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An aluminum-based rat model for Alzheimer's disease exhibits oxidative damage, inhibition of PP2A activity, hyperphosphorylated tau, and granulovacuolar degeneration

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Abstract

In Alzheimer's disease (AD), oxidative damage leads to the formation of amyloid plaques while low PP2A activity results in hyperphosphorylated tau that polymerizes to form neurofibrillary tangles. We probed these early events, using brain tissue from a rat model for AD that develops memory deterioration and AD-like behaviors in old age after chronically ingesting 1.6 mg aluminum/kg bodyweight/day, equivalent to the high end of the human dietary aluminum range. A control group consumed 0.4 mg aluminum/kg/day. We stained brain sections from the cognitively-damaged rats for evidence of amyloid plaques, neurofibrillary tangles, aluminum, oxidative damage, and hyperphosphorylated tau. PP2A activity levels measured 238.71 ± 17.56 pmol P_i/μg protein and 580.67 ± 111.70 pmol P_i/μg protein ($p < 0.05$) in neocortical/limbic homogenates prepared from cognitively-damaged and control rat brains, respectively. Thus, PP2A activity in cognitively-damaged brains was 41% of control value. Staining results showed: (1) aluminum-loading occurs in some aged rat neurons as in some aged human neurons; (2) aluminum-loading in rat neurons is accompanied by oxidative damage, hyperphosphorylated tau, neuropil threads, and granulovacuolar degeneration; and (3) amyloid plaques and neurofibrillary tangles were absent from all rat brain sections examined. Known species difference can reasonably explain why plaques and tangles are unable to form in brains of genetically-normal rats despite developing the same pathological changes that lead to their formation in human brain. As neuronal aluminum can account for early stages of plaque and tangle formation in an animal model for AD, neuronal aluminum could also initiate plaque and tangle formation in humans with AD.

Keywords: Aluminum; Alzheimer's disease; PP2A; Oxidative damage; Hyperphosphorylated tau; Animal model; Amyloid; Neurofibrillary tangles; Granulovacuolar degeneration

1. Introduction

Studies of Alzheimer's disease (AD) incidence in identical twins have shown that AD causality is best explained as an interaction between environmental and genetic factors (e.g., [1]). Amyloid plaques and neurofibrillary tangles are the two most widely recognized hallmarks of AD and molecular events pertaining to their formation have been under intensive study for over a decade. Despite considerable progress, insufficient attention has been directed towards identifying the environmental factor(s) and susceptibility genes that cause these formative events in plaque and tangle formation in the majority of Alzheimer patients. For example, oxidative stress is now known to precede and lead to the appearance of β-amyloid deposits in brains of humans with sporadic AD [2] and in brain tissue of a transgenic mouse model for AD amyloidogenesis [3]; however, the cause of this oxidative damage is unknown. In order to understand AD etiology, it will be necessary to identify the prime source(s) of oxidative stress in AD.

Similarly, hyperphosphorylation of tau precedes and leads to the formation of neurofibrillary tangles (NFTs)

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in AD brains [4,5]. Tau is a microtubule-associated protein that requires phosphate for its function. However, if tau is hyperphosphorylated, it loses its biological activity [6]. Protein phosphatase 2A (PP2A), a serine/threonine protein phosphatase, is the major phosphate-removing enzyme in the brain active against tau and neurofilament hyperphosphorylation and, hence, against tau accumulation and NFT formation [7–10]. In AD, both the activity of PP2A [11] and its expression [12] are reduced in memory-processing regions of the brain. Consequently, tau can become abnormally phosphorylated at more than 30 of its serine and threonine phosphorylation sites [13]. Although low PP2A activity is regarded as the main reason for tau hyperphosphorylation, the cause of low PP2A activity in the AD brain remains unknown.

Aluminum, a neurotoxin that occurs in human brain [14–17], has been associated with AD causality for decades. The present study is based on an aluminum-based animal model for AD that exhibits progressive memory deterioration and AD-like behaviors in old age [18]. The cognitively-damaged rats in this study had consumed aluminum throughout their middle age and old age in amounts equivalent to the high end of the human range for dietary aluminum. Here we examined whether their brains do in fact develop pathological changes that lead in human brains to the formation of plaques and tangles. These early AD changes are: oxidative damage, inhibited PP2A activity and hyperphosphorylated tau.

2. Materials and methods

2.1. Animals, tissue preparation, and microscopy

The protocol for the aluminum-based rat model for AD is described elsewhere [18] and for replication of this experiment that reference should be consulted. Briefly, ethics approvals for the protocol were given by the Animal Care and Ethics Committee of the Commonwealth Scientific and Industrial Research Organisation (Prospect, NSW, Australia). The brain tissue used in the current study was obtained from two groups of memory-trained male Wistar rats that consumed measured amounts of aluminum from 6 months of age until their death in old age. From age 12 months onwards, the lower aluminum dose group ingested 0.4 mg/kg bodyweight/day and the higher aluminum dose group ingested 1.6 mg/kg bw/day. The latter value is equivalent in amount to the high end of the dietary aluminum range consumed by humans living in contemporary urban society [19] whereas the former is in the low-mid part of the range. The basal diet was a low protein/low fat maintenance diet (Gordon Stockfeeds, Young, Australia) measured to contain 9 mg aluminum/kg. The low aluminum group drank purified water without added aluminum whereas the water drunk by the other group contained 20 mg aluminum/L in the form of aluminum chloride. Ingestion of this species allows aluminum increase with minimal perturbation to the gastric fluid composition.

From 6 months of age, the rats were trained in a continuous rewarded alternation memory task that is hippocampus-dependent [20] and tested almost weekly for the remainder of their lives. At around 26 months of age, a substantial proportion of the rats that chronically consumed the higher aluminum level developed statistically-significant decline in memory scores and other behavioural changes associated with dementia as in the smaller pilot study [18]. The full behavioural results for these groups will be reported elsewhere. Otherwise, the rats exhibited good physical health and their longevity averaged 30 months as in the pilot study.

All rats were euthanized upon developing their terminal condition and their brains were rapidly removed. One hemisphere was fixed in 10% buffered formalin for paraffin-embedding and the other was snap-frozen. Paraffin-embedded hemi-brains from 8 cognitively-damaged rats and 8 cognitively-intact rats were sectioned at 10 μm for histological staining, and at 6 μm for immunostaining. Some rat brain sections were stained by the Pathology Department, Sydney University (NSW, Australia) with Bennhold's Congo red method [20] to disclose fibrillar β-amyloid under polarized light, if present, and silver-stained with Bodian's method [21] to reveal NFTs. Other sections were processed either with hematoxylin and eosin, a modified stain for aluminum or immunostained as described below. In order to learn whether aluminum-loading could be associated with oxidative damage or hyperphosphorylated tau, groups of neurons in the aluminum-stained rat brain sections were examined to determine their stage of aluminum-loading. Then, comparable neurons were identified in the same regions of adjacent sections immunostained with the HNE or PHF-1 antibody markers for oxidative damage and hyperphosphorylated tau, respectively. Microscopy and photography were performed with a Leica DMM LB 30 research microscope with Kohler illumination (Leica Instruments, Wetzlar, Germany).

2.2. Modified Walton stain for aluminum

In aged neural tissue processed with the Walton histological stain, aluminum is readily distinguishable in neurons and glia, appearing from purple to magenta with bright field microscopy and from yellow to gold under fluorescence. As originally described, the Walton stain for aluminum incorporated hematoxylin plus its associated acid alcohol and bluing solution steps to counterstain nuclei [22]. We have since simplified this procedure and brightened the stain. The hematoxylin steps have been replaced with immersion in Einarson's reagent [23] which is prepared by dissolving 5 g chromium potassium sulphate and 0.15 g gallocyanin (C.I. No. 51030, Sigma–Aldrich Corp., St. Louis, MO) in 100 ml distilled water, slowly heating the solution to the boiling point, boiling it for 15–20′ with frequent stirring, and then slowly cooling to room temperature and filtering before use. Other components of the Walton staining method are merely diluted