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Brief communication

Expression of cortical and hippocampal apoptosis-inducing factor (AIF) in aging and Alzheimer's disease☆

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Abstract

Apoptosis-inducing factor (AIF) is a mitochondrial oxidoreductase originally identified for its role in caspase-independent programmed cell death (PCD). In this study, we investigated AIF protein expression levels in frontal and temporal cortices of normal subjects of various ages, as well as in subjects with Alzheimer's disease (AD). AIF levels were also measured in the hippocampus of age-matched elderly and AD subjects. Amounts of all three AIF isoforms increased significantly with age in both cortical areas. Interestingly, AIF expression levels in the cortex (but not hippocampus) were consistently lower in AD compared to age-matched controls. The up-regulation of cortical AIF in normal aging is consistent with its previously hypothesized role as a free radical scavenger, and may thus represent an adaptive cellular response to compensate for the steady increase in oxidative stress occurring with age.

Keywords: Apoptosis-inducing factor; Cerebral cortex; Hippocampus; Human; Aging; Alzheimer's Disease; Oxidative stress

1. Introduction

Classical caspase-dependent apoptosis, the most studied form of programmed cell death (PCD), is thought to contribute significantly to neuronal losses occurring in normal aging and Alzheimer's disease (AD)-associated neurodegenerative processes. This view is supported by several studies describing the presence of classical apoptotic markers in various areas of AD brains [reviewed in 10], but remains controversial in the case of normal aging [12]. Although the underlying mechanisms remain to be fully understood, it is believed that cumulative oxidative stress contributes significantly to apoptotic cell demise [13]. However, alternative forms of PCD initially described in non-neuronal cell types are likely contributors to neuronal loss [see 17]. Among key players involved in caspase-independent PCDs, the apoptosis-inducing factor (AIF) has recently attracted considerable interest [6,16].

AIF is a ubiquitously expressed flavoprotein synthesized as a cytoplasmic ~67 kDa precursor giving rise to a mature ~62 kDa protein located in the mitochondrial intermembrane space [11]. In mitochondria, AIF is involved in oxidoreduction [14,15] and is considered as a putative reactive oxygen

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species (ROS) scavenger [8]. Upon pathological permeabilization of mitochondria, however, AIF is further processed to a ~57 kDa form, then released and translocated to the nucleus where it is associated with chromatin condensation and large-scale DNA fragmentation (>50 kpb) [14]. Interestingly, both mitochondrial and nuclear effects of AIF have been observed in neuronal death associated with rodent brain aging and acute traumatic injuries [8,19]. However, a possible role for AIF in either normal human brain aging or AD-associated neurodegeneration has not been studied thus far. The present study was undertaken to determine AIF protein levels in cortical areas of the human brain at various ages and in AD.

2. Materials and methods

2.1. Subjects and tissue samples

All experiments were approved by the Douglas Hospital Research Ethics Board. Human cortical tissues from a total of 51 subjects were obtained from the Douglas Hospital Research Centre Brain Bank and the Douglas Hospital Suicide Brain Bank (Montreal, Canada). Tissues from 35 control subjects, without any evident neurological disorders, were divided into three age groups, namely young (19–39 years old; $n = 12$), middle-aged (40–64 years old; $n = 7$) and elderly (65–88 years old; $n = 16$) (Table 1). An additional group ($n = 16$; Table 1) was constituted of subjects afflicted with advanced AD, as determined from clinical diagnosis and neuropathological examination. Blocks of frozen frontal and temporal cortices, corresponding respectively to Brodmann areas 9 and 20, were selected with the aid of a human brain photographic atlas [2]. Blocks of hippocampi were sampled in the same fashion, but were only available for a limited number of subjects from the elderly and AD groups ($n = 10$ per group).

2.2. Western blotting

Unless stated otherwise, all reagents were from Sigma Chemicals or Fisher Canada (Montreal, Canada). Tissues were processed for Western blotting according to a standard protocol previously described [5]. In brief, total soluble protein extracts were adjusted at 5 μg/sample with the BCA Protein Assay Kit (Pierce, Rockford, IL), resolved through 4–20% SDS-PAGE (Invitrogen; Burlington, Canada) and transferred to nitrocellulose membranes. The membranes were then incubated overnight with purified goat polyclonal antibody (D-20; 1:1000; Santa Cruz Biotechnology; Santa Cruz, CA) or mouse monoclonal antibody (E-1; 1:1500; Santa Cruz Biotechnology), respectively, raised against the C- and N-terminal regions of human AIF. After incubation with either donkey secondary peroxidase (POD)-conjugated anti-goat IgG antibody or goat anti-mouse IgG antibody (both diluted 1:6000, and from Santa Cruz Biotechnology) for D-20 and E-1, respectively. POD activity was visualized

Table 1
Summary of cases

Case	Ctrl/AD	Age	Gender	PMI	Cause of death
Young ($n = 12$)					
1	Control	19	M	32	Road accident
2	Control	22	M	24	Hanging
3	Control	27	M	20.3	Heart attack
4	Control	28	M	16	Hanging
5	Control	29	M	26.3	Hanging
6	Control	29	M	27	Road accident
7	Control	30	M	26.3	Stabbed
8	Control	31	M	32.3	Hanging
9	Control	31	M	29.3	Road accident
10	Control	34	M	31	Hanging
11	Control	35	M	24	Hanging
12	Control	36	M	25	Hanging
	Mean	29		26.1	
	S.E.M.	1		1.3	
Middle-aged ($n = 7$)					
13	Control	41	M	14.5	Pneumonia
14	Control	50	F	11.5	Heart attack
15	Control	51	M	11	Road accident
16	Control	61	M	15.75	Pneumonia
17	Control	61	M	17.5	Heart attack
18	Control	61	M	6.5	Heart attack
19	Control	64	M	20.75	n.a
	Mean	56		13.9	
	S.E.M.	3		1.6	
Elderly ($n = 16$)					
20	Control	66	M	20.5	Pneumonia
21	Control	67	M	24.75	Peritonitis
22	Control	68	F	22.75	Pneumonia
23	Control	69	M	32.5	Heart attack
24	Control	70	F	37	Heart attack
25	Control	72	M	46	Hanging
26	Control	72	F	13.5	Heart attack
27	Control	72	M	24.5	Heart attack
28	Control	75	F	10.75	Heart attack
29	Control	75	F	6.5	Heart attack
30	Control	76	M	11	Pneumonia
31	Control	76	F	10.5	Heart attack
32	Control	78	F	19.75	n.a
33	Control	79	M	14.75	Heart attack
34	Control	86	F	23.25	Pneumonia
35	Control	88	M	16	Heart attack
	Mean	74		20.9	
	S.E.M.	2		2.6	
AD ($n = 16$)					
36	AD	64	M	26.5	n.a
37	AD	66	M	8.5	Tumor metastasis
38	AD	66	M	13.75	Pneumonia
39	AD	68	F	16.5	Pneumonia
40	AD	69	F	18	Tumor metastasis
41	AD	70	M	12.75	Heart attack
42	AD	71	M	16.75	Pneumonia
43	AD	79	F	10.75	Pneumonia
44	AD	79	M	30	Pneumonia
45	AD	80	F	8.25	Heart attack
46	AD	84	F	11.75	n.a
47	AD	85	F	11.25	n.a

Table 1 (*Continued*)

Case	Ctrl/AD	Age	Gender	PMI	Cause of death
48	AD	85	M	35.5	Pneumonia
49	AD	87	F	26.5	Heart attack
50	AD	87	F	13.75	n.a
51	AD	87	F	39.25	Pneumonia
	Mean	77		18.7	
	S.E.M.	2		2.4	

using Western Blot Chemiluminescence Reagent Plus (Perkin-Elmer, Toronto, Canada). Equal protein loading was confirmed after stripping and re-probing blots with a 1:2500 dilution of a mouse monoclonal antibody directed against β-actin (Sigma; St. Louis, MO, USA). Densitometric quantification of immunopositive bands was performed from photographic impressions of immunoblots using the MCID image analysis system software (Imaging Research, St. Catherines, Canada). Level of AIF protein expression was expressed as the ratio of densitometric intensity measured for AIF-immunolabeled band over that of β-actin. The 67 and 62 kDa AIF isoforms were thus quantified after detection with both antibodies. The 57 kDa isoform, not readily detected with the monoclonal E-1, was quantified solely after detection with the D-20 antibody. Controls included pre-incubation of D-20 antibody with its blocking peptide (D-20P, Santa Cruz) in 100-fold excess, and omitting the D-20 or E-1 primary antibodies (Supplementary Fig. 1). Furthermore, Western blots were performed with extracts from purified mitochondria and with mitochondria treated with the permeability-transition-pore-opening agent atractyloside, containing, respectively, the 62 and 57 kDa AIF isoforms [18]. The effects of different (0–48 h) post-mortem intervals (PMI) on AIF and β-actin degradation were evaluated with rat brain extracts, both at room temperature and 4 °C, and found to be negligible for both proteins (Supplementary Fig. 2). For statistical analysis, the mean value for each sample was determined from four repeated immunoblots and analyzed with the GraphPad Prism 4.0 software computer program (San Diego, CA) After demonstration of global significance by one-way ANOVA, post-test comparisons between group means were made using the Bonferroni test for multiple comparisons.

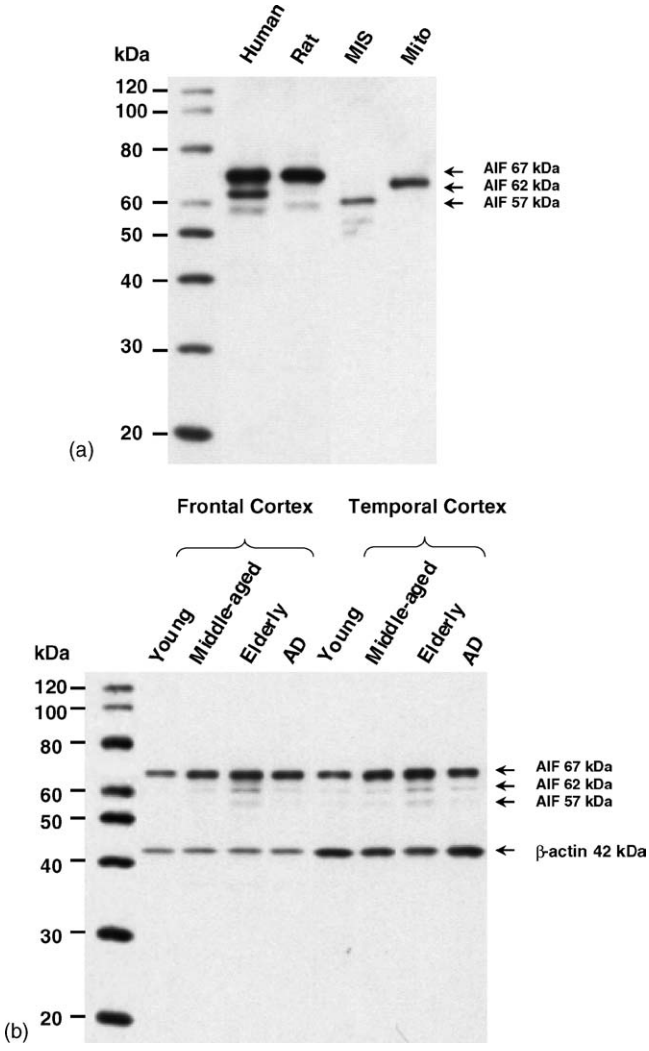

Fig. 1. Three AIF isoforms are detected in human brain extracts. (a) AIF detection with the D-20 polyclonal antibody from extracts of human temporal cortex, rat frontal cortex, mitochondrial intermembrane space (MIS; mitochondrial extracts after treatment with atractyloside as described previously [18]) and purified entire mitochondria (Mito). MIS and Mito extracts display 57 and 62 kDa isoforms, respectively [18]. (b) Illustration of representative variations in expression of AIF isoforms measured between young, middle-aged, elderly and AD subjects corresponding to the case numbers 2, 14, 28 and 37, respectively (Table 1). In this experiment, antibodies directed against AIF and β-actin were added and revealed simultaneously. Arrows point to the molecular weight of different AIF isoforms and β-actin.

3. Results

The specificities of both goat polyclonal (D-20) and mouse monoclonal (E-1) anti-AIF antibodies were demonstrated by pre-adsorption and omission controls (Supplementary Fig. 1) the detection of 57 and 62 kDa bands from different mitochondrial extracts (Fig. 1a) and by the finding that both antibodies yielded comparable expression profiles with human cortical extracts (Fig. 2). The profiles of expression were very similar in the temporal and frontal cortices for all three isoforms. Average levels were always higher in the temporal cortex of elderly versus young subjects (Figs. 1b and 2a–e). AIF levels in young and middle-aged groups were never significantly different. Expression of the 67 kDa isoform was almost doubled ($p < 0.001$) in the elderly versus young group when using the D-20 antibody (Fig. 2a). The overall profile of expression obtained with the E-1 monoclonal antibody (Fig. 2b) was similar to that seen with the D-20 antibody, except that the increase between middle-aged and elderly groups reached significance ($p < 0.05$). AIF expression in the AD group was consistently lower than in the age-matched elderly group, although this difference was not statistically significant. However, AIF expression in the AD group was significantly different from young for the 62 kDa isoform as

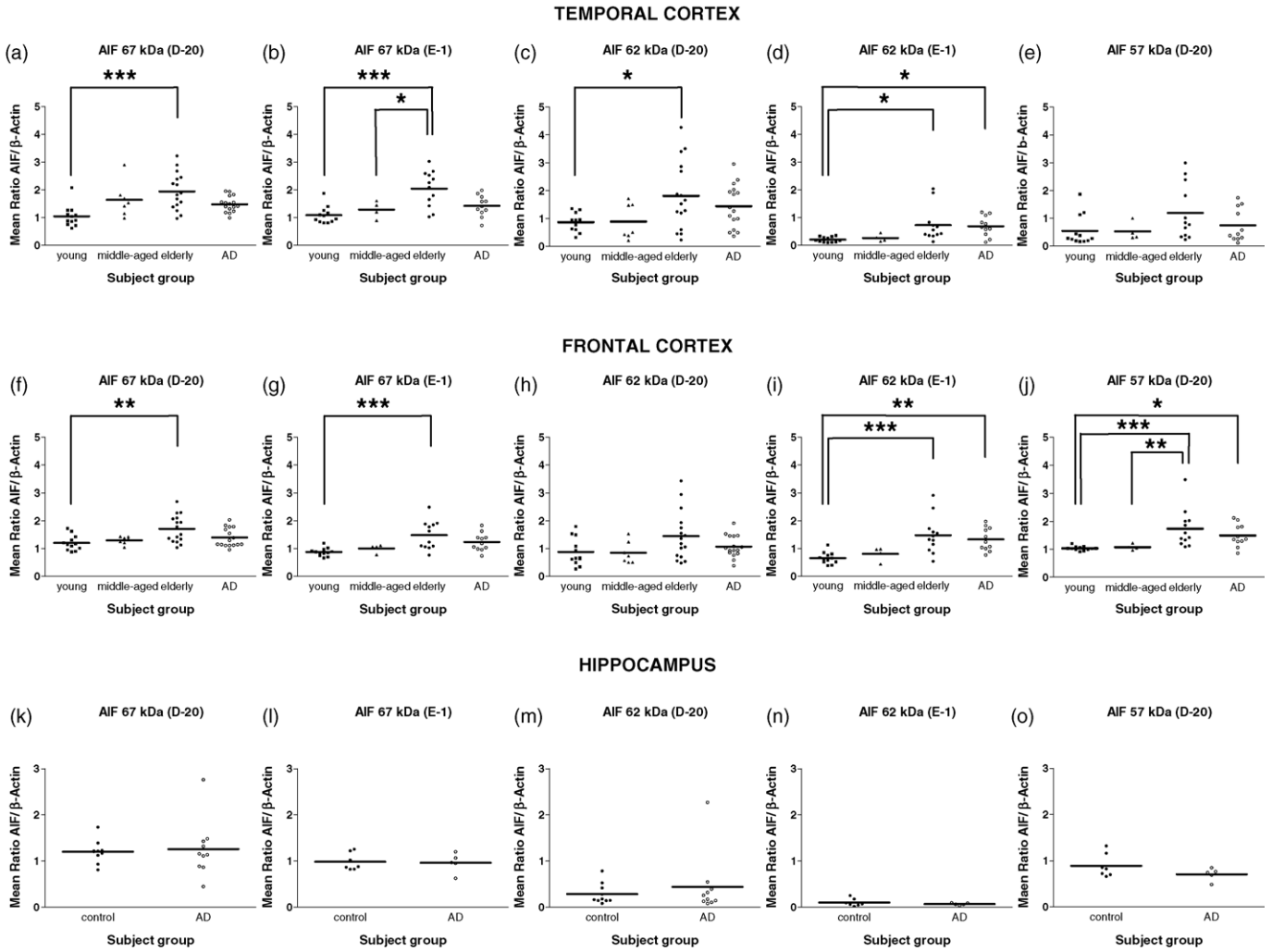

Fig. 2. AIF expression levels in human cerebral cortex and hippocampus with aging and in AD. The expression levels of 67 kDa (a, b, f, g), 62 kDa (c, d, h, i) and 57 kDa (e, j) AIF isoforms were investigated in temporal and frontal cortices of young (19–39 years old), middle-aged (40–64 years old), elderly (65–88 years old) and AD (64–87 years old) subjects, using the D-20 polyclonal (a, c, e, f, h, j) and E-1 monoclonal (b, d, g, i) anti-AIF antibodies. Values are consistently higher in the elderly group. The 67 kDa (k, l), 62 kDa (m, n) and 57 kDa (o) AIF isoforms were also investigated in the hippocampus of AD and age-matched control subjects, using the D-20 polyclonal (k, m, o) and E-1 monoclonal (l, n) anti-AIF antibodies. Each value represents the mean from four repeated immunoblots. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

revealed using the E-1 antibody (Fig. 2d). Expression of the 57 kDa isoform followed the same general trend, but the age-related increase failed to reach significance (Fig. 2e).

AIF expression levels in the frontal cortex were similarly increased with age, albeit less dramatically than in the temporal cortex (Figs. 1b and 2f–j). For example, using the D-20 antibody, mean values for the 67 kDa isoform in the elderly group were 32 and 42% ($p < 0.01$) greater than corresponding data for middle-aged and young groups (Fig. 2f). As in the temporal cortex, AIF levels in the AD group were lower (18%) than in the elderly, age-matched group and not significantly different from those of the young or middle-aged groups (Fig. 2f). A similar pattern was observed using E-1 antibody (Fig. 2g) and 62 kDa AIF expression profile (Fig. 2h,i) resembled that seen for its precursor (Fig. 2f,g). Interestingly, expression levels of 62 kDa AIF were significantly higher (103%; $p < 0.01$) in AD versus young brains (Fig. 2i). In the frontal cortex, 57 kDa expression in the elderly increased with strong significance from the values measured for the young and middle-aged groups ($p < 0.001$ and $p < 0.01$, respectively; Fig. 2j). Furthermore, the value for the AD group was also significantly higher than for the young group ($p < 0.05$; Fig. 2j). In the hippocampal formation, AIF expression levels were similar between AD and age-matched groups (Fig. 2k–o).

4. Discussion

This is the first study on the expression of AIF in the human brain either during normal or pathological aging. The major findings are (a) that in temporal and frontal cortices, AIF levels increase with age and (b) that these increases are somewhat surprisingly mostly absent in subjects with AD. Increased amounts of AIF likely reflect an age-related change in protein transcription and/or synthesis rather than in mitochondrial maturation, since levels of precursor and mature AIF isoforms increased in parallel. Furthermore, the observation that the "apoptotic-inducing" 57 kDA isoform is similarly increased suggests that the prevalence of AIF-dependent programmed cell death in cerebral cortex also increases with age. Hippocampal tissues from younger subjects were unfortunately not available to us. Hence, the impact of aging on AIF expression could not be examined in this region.

The up-regulation of AIF with age may represent a defense mechanism aimed at maintaining the integrity and function of the respiratory chain against age-related changes in brain metabolism, such as the increase in ROS production and associated oxidative stress [3,7]. It has been proposed that AIF serves as a ROS scavenger based on experiments showing that cerebellar granule cells from mutant mice with decreased AIF expression are more sensitive to peroxide-induced cell death than their wild-type counterparts, but can be rescued by overexpression of wild-type AIF [8]. Accordingly, the increased expression of AIF observed here could help to counteract increases in oxidative stress known to occur in the aging brain [1]. In contrast to the situation observed during normal brain aging, AIF expression levels were generally not increased in AD, at least not in the three regions under study. In fact, AIF levels in frontal and temporal cortices were always lower in AD than in normal aged-matched controls. This may suggest a loss of capacity of the AD brain to maintain adequate anti-oxidative activities, in accordance with earlier report [4]. AIF could thus represent a novel target to increase endogenous anti-oxidant capacities of the aged and pathological brain.

Finally, data showing that the expression of the "apoptosis-inducing" 57 kDa isoform is also down-regulated in AD brains compared to age-matched controls suggests that effectors other than AIF might be direct PCD triggers in AD. This may seem at odds with the age-related increase in the expression of the 57 kDa isoform observed in non-AD subjects. Additional studies will be required to determine whether PCD phenotypes different from classical apoptosis (mediated by cytochrome c; reviewed in [10]) and apoptosis-like PCD (mediated by AIF) are involved in the pathogenesis of AD, and if their prevalence differs from that seen in normal aging. In that context, the involvement of necrosis-like PCD (mediated by yet unknown mediators) appears as an attractive hypothesis [9].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2006.01.003.

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