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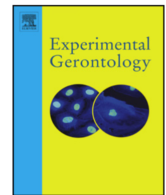
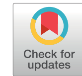


Regional vulnerability to lipoxidative damage and inflammation in normal human brain aging



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ABSTRACT

Oxidative damage and inflammation coexist in healthy human brain aging. The present study analyzes levels of protein adduction by lipid peroxidation (LP) end-products neuroketal (NKT) and malondialdehyde (MDA), as markers of protein oxidative damage, cycloxygenase-2 (COX-2) levels, as a marker of inflammation, and cytochrome P450 2J2 (CYP2J2), responsible of generation of neuroprotective products, in twelve brain regions in normal middle-aged individuals (MA) and old-aged (OA) individuals. In addition, levels of these markers were evaluated as a function of age as a continuous variable and correction for multiple comparisons. Selection of regions was based on their different vulnerability to prevalent neurodegenerative diseases in aging. Our findings show region-dependent LP end-products, COX-2 and CYP2J2 changes in the aging human brain. However, no clear relationship can be established between NKT, MDA, COX-2 and CYP2J2 levels, and regional vulnerability to neurodegeneration in old age.

1. Introduction

Brain aging, particularly in humans, is characterized by structural changes including gray and white matter volume reductions, modifications in the number and types of neurons and glial cells, altered synapses with apparently specific patterns of vulnerability (Hof and Morrison, 2004; Walhovd et al., 2005; Mattson and Magnus, 2006; Long et al., 2012).

Several processes are implicated in brain aging including oxidative stress and neuroinflammation (Kirkwood, 2005; Esiri, 2007). The imbalance between increased reactive oxygen species (ROS) production and decreased antioxidant response mechanisms can cause oxidative damage by interacting with macromolecules (Smith et al., 1991; Agarwal and Sohal, 1994; Sastre et al., 2000; Floyd and Hensley, 2002; Hagen, 2003; Poon et al., 2004; Barja, 2004; Mariani et al., 2005; Dröge and Schipper, 2007; Nunomura et al., 2012). Lipid peroxidation (LP) is a common modification caused by ROS which may occur non-enzymatically or enzymatically through lipid mediators (Massey and Nicolaou, 2013). As a result, a variety of reactive aldehydes are produced which are important source of secondary ROS-mediated injury by covalently binding to proteins altering their structure and function (Jové et al., 2014). In the human brain, cell membranes present a high concentration of poly-unsaturated fatty acids (PUFA) susceptible to LP which increases with the number of double bonds (O'Brien and Sampson, 1965; Naudí et al., 2015). Among them, docosahexaenoic acid (DHA; C22:6 ω3) and arachidonic acid (AA; C20:4 ω6) are highly represented with particular regional distributions (Naudí et al., 2012). LP of these PUFA produces toxic and reactive end-products such as neuroketals (NKT) derived from DHA (Bernoud-Hubac et al., 2001) and malondialdehyde (MDA) and 4-hydroxynonenal (HNE) derived from AA (Romero et al., 1998). LP end-products increase in the brain with aging (Calabrese et al., 2010; Domínguez et al., 2016).

On the other hand, low levels of neuroinflammation occur during brain aging (Yankner et al., 2008; Godbout and Johnson, 2009; Lucin and Wyss-Coray, 2009; Lynch et al., 2010; Chung et al., 2011; Pizza et al., 2011; Michaud et al., 2013; Matt and Johnson, 2016). It has been suggested that oxidative damage and neuroinflammation are bidirectional intertwined processes. This idea is supported by findings linking oxidized lipid by-products and chronic neuroinflammation (Barger et al., 2007; Kim et al., 2012). Inflammation and arachidonic acid

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cascade are linked in the human frontal cortex in aging (Keleshian et al., 2013).

Among the several signaling molecules and enzymatic pathways involved in neuroinflammation, cyclooxygenases (COX) or prostaglandin endoperoxide synthases, which catalyze the formation of prostanoids, prostaglandins, prostacyclin and thromboxanes, are prevalent (Minghetti, 2004). COX-2 can be rapidly induced by several stimuli such as growth factors, low-density lipoproteins cytokines and pro-inflammatory molecules (Smith et al., 1996; Minghetti et al., 1997; Uchida, 2008). COX-2 expression increases in aged astrocytes (Bellaver et al., 2017). Several lines of evidence suggest that COX-2 has detrimental effects as over-expression of COX-2 is associated with neurodegenerative diseases in aging (Minghetti, 2004; Meraz-Ríos et al., 2013).

CYP2J2 cytochrome P450 family 2 subfamily J member 2 (also named cytochrome P450 epoxygenase), is a monooxygenase which catalyzes many reactions involved in the synthesis of lipids and cholesterol and has epoxygenase activity on particular polyunsaturated fatty acids from series n-3 and n-6 leading to the generation of neuroprotective products based on their anti-inflammatory and anti-apoptotic properties (Xu et al., 2013).

The present study was designed to learn about regional differences in the generation of LP-end-products as revealed by NKT and MDA protein adducts in combination with COX2 and CYP2J2 expression levels in twelve brain regions in two groups of individuals. Assessed regions, including cerebral cortex (frontal area 8, parietal area 7, inferior temporal area 20, occipital areas 17–18, cingulate area 24, entorhinal cortex and hippocampus), striatum (caudate and putamen), thalamus, substantia nigra and upper vermis, were selected on the basis of their variable vulnerability to neurodegenerative diseases in aging. Middle-aged individuals had no clinical and neuropathological alterations, whereas old-aged individuals had no clinical symptoms and neuropathological alteration restricted to stage I-II of neurofibrillary degeneration. Since the majority of human beings aged 65 years have stages I-II of neurofibrillary tangle pathology (Braak et al., 2011; Ferrer, 2012), the old-aged group was considered representative of normal brain aging.

2. Materials and methods

2.1. Human samples

Brain tissue was obtained from the Institute of Neuropathology Brain Bank (HUB-ICO-IDIBELL Biobank) following the Spanish legislation and the approval of the local ethics committee.

One hemisphere was immediately cut in coronal sections, 0.5 cm thick, and selected areas of the encephalon were rapidly dissected, frozen on metal plates over dry ice, placed in individual air-tight plastic bags, numbered with water-resistant ink and stored at −80 °C. The other hemisphere was fixed by immersion in 4% buffered formalin for 3 weeks for morphological studies. Procedures were carried out to preserve post-mortem material under optimal conditions for morphological and biochemical studies (Ferrer et al., 2008). Post-mortem delay was between 2 h and 14 h 40 min (6:15 ± 2:48).

Neuropathological examination was carried out on de-waxed paraffin sections, 4-μm-thick, from twenty selected regions which were stained with haematoxylin and eosin, Klüver-Barrera and processed for immunohistochemistry to phosphorylated tau, β-amyloid, α-synuclein, TDP-43, ubiquitin, glial fibrillary acidic protein, IBA-1 and CD68.

For biochemical studies, twelve regions were used in the present study: frontal cortex area 8 (FC), parietal cortex area 7 (PC), inferior temporal cortex area 20 (TC), occipital cortex 17–18 (VC), cingulate gyrus area 24 (CG), entorhinal cortex (EC), hippocampus (H), head of the caudate (CN), anterior putamen (P), thalamus (T), substantia nigra (SN) and upper vermis (V). Samples were from individuals with no neurological symptoms and without focal and systemic infectious, inflammatory and autoimmune diseases. Cases with metabolic syndrome, disseminated malignant diseases and drug abuse, most particularly excessive ethanol consumption, were not included. Special care was also taken to not include cases with prolonged agonal state (patients subjected to intensive care or experiencing hypoxia). After neuropathological examination, cases with neurodegenerative and vascular diseases were excluded excepting those with stages I-II of neurofibrillary tangle pathology. Cases with associated neurodegenerative processes (i.e. α-synucleinopathy, argyrophilic grain pathology, TDP-43 proteinopathy) were excluded. Only cases with minor changes consistent with small blood vessel disease were acceptable in the present study.

Table 1
Summary of cases.

Case	Age	Group	Gender	P. delay	Neuropathology	NA
1	40	MA	W	8 h 45 m	NL	
2	40	MA	M	5 h 10 m	NL	
3	44	MA	M	6 h 40 m	NL	SN
4	45	MA	M	4 h 5 m	CRIB	
5	46	MA	W	7 h 15 m	CRIB	
6	48	MA	W	4 h 5 m	NL	EC
7	52	MA	M	9 h 30 m	NL	
8	52	MA	M	4 h 40 m	NL	EC
9	57	MA	M	5 h 20 m	NL	
10	61	OA	M	4 h 30 m	I–II	SN
11	65	OA	M	3 h 15 m	I–II	TC
12	66	OA	M	6 h 25 m	I–II	
13	67	OA	M	14 h 40 m	I–II	EC, SN
14	70	OA	M	2 h	I–II	SN
15	75	OA	W	6 h 10 m	I–II + CRIB	
16	76	OA	M	6 h 30 m	I–II	
17	77	OA	M	7 h	I–II + CRIB	
18	79	OA	W	6 h 25 m	I–II + CRIB	

MA: middle-aged; OA: old-aged; W: woman; M: man; P. delay: post-mortem delay; NL: no lesions; CRIB: status cribrosus; I–II neurofibrillary tangle pathology stage I–II of Braak and Braak; NA: not available; SN substantia nigra, EC: entorhinal cortex; TC: temporal cortex.

Middle-aged subjects (n = 9; 6 men, 3 women) were 47.1 ± 5.7 years and they had no clinical and neuropathological lesions including first stages of neurofibrillary tangle (NFT) pathology (Braak and Braak, 1991). Old-aged individuals (n = 9; 7 men, 2 women) were 70.6 ± 6.3 years and they had no clinical symptoms and scored stage I–II of NFT pathology at post-mortem examination. β-Amyloid deposition was absent in every case. A summary of cases and regions analyzed is shown in Table 1.

2.2. Sample preparation and Western-blot analyses

Frozen tissue from every region was homogenized as previously described (Domínguez et al., 2016). Protein concentration was determined by Bradford assay with bovine serum albumin (Sigma-Aldrich) as standard. For every region, equal amount of total protein (10 μg) in Laemmli buffer was loaded onto 10% SDS-polyacrylamide gels and electrophoresed in Tris-glycine running buffer. Proteins were transferred into nitrocellulose membranes using the Trans-Blot® Turbo™ blotting system (Bio-Rad). Membranes were blocked with 5% skimmed milk in TBS-T buffer (100 mM Tris-HCl, 140 mM NaCl and 0.1% Tween 20, pH 7.4) at room temperature for 1 h and incubated at 4 °C overnight with one of the primary antibodies diluted in 3% bovine serum albumin in TBS-T. Primary antibodies used were anti-β-actin (Sigma-Aldrich, reference A5316), anti-COX-2 (Cayman, reference 160,126), anti-MDA (AcademyBIOM, reference MD20a-R1a), anti-CYP2J2 (Abcam, reference ab139160) and anti-NKT (Merck Millipore, reference ab5611). Following incubation with the primary antibodies, the membranes were washed for 10 min three times in TBS-T, and later incubated at room temperature for 1 with the appropriate secondary

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