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Research Report

An okadaic acid-induced model of tauopathy and cognitive deficiency

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disease that causes cognitive and behavioral deterioration in the elderly. Neurofibrillary tangles (NFTs) are one of the pathological hallmarks of AD that has been shown to correlate positively with the severity of dementia in the neocortex of AD patients. In an attempt to characterize an *in vivo* AD tauopathy model, okadaic acid (OA), a protein phosphatase inhibitor, was microinfused into the right lateral dorsal hippocampus area of ovariectomized adult rat. Cognitive deficiency was seen in OA-treated rats without a change in motor function. Both silver staining and immunohistochemistry staining revealed that OA treatment induces NFTs-like conformational changes in both the cortex and hippocampus. Phosphorylated tau as well as cyclin-dependent kinase 5 (cdk5) and its coactivator, p25, were significantly increased in these regions of the brain. Oxidative stress was also increased with OA treatment as measured by protein carbonylation and lipid peroxidation. These data suggest that the unilateral microinfusion of OA into the dorsal hippocampus causes cognitive deficiency, NFTs-like pathological changes, and oxidative stress as seen in AD pathology via tau hyperphosphorylation caused by inhibition of protein phosphatases.

1. Introduction

Neurofibrillary tangles (NFTs), a major hallmark of Alzheimer's disease (AD), are highly prevalent in the aging hippocampus of AD patients (Lace et al., 2009). Tau proteins are a group of microtubule-associated proteins that are abundant in neurons and play a key role in microtubules stabilization, axonal transportation, and neurite outgrowth under physiological conditions (Avila et al., 2004; Devred et al., 2004; Johnson and Stoothoff, 2004; Weingarten et al., 1975). On the other hand, deposits of abnormally hyperphosphorylated tau protein are found in many neurodegenerative diseases such as AD (Avila et al., 2004; Grundke-Iqbal et al., 1986a,b; Johnson and Stoothoff, 2004; Lace et al., 2009).

Clinical studies have suggested that the severity of dementia in AD patients is positively correlated with the

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Abbreviations: OA, okadaic acid; cdk5, cyclin-dependent kinase 5; GSK-3, glycogen synthase kinase-3; NFTs, neurofibrillary tangle

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numbers of NFTs in the cortex (Arriagada et al., 1992; Nagy et al., 1995). The tau hypothesis, which proposes that tau protein abnormalities initiate the AD cascade, is also supported by the observation that NFTs are found in the neurons and their aggregates disrupt the neuron's transport system ultimately resulting in neuronal death (Brandt et al., 2005; Hernandez and Avila, 2007; Lovestone and Reynolds, 1997; Mudher and Lovestone, 2002). Although the tau hypothesis is supported by many experimental studies, especially studies from transgenic mice (Duff and Planel, 2005; Santacruz et al., 2005), it remains unclear whether NFTs are the initiating factors or merely markers of the disease process and whether NFTs crosstalk with other AD-related hallmarks (Castellani et al., 2008).

Several protein kinases, including glycogen synthase kinase-3 (GSK-3), mitogen-activated protein kinase (MAPK), and cyclin-dependent kinase 5 (cdk5), have been reported to phosphorylate tau protein at various sites that are found in AD hyperphosphorylated tau (Correas et al., 1992; Drewes et al., 1992; Hanger et al., 1992; Liu et al., 2008; Lucas et al., 2001), whereas its dephosphorylation is mainly catalyzed by protein phosphatase (PP) 1, 2A, 2B and 5, with PP2A as the major player (Arendt et al., 1998b; Gong et al., 1994a,b,c; Liu et al., 2005). An imbalance between tau phosphorylation and dephosphorylation is critical to AD tauopathy (Arendt et al., 1998a; Gong et al., 2006). Selective inhibition of PP 2A by okadaic acid (OA) can induce an Alzheimer-like hyperphosphorylation and accumulation of tau both *in vivo* (Arendt et al., 1998a; Gong et al., 2006) and in vitro (Alvarez-de-la-Rosa et al., 2005; Zhang and Simpkins, 2010).

It is well established that the prevalence and incidence of AD in women dramatically increases following post-menopause (Filley, 1997) and that estrogens are potent neuroprotectants (Singh et al., 2006). Herein, we developed an experimental tau model via chronic microinfusion of OA, a serine/threonine phosphatase inhibitor, into the dorsal hippocampus of ovariectomized adult Sprague–Dawley (SD) rat, which would mimic the estrogen deficiency of the postmenopausal state. Chronic infusion of OA induced a progressive cognitive deficiency, NFTs-like conformational changes in the brain, and oxidative damage in both the cortex and hippocampus. Inhibition of serine/threonine phosphatases also increased phosphorylation of tau and protein expression of cdk5 and p25. Our findings suggest that chronic infusion of OA induces an *in vivo* tauopathy model; and cdk5 plays a role in tau hyperphosphorylation induced by the inhibition of protein phosphatases.

2. Results

2.1. Effect of OA infusion on behavioral performance

To examine the effect of serine/threonine phosphatase inhibition on NFTs in the hippocampus of ovariectomized adult rat, which would theoretically mimic the postmenopausal condition in the human female, OA was unilaterally microinfused into the right dorsal hippocampal area via an osmotic pump. Rats were separated into three groups: vehicle control, low-dose OA, and high-dose OA. Following a 14-day infusion period, each rat was subjected to a spatial learning and memory test using the Morris water swim maze and a motor function test using the rotarod.

2.1.1. Body weight

The body weight of each rat was monitored daily throughout the treatment period and during the behavioral testing period (data not shown). As expected, each rat exhibited a steady increase in body weight and no rats died during the experimental period. There were no effects of OA infusion on the rate of weight gain or on the absolute body weight among the different groups. These data suggest that microinfusion of OA into unilateral dorsal hippocampus did not exert a generalized toxicity.

2.1.2. Spatial learning and memory

During the first three training sessions of acquisition (sessions 1–5), rats from the control group and the low-dose group learned to locate the hidden platform efficiently as evidenced by decrease in path length over sessions (Fig. 1A). In contrast, the high-dose group showed little improvement in behavior over the 5 sessions (Fig. 1A).

The retention sessions (sessions 6 and 7) were started 2 days after the acquisition training. In the control group and low-dose OA group, the rats swam the same and even shorter distances to find the platform as in acquisition sessions, but there are no significant changes in the high-dose OA group (Fig. 1A).

2.1.3. Visible MWM

After regular MWM tests, the rats were retested in the Morris water maze with a visible platform. This test permits the assessment of motivational and/or sensorimotor factors rather than spatial learning per se. The rats from all three treatment groups learned the path to the platform with the training. There were no differences in the ability of the different treatment groups to locate the visible escape platform in any single session (Fig. 1B).

2.1.4. Rotarod

To rule out group differences in motor coordination, all the rats were subjected to rotarod tests. Learning of coordinated running was measured by the latency to fall over the three training sessions and maximum performance was estimated by performance on the final session (Fig. 1C). A one-way ANOVA on latency to fall for the final session failed to indicate any effect of OA treatment on motor function.

2.2. Pathological changes in OA-induced tauopathy model in female rats

To characterize the pathological changes of our experimental AD model induced by OA in female rats, brain sections were stained with silver nitrate to assess NFT-like conformational changes and probed with antibodies against phosphorylated tau.

2.2.1. Silver staining of pathological changes in OA-induced tauopathy model

After this series of behavioral tests, half of the rats were submitted to prepare paraffin sections of brain samples; the other half of the rats was subjected to oxidative stress assays.