et al., 1994, Richardson, 1993) in AD. Taken together, these studies do not demonstrate the specific deficit of SOD isoenzymes in AD,

which is consistent with the results of Cu,Zn- and Mn-SOD gene expression analysis in this study.

Studies of peroxidation-handling enzymes (CAT, GSH-Px, and GSSG-R) in AD are not consistent (Markesbery, 1997; Marcus et al., 1998). Our findings of relatively increased levels of CAT, GSH-Px, and GSSG-R mRNAs in vulnerable regions of the AD brain are consistent with changes of activity of these enzymes in the hippocampus and neocortex of AD patients reported by Lovell and coauthors (1995).

Changes of antioxidant enzyme-specific gene expression we report here coincide with the regional distribution of oxidative damage in AD brain, as it was reported in several studies (Lovell et al., 1995, Hensley et al., 1995). It is likely that these changes are secondary to the increased peroxidation in neural cells and, in part, reflect the activation and propagation of glia in the brain areas affected by AD pathology. We did not observe any specific deficiencies of oxidative stress handling enzyme expression on the transcriptional level in AD. However, our results suggest that the general decrease of mRNA production can make the protective induction of several key oxidative stress handling genes insufficient to compensate for the increased oxidative damage in vulnerable areas of the AD brain.

The results of this study suggest that region-specific differences of the grade of ROS-mediated injury rather than primary deficits of oxidative stress handling gene transcription are likely to contribute to varying intensities of neurodegeneration in different regions of the AD brain. Our study does not rule out the possibility that the specific changes of antioxidant enzyme-mediated cell responses may occur on the posttranscriptional level in brain areas, which are selectively affected by AD pathology. Additional studies of the post-transcriptional regulation of antioxidant enzyme production are needed to answer this question.

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