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Region-specific vulnerability to lipid peroxidation and evidence of neuronal mechanisms for polyunsaturated fatty acid biosynthesis in the healthy adult human central nervous system

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ABSTRACT

Lipids played a determinant role in the evolution of the brain. It is postulated that the morphological and functional diversity among neural cells of the human central nervous system (CNS) is projected and achieved through the expression of particular lipid profiles. The present study was designed to evaluate the differential vulnerability to oxidative stress mediated by lipids through a cross-regional comparative approach. To this end, we compared 12 different regions of CNS of healthy adult subjects, and the fatty acid profile and vulnerability to lipid peroxidation, were determined by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS), respectively. In addition, different components involved in PUFA biosynthesis, as well as adaptive defense mechanisms against lipid peroxidation, were also measured by western blot and immunohistochemistry, respectively. We found that: i) four fatty acids (18.1n-9, 22:6n-3, 20:1n-9, and 18:0) are significant discriminators among CNS regions; ii) these differential fatty acid profiles generate a differential selective neural vulnerability (expressed by the peroxidizability index); iii) the cross-regional differences for the fatty acid profiles follow a caudal-cranial gradient which is directly related to changes in the biosynthesis pathways which can be ascribed to neuronal cells; and iv) the higher the peroxidizability index for a given human brain region, the lower concentration of the protein damage markers, likely supported by the presence of adaptive antioxidant mechanisms. In conclusion, our results suggest that there is a region-specific vulnerability to lipid peroxidation and offer evidence of neuronal mechanisms for polyunsaturated fatty acid biosynthesis in the human central nervous system.

1. Introduction

The human nervous system makes a broad range of motor, sensory, regulatory, behavioral, and cognitive functions which are supported by a complex organization of groups of diverse neural cell populations. From this setting of cellular diversity there emerges the concept of selective neuronal vulnerability (SNV). SNV can be defined as the differential sensitivity of neural cell populations (and particularly neurons) to stresses that originate cell dysfunction or death leading, in this last case, to neurodegenerative processes [1–3].

One of these cell-damaging processes is oxidative stress [3]. Oxidative stress is a basic mechanism in the aging process of the nervous system [3,4], whilst excessive oxidation-derived molecular damage is considered as an etiopathogenic or physiopathological mechanism for neurodegeneration [5]. Oxidative stress, the result of a homeostatic imbalance between generation of free radicals and the adaptive antioxidant mechanisms evolved against these highly reactive species, is detrimental to cells because reactive species chemically damage all cell components [6]. So it is relevant to determine the vulnerability of

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the different neural populations in terms of susceptibility to oxidative stress in physiological conditions in order to extend our understanding of how this particular form of cell vulnerability causes selective neuronal dysfunction or loss in the nervous system, and to reveal potential molecular and cellular mechanisms that bring about relative resistance or sensitivity of neurons to stresses.

The natural history of lipids is closely related with the evolution of life and multicellularity. As a result, all living organisms have lipid membranes [7]. Neural cells (neurons and glial cells) are no exception. Indeed, lipids stimulated and favored the evolution of the brain [8,9]. Among the tissues of the human body, nervous tissue is the richest in lipid content (with the permission of adipose tissue). It is well-established that lipids represent about 10–12% of the fresh weight and half the dry matter of the brain [10]. Apart from their quantitative importance, the lipids of the nervous system show a wide structural and functional diversity [11]. All lipid classes are represented in neural cells, and the wide spectra of lipid molecular species is an expression of the different needs and functions ascribed to them. In addition, the demonstration that lipids have broad information-carrying roles in the nervous system has stirred renewed interest in this particular cell component. In this context, it is postulated that the morphological and functional diversity among neural cells is also projected and achieved through the expression of particular lipid profiles.

Fatty acids are prime components in the structural diversity of lipids that play a key role in determining functional properties of lipids in brain, including the structural and functional integrity of the neural cell membranes, the generation of lipid mediators, and the chemical reactivity of the acyl chains [11]. The chemical reactivity of the fatty acids that compose the membrane determines their susceptibility to oxidative damage. So, PUFA side chains (with two or more double bonds) are much more easily attacked by reactive species than saturated fatty acid (SFA, no double bonds) or monounsaturated fatty acid (MUFA, one double bond) side chains [12–17]. Consequently, neural cell membrane lipids become targets of oxidative stress with the subsequent activation of the lipid peroxidation process.

Lipid peroxidation generates reactive intermediates called reactive carbonyl species (RCS) of 3 to 9 carbon atoms in length [15,18]. Malondialdehyde (MDA) and glyoxal are significant aldehydic products of lipid peroxidation of PUFAs, among others, detected, characterized, and located in the human brain. Carbonyl compounds exhibit a wide range of molecular and biological effects, ranging from cellular component damage to signalling pathway activation and/or alteration. Thus, these compounds have specific physiological signaling roles inducing adaptive responses to decrease oxidative damage through the activation of antioxidant defenses [15,19]. One of these mechanisms is the activation of the antioxidant response signaling Nrf2 pathway that includes the expression of antioxidants and detoxifying enzymes specifically designed to detoxify RCS [20,21]). By contrast, RCS also react with nucleophilic groups in macromolecules (lipoxidation reactions) like proteins [22], DNA [23], and aminophospholipids [24], resulting in their chemical, and irreversible modification, and the formation of a variety of adducts and crosslinks collectively named Advanced Lipoxidation Endproducts (ALEs) [22,25]. The physico-chemical and biological effects resulting from ALE formation include protein changes in physico-chemical properties (spatial conformation, charge, hydrophobicity, and solubility), formation of intra- and inter-molecular protein crosslinks and aggregates, loss of enzymatic activity, and DNA damage and mutagenesis, among several others [11,15,25].

In the present work, we proposed to evaluate the differential vulnerability to oxidative stress mediated by lipids through a cross-regional comparative approach. To this end, we compared 12 different regions of the central nervous system (CNS) of healthy adult subjects, and the fatty acid profile, vulnerability to lipid peroxidation, and concentration of lipoxidation-derived protein damage were measured. In addition, different components involved in PUFA biosynthesis pathways, as well as adaptive defense mechanisms against lipid peroxidation, were also measured. Our results demonstrate that there is a region-specific vulnerability to lipid peroxidation and offer evidence of neuronal mechanisms for polyunsaturated fatty acid biosynthesis in the human CNS.

2. Methods and materials

2.1. Chemicals

Unless otherwise specified, all reagents were from Sigma-Aldrich, and of the highest purity available.

2.2. Human samples

Brain samples were obtained from the Institute of Neuropathology Brain Bank following the guidelines of the local ethics committee, and in accordance with recently published criteria of sample quality [26]. The selection of cases examined in the present study corresponded to a consecutive series of donations having in common (i) lack of neurological symptoms and signs, (ii) lack of known hepatic or renal function impairment, and (iii) lack of evidence of prolonged agonal state. The brains of healthy adult subjects were obtained from 3 to 17 h after death, and were immediately prepared for morphological and biochemical studies (see Table S1).

At autopsy, one hemisphere was fixed in 4% buffered formalin for about three weeks while the other hemisphere was cut into coronal Sections 1 cm thick; selected samples of the brain were dissected and kept in labelled plastic bags, immediately frozen on dry ice, and stored at −80 °C until use. The neuropathological study was carried out on formalin-fixed, paraffin-embedded samples of the frontal, primary motor, primary sensory, parietal, temporal superior, temporal inferior, anterior cingulated, anterior insular, and primary and associative visual cortices; entorhinal cortex and hippocampus; caudate, putamen and globus pallidus; medial and posterior thalamus; subthalamus; Meynert nucleus; amygdala; midbrain (two levels), pons and medulla oblongata; and cerebellar cortex and dentate nucleus. De-waxed sections, 5 μm thick, were stained with haematoxylin and eosin, and Klüver Barrera, or processed for immunohistochemistry to β-amyloid, phosphorylated tau, α-synuclein, ubiquitin, p62, TDP43, glial fibrillary protein, and microglia markers.

Selected cases did not show lesions on neuropathological examination including any kind of β-amyloid, tau, hypoxic, or vascular pathology. Following initial screening, the present series included 28 cases: 19 men and 9 women, with an age ranging from 43 to 68 years (Table S1). The grey matter of frozen samples from spinal cord (n = 5), medulla oblongata (n = 6), cerebellum (n = 6), substantia nigra (n = 7), thalamus (n = 7), amygdala (n = 6), striatum (n = 7), entorhinal cortex (n = 8), hippocampus (n = 5), temporal cortex (n = 6), occipital cortex (n = 6), and frontal cortex (n = 8) were dissected and used for biochemical analysis.

For biochemical studies, 50–100 mg of tissue samples were homogenized in a buffer containing 180 mM KCl, 5 mM MOPS, 2 mM EDTA, 1 mM diethylenetriaminepentaacetic acid, and 1 μM butylated hydroxytoluene, pH 7.4, with a Ultra-Turrax (3,420,000 IKA, Germany) device, at 4 °C. For western blot, a cocktail of both protease (80-6501-23 Amersham Biosciences, Madrid, Spain) and phosphatase (1 mM sodium orthovanadate and 1 mM sodium fluoride) inhibitors were added to the previous buffer. After a brief centrifugation (500 × g, at 4 °C for 1 min) to pellet cellular debris, protein concentrations were measured in the supernatants using the Bradford assay [27]. Before analyses, samples were randomized.

2.3. Fatty acid profile and global fatty acid indexes

Total lipids from human brain region homogenates were extracted with chloroform/methanol (2:1, v/v) [28] in the presence of 0.01% butylated hydroxytoluene, and containing 1,2-dinonadecanoyl-sn-