Apoptotic gene expression in Alzheimer disease: a preliminary report

Author:
Associate Professor Stephen A MARGOLIS MBBS MD
School of Medicine
University of Queensland
Australia

Professor Kate D. HAMMOND BSc (Hons) PhD
and Mr M M Qureshi
Department of Biochemistry
UAE University
United Arab Emirates

Corresponding:
Dr Stephen Margolis
Associate Professor and Head
Rural Clinical Division- Central Queensland
University of Queensland
PO Box 4143
Rockhampton 4700
Queensland - Australia
Phone: +61 7 49207390
Fax: +61 7 49207395

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ABSTRACT

Background: This preliminary study examined the pattern of apoptotic gene expression in Alzheimer Disease [AD] using RNA from whole blood. Methods: Venous blood samples were collected from 8 Gulf Arab people aged 60+ years with AD and 12 ethnically similar controls. RNA was isolated using the PAXgene blood RNA procedure and analysed using a GEArray system, profiling 96 key apoptotic genes. Results: In all samples, TNFRSF1B (TNF receptor family TNFR2) was the most highly expressed; BCL2L1 (BCL2 family bcl-x) and CASP14 (caspase family) were also of relatively high intensity. In AD, BCL2L1 was significantly lower than in controls [p=0.022]. TNFSF14 (HVEM-L) was significantly higher in males than females [p=0.025]. There was no difference in expression of the other apoptotic genes observed. Conclusion: The findings suggest a possible role for the anti-apoptotic gene BCL2L1 in AD and a possible differential role between sexes in ageing for TNFSF14.

INTRODUCTION

Apoptosis is a genetically controlled mechanism for

programmed cell death [1]. The key enzymes orchestrating apoptosis are the caspases, proteases participating in a cascade of events leading to cleavage of specific proteins, disruption of the nucleus and dismantling of the cell. Activation of caspases may be through extrinsic or intrinsic pathways. The extrinsic pathway requires activation of membrane receptors included among which are Fas and members of the tumour necrosis factor receptor (TNFR) family. The signal for activation of the intrinsic pathway leads to release of pro-apoptotic molecules and cytochome c from the mitochondria. Initiation of apoptosis is tightly regulated by factors such as the Bcl-2 proteins [2-3].

Apoptosis has been implicated as having a possible role in a wide range of conditions, including aging and dementia, although the relationship remains somewhat controversial [1, 4 - 7]. Dementia is the name of a progressive syndrome characterised by a persistent loss of memory and at least one other type of cognitive deficit [8]. Alzheimer disease (AD) is the most common cause of dementia, 7 - 8% of all people aged 65+ years [9].

Inappropriate activation of apoptotic pathways may contribute to dementia [10 - 11]. The progressive loss of cognitive function seen in dementia is due to loss of nerve cells, which appears to be due to apoptotic mechanisms [12]. Amyloid ß, which plays a key role in the pathogenesis of AD in vivo, has been shown to induce apoptosis to nerve cells in vivo [13].

The aim of this preliminary study was to use a microarray procedure to examine the expression profiles of genes encoding caspases, proteins of the intrinsic and extrinsic apoptotic pathways and regulatory factors in blood from aging Gulf Arab subjects, with and without AD.

METHODS

Study design and patients

In this cross-sectional survey, all older people aged 60+ years old in six of the seven institutions providing aged care in the United Arab Emirates [UAE] were assessed for evidence of AD using the NINCDS-ADRDA criteria [14]. Diagnosis was based on clinical criteria in conjunction with X ray and laboratory data by a clinician experienced in geriatric care. For each of the 24 people identified, relatives were contacted to provide written consent to participate. Of the 7 male and 10 female residents whose relatives provided consent, a stratified random sample of four male and four female Gulf Arabs were chosen to participate in this study. The control group was randomly selected from a cohort of older people who lived in the community in the UAE described in an earlier study [15]. Only those of similar ethnicity without cognitive impairment or cerebro-vascular disease and aged 60+ years were considered. Using a random number generator, seven male and five female people were chosen.

Isolation of RNA

For each subject, analysis was completed on a single independent sample of venous blood collected directly into PAXgene RNA tubes and RNA isolated using the PAXgene blood RNA system [16]. The procedure involves centrifugation to pellet nucleic acids, treatment of the pellet with proteinase K to digest proteins and application of the sample to a spin column. RNA is eluted from the column with an optimized buffer. Concentration of RNA was determined by measuring absorbance at 260nm in 10mM Tris-Cl buffer, pH 7.5. The purity of the RNA sample was confirmed by measuring the ratio of the absorbance readings at 260nm and 280nm. Integrity of RNA was checked by denaturing agarose gel electrophoresis and ethidium bromide staining. These methods were as described in the PAXgene handbook [16].

Apoptotic gene expression analysis

The isolated RNA was used as a template for biotin-labelled cDNA probe synthesis, through a reverse transcriptase reaction. The probe was hybridized with a GEArray membrane [17]. A chemiluminescent detection method was used; the membrane was incubated with streptavidin-AP conjugate, the image developed with CDP-Star substrate and recorded on X-ray film. Negative controls showed no chemiluminescence. Data analysis was semi-quantitative. Intensities of standard reference genes included on the arrays, RPL13A, ACTB and GAPD, were assigned the arbitrary values 3, 4 and 3, respectively, in all cases. For each film, the intensity of the each square on the grid corresponding to a specific gene was assessed relative to standards on a scale 0 [not visible] through 6 [maximum intensity observed]. Only those genes which were expressed with relatively high intensity, for which reliable results could be obtained, were analysed. Repeat analysis of the isolated RNA gave consistent results.

Statistical analysis

The Statistical Package for the Social Sciences was used [18]. Simple frequency analysis was used to describe demographics. Comparative statistics were calculated using chi-square analysis or independent sample t test. General Linear Model Univariate analysis [GLM] was utilised to statistically account for the differences in age between the control and AD groups. The level of statistical significance was defined as $p<0.05$.

Ethical Approval

Ethical approval was obtained from the Research Ethics Committee of the Faculty of Medicine and Health Sciences, UAE University.

RESULTS AND DISCUSSION

RNA was successfully extracted from all participants with an average yield of 4.38 +/- 1.46 ug/ml. The PAXgene system overcomes the instability of RNA in vitro, providing a rapid and efficient procedure for isolation of intact RNA. Of the RNA found in the blood, rRNA is 80% and mRNA only 5%.

Changes occurring in nervous tissue in AD may be reflected in the blood and indeed certain factors produced by blood cells may be associated with initiation of the apoptotic process. Furthermore, substances may be released from damaged or abnormal tissue into the blood via the intracellular fluid. These phenomena create the potential for clinically accessible peripheral tissues to be used to identify diagnostic markers that could be used for selecting treatment or monitoring therapeutic effectiveness, as has been suggested by Gibson and Zhang [19].

Participant demographics are detailed in **Table 1**. There was no significant difference in age between males and females, although those with AD were significantly older [p=0.002].

In the present preliminary study, we used a multidimensional microarray approach to analyse interactive pathways of apoptosis in RNA isolated from whole blood. The GEArray system profiles the expression of ninety-six key genes. These genes are grouped into categories according to their functional and structural features, and include TNF ligands, TNF receptors, caspases, Bcl-2 proteins, death domain family members, death effector domain family members, as well as genes involved in the p53 and ATM pathways. A representative array showing genes expressed in this study group is given in **Figure 1**. Members of the TNF receptor, TNF ligand, caspase and BCL2 families can be seen. The relative expression of these genes is displayed in **Table 2**.

Different cell types express different combinations of apoptotic proteins depending on conditions and stage of development. There are reported to be at least nineteen TNF proteins signalling through twenty-nine receptors [20-21]. Genes encoding several members of these families were detected in the present study. In all samples, TNFRSF1B (TNFR2) was apparent and had the highest intensity. The decoy receptor, TNFRSF10C (DcR1), a negative regulator of cell death, was expressed, although at relatively low levels, in some subjects. Death receptors TNFRSF10A (DR4) and TNFRSF5 were observed only occasionally and their intensities were very low. The ligand TNFSF9 was present in many samples, while TNFSF14 was observed in some cases but not in others.

An intriguing observation in almost all cases was the relatively high intensity of caspase 14, a recent addition to the family of aspartate proteases involved in the execution of the death programme; the precise function of this enzyme, however, remains unclear. [22]. Another gene expressed at a relatively high level was the anti-apoptotic regulator BCL2L1 (Bcl-x).

The relative proportions of the caspase 14 and BCL2L1 varied between individuals.
The expression of BCL2L1 was significantly lower in AD, independent of age [p=0.022, GLM Univariate analysis]. The ratio of [CASP14 - BCL2L1] / CASP14 was significantly higher in AD, independent of age [p=0.015, GLM Univariate analysis]. This suggests that the fine balance between survival and death may be disturbed in these AD patients and that BCL2L1 could be a key factor in this disease. Interestingly, of the thirteen genes of the Bcl-2 family tested, only BCL2L1 was expressed to any significant degree in our subjects. Similarly, of the twelve caspases, it was only caspase 14 that was seen to any measurable degree.

The expression of TNFSF14 (HVEM-L) was significantly higher in males, independent of the presence of AD [p=0.025, GLM Univariate analysis]. The importance of the increase in expression of TNFSF14 in males is unclear at this stage. The precise role of TNFSF14 is not yet well defined; it appears to induce apoptosis, presumably through a death receptor, but there is relatively little knowledge as to the way in which it is regulated [23].

We have demonstrated that apoptotic gene expression in blood can be analysed effectively using a microarray technique. The apoptotic functions of the proteins encoded by the genes expressed in our subjects are by no means fully understood, and whether they have a specific role in AD and in aging populations remains to be determined. Further studies with a separate cohort of older people and comparison with a younger group would be worthwhile. The change in BCL2L1 is particularly interesting and could prove to be of clinical significance. The possibility arises that altered expression of this gene may be a factor in the development of AD, the decrease being indicative of altered regulation and increased apoptosis; this needs further investigation. The increased expression of TNFSF14 in males, as compared with females, could be associated with increased apoptosis, and perhaps a lower life expectancy? Our findings need to be confirmed quantitatively and studies carried out to see whether the differences in BCL2L1 and TNFSF14 are reflected in the expression of the respective proteins.

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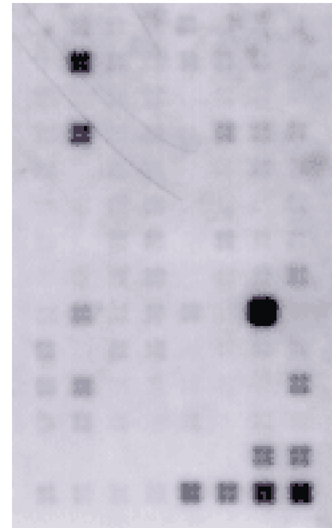
FIGURE 1
A representative example of a GEArray showing apoptotic gene expression.



There are eight columns and fourteen rows, designated C1 – C8 from left to right and R1 – R14 from top to bottom, respectively. The genes are identified as follows: C2R2 BCL2L1: C2R4 CASP14: C8R8 TNFRSF10A: C2R9 TNFRSF10C: C7R9 TNFRSF1B: C1R10 TNFRSF5: C2R11 TNFSF14: C8R11 TNFSF9. Positive controls: C7R13 and C8R13 GAPD, C5R14 and C6R14 RPL13A, C7R14 and C8R14 ACTB. Negative controls and blanks: C1 – C6R13

TABLE 1
Participant demographics

	Age Mean +/- sd	n
All		
Male	74.4 +/- 8.9	11
Female	73.0 +/- 7.0	9
Alzheimer Disease		
Male	84.8 +/- 5.4	4
Female	75.3 +/- 10.2	4
Aged Controls		
Male	68.4 +/- 2.0	7
Female	71.2 +/- 3.4	5

TABLE 2. Apoptotic gene expression

	TNFRSF1B		BCL2L1		CASP14		TNFSF9		TNFRSF10C		TNFSF14	
	[TNF receptor family TNFR2]		[BCL2 family bcl-x]		[Caspase family]		[TNF ligand family]		[TNF receptor family DcR1]		[TNF ligand family HVEM-L]	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
AD*	6	All 6	0	0 -3	4	3 - 5	2	0 - 3	0	0 - 3	0	0 - 2
Control	6	All 6	3	0 -4	4	0 - 5	1.5	0 - 4	0	0 - 3	0	0 - 2
Male	6	All 6	1	0 -4	4	0 - 5	3	0 - 3	0	0 - 3	0	0 - 2
Female	6	All 6	2	0 -4	4	3 - 5	1	0 - 4	0	0 - 3	0	All 0

Recorded as intensity, relative to controls, on an arbitrary scale of 0 (not detected) to 6 (highest intensity observed)
*AD: Alzheimer disease.