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Journal of the Neurological Sciences 267 (2008) 120–124

Journal of the Neurological Sciences

www.elsevier.com/locate/jns

Oxidative-stress-induced apoptosis in PBLs of two patients with Parkinson disease secondary to alpha-synuclein mutation

Carla Battisti, Patrizia Formichi, Elena Radi, Antonio Federico *

Department of Neurological and Behavioural Sciences, O.U. of Neurometabolic Disease, University of Siena, Siena, Italy

Received 5 July 2007; received in revised form 26 September 2007; accepted 4 October 2007
Available online 3 December 2007

Abstract

Alpha-synuclein has been implicated in the pathology of certain neurodegenerative diseases, including Parkinson disease (PD). Although the precise physiological and pathological role of alpha-synuclein is unclear, overexpression of the protein or its mutants may reduce cell viability. In this study we evaluated the apoptotic response to oxidative stress induced by 2-deoxy-D-ribose (dRib) in peripheral blood lymphocytes (PBLs) of two siblings with Parkinson disease secondary to A53T alpha-synuclein mutation. PBLs exposed to oxidative stress showed a higher percentage of apoptotic cells in PD patients than in controls. However in cells of PD patients, the increase of apoptotic response was lower than in controls, suggesting that cells of PD patients have greater "resistance" to oxidative stress. We conclude that other environmental agents could play a key role in inducing programmed cell death in cells of PD patients with mutant alpha-synuclein.

Keywords: Alpha-synuclein; Parkinson; Apoptosis; Oxidative stress; dRib; Flow cytometry

1. Introduction

Cells including neurons may respond adaptively to oxidative stress, activating a programmed cell death pathway called apoptosis. Synucleinopathies are a group of neurodegenerative proteinopathies characterized by formation of aggregates that cause cytotoxicity in selected populations of neurons and glia [1]. Alpha-synuclein, a synaptic member of the synuclein protein family, is a major component of neuronal cytoplasmic protein aggregates known as Levy bodies (LBs), characteristic of several neurodegenerative diseases, including idiopathic and familial forms of Parkinson disease (PD) [2]. In spite of extensive studies, the exact function of alpha-synuclein is still unclear as well as the etiological mechanism by which over-expression of this protein or its mutant forms causes selective dopaminergic neuronal death in PD.

Studies on the pathogenic effects of alpha-synuclein, overexpressed, mutant or wild-type (*wt*), support a role of this protein in apoptotic process [3]; studies on brain tissues from PD patients provide evidence that apoptosis of neurons occurs in this disorder [4]. Several studies agree that *wt* synuclein play a physiological role in neuroprotection and/or as an anti-apoptotic molecule [5]. This role may be partly lost in A30P and A53T mutants, or when *wt* synuclein is

Table 1
Clinical pattern of the two siblings belonging to Contursi kindred

	Sex	Age	Genetic pattern	Age of onset (year)	Phenotypic pattern	Response to levodopa
S.A.	M	44	Alpha-synuclein mutation (A53T)	29	Bradykynesia, postural instability, rigidity-dementia	Positive
S.G.	M	33	Alpha-synuclein mutation (A53T)	25	Rigidity, bradykynesia, postural instability	Positive

* Corresponding author. Dipartimento di Scienze Neurologiche e del Comportamento, U.O. Malattie Neurometaboliche, Policlinico "Le Scotte", V.le Bracci, 2, 53100 Siena, Italy.
E-mail address: federico@unisi.it (A. Federico).

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doi:10.1016/j.jns.2007.10.012

overexpressed [6]. On the other hand, experimental data from studies with the yeast *Saccharomyces cerevisiae* suggest that *wt* and mutant forms of the protein play a proapoptotic role [7].

Here we evaluated oxidative-stress-induced apoptosis in peripheral blood lymphocytes (PBLs) of two patients with PD of the Contursi kindred with mutation in the alpha-synuclein gene.

2. Materials and methods

2.1. Patients

We analysed PBLs from two siblings with PD carrying the A53T missense mutation in the alpha-synuclein gene, and five healthy sex and age matched controls. Clinical parameters of the patients are shown in Table 1.

2.2. Cell culture, treatment and apoptosis analysis

PBLs from patients and controls were cultured according to Battisti et al. [8]. PBLs were harvested after 1, 24, 48 and 72 h of culture and analysed by flow cytometry [9] and 2% agarose gel electrophoresis [8]. Cells collected at each time point were also seeded on microscope slides and analysed for alterations of mitochondrial membrane potential by JC1 [10], phosphatidylserine (PS) plasma membrane translocation with Annexin V [11] and 3- and 7-caspase activation with Carboxyfluorescein FLICA Apoptosis Detection Kit Caspase Assay" (Immunochemistry Technologies, LLC). Untreated cells, collected at the same times, served as control.

Cytofluorimetric analysis was performed only after 1, 24 and 48 h of incubation with dRib; after 72 h of incubation, cells loss was so high that it was impossible to obtain significant results.

3. Results

3.1. Flow cytometry

Flow cytometric analysis showed the DNA content in the sub-G1 region of PBLs from controls and PD patients at different times of incubation with dRib. PBLs cultured without dRib showed a higher percentage of apoptotic cells in patients than in controls and the difference between the two groups was statistically significant at each time point (Fig. 1a, b). After 24 and 48 h of culture with dRib the percentage of apoptotic cells in both groups was much higher than in untreated cells ($p<0.05$) (Fig. 1c). However the percentage of apoptotic cells was much higher in PBLs from PD patients than controls and the difference between the two groups was statistically significant at each time point ($p<0.05$). Moreover, after 24 and 48 h of culture the number of apoptotic cells in patients was nearly twice than that of controls (Fig. 1c).

3.2. Agarose gel electrophoresis

Fig. 1d, e shows DNA agarose gel electrophoresis of PBLs from controls (Fig. 1d) and PD patients (Fig. 1e). Under basal conditions, PBLs from controls showed DNA fragmentation (smearing) but not ladder pattern (Fig. 1d), whereas PBLs from PD patients showed a weak ladder pattern representing apoptotic cell death after 72 h of culture (Fig. 1e, lane 4). PBLs from controls incubated with dRib for 1, 24 and 48 h showed DNA fragmentation (Fig. 1d, lane 5, 6 and 7) and a weak ladder pattern was detected after 72 h of incubation (Fig. 1d, lane 8). In PBLs from patients, the ladder pattern was detectable even after 24 h of incubation with dRib (Fig. 1e, lane 6) and reached the maximum intensity after 48 h (Fig. 1e, lane 7).

3.3. JC1

After 1 h of incubation with dRib, PBLs from controls and PD patients showed many intact, brightly-stained mitochondria. After 24, 48 and 72 h of culture with dRib, PBLs of both groups showed an increase in fluorescent green mitochondria, reflecting a fall in $\Delta\Psi m$. PBLs from patients (Fig. 1f) showed more evident green fluorescence than PBLs from controls (Fig. 1g), demonstrating a higher degree of mitochondrial membrane depolarization. PBLs from patients also showed a higher degree of mitochondrial depolarization under basal condition (data not show).

3.4. Annexin V

After 1 h of incubation with dRib, PBLs from controls and PD patients, double stained with AnnVCy3 and 6-CFDA showed the typical pattern of living cells: intense green fluorescence and no red fluorescence. After 24, 48 and 72 h of incubation with dRib, PBLs of both groups showed less green fluorescence and many red fluorescent cells (double-stained cells were apoptotic). Apoptotic PBLs were more numerous in PD patients (Fig. 1h) than in controls (Fig. 1i).

3.5. FLICA

After 1 h of incubation with dRib, PBLs from controls and PD patients lacked caspase-3 and 7 activity as demonstrated by the absence of green fluorescence. In both group an evident and progressive increase in caspase-3 and 7 activity was found after 24, 48 and 72 h of incubation with dRib. Apoptotic cells were more numerous in PD samples (Fig. 1l) than in controls (Fig. 1m).

4. Discussion

The first locus for autosomal dominant inherited PD was mapped to chromosome 4q21–q23 in the Contursi kindred in 1996 [12]; 1 year later, a missense mutation leading to an amino acid substitution (Ala53Thr) in the alpha-synuclein