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AGE-DEPENDENT EFFECTS OF VALPROIC ACID IN ALZHEIMER'S DISEASE (AD) MICE ARE ASSOCIATED WITH NERVE GROWTH FACTOR (NGF) REGULATION

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Abstract—Alzheimer's disease (AD) is a progressive neurodegenerative disease that causes cognitive impairment. Major pathophysiological AD characteristics include numerous senile plaque, neurofibrillary tangles, and neuronal loss in the specific regions of patients' brains. In this study, we aimed to understand disease stage-dependent regulation of histone modification for the expression of specific markers in plasma and the hippocampus of *in vivo* AD model. Since the control of histone acetylation/deacetylation has been studied as one of major epigenetic regulatory mechanisms for specific gene expression, we detected the effects of histone deacetylase (HDAC) inhibitor on marker expression and neuroprotection in *in vivo* AD model mice. We determined the effects of valproic acid (VPA, HDAC inhibitor), on the levels of cytokines, secreted form of APP (sAPP), nerve growth factor (NGF), and cognitive function in Tg6799 AD mice in three different disease stages (1 month: pre-symptomatic; 5 months: early symptomatic; and 10 months: late-symptomatic stages). VPA decreased the mRNA levels of nuclear factor kappaB (NF-κB) and IL-1ß in the plasma of Tg6799 mice compared to vehicle control at 10 months of age. VPA increased the protein levels of NGF in the hippocampus of Tg6799 mice at 5 and 10 months of age. In addition, VPA decreased escape latencies of Tg6799 mice at 5 and 10 months of age in Morris water maze assessment. Taken together, HDAC inhibition is a promising therapeutic target for AD and it needs to be considered in n age-dependent and/or stage-dependent manner.

Key words: AD, VPA, aging, cognitive improvement, NGF, sAPP.

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Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, amyloid β; ChAT, choline acetyltransferase; EDTA, ethylenediamine tetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GSK, glycogen synthesis kinase; HDAC, histone deacetylase; HRP, horseradish peroxidase; LM, littermates; MT, mutant type; NAM, nicotinamide; NF-κB, nuclear factor kappaB; NGF, nerve growth factor; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PHF1, paired helical filaments; RT-PCR, reverse transcription polymerase chain reaction; sAPP, secreted form of APP; TLR, toll-like receptor; TSA, trichostatin A; VPA, valproic acid.

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INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia. Major pathophysiological AD characteristics include the presence of numerous senile plaque and neurofibrillary tangles and neuronal loss in the brain, particularly in the hippocampus and cerebral cortex (Qing et al., 2008; Blanco et al., 2010). Cholinergic neurons that innervate the cortex and hippocampus play a key role in cognitive functions, which dysfunction is one of the major pathological symptoms of AD. Neuroprotection of cholinergic neurons has been studied as potential therapeutic target for AD including protective function of several trophic factors. Nerve growth factor (NGF) is synthesized and secreted from cells in the cortex and hippocampus, which contain TrkA (high-affinity) and p75 neurotrophin receptor (low-affinity). NGF from target cells activates TrkA on axon terminals and induces activation of PI3K/Akt, MEK/ERK, and PLCγ signaling, which signal retrograde transportation along the axon to the cell body and promote neural survival. While TrkA activation promotes neural survival, p75 (NTR) activation leads to apoptotic cell death. NGF dysfunction and NGF receptors have been proposed to elucidate the selective degeneration of cholinergic neurons in AD. AD is related to an imbalance of amyloid β (Aβ) production, hyperphosphorylated tau accumulation, apolipoprotein E, trophic responses, cytoskeletal disarrangement, and mitochondrial dysfunction, which eventually lead to neuronal and/or synaptic degeneration.

Recent studies have indicated that an epigenetic mechanism has an important role in AD development (Udan et al., 2008). Several characteristics of sporadic AD are associated with epigenetic components. Histone modifications regulate chromatic structure and serve in a signaling role by integrating responses to multiple biochemical cascades, which mediate epigenetic changes in gene expression (Abel and Zukin, 2008). Modifications include acetylation, methylation, phosphorylation, ubiquitination, sumoylation, biotinylation, adenosine diphosphate ribosylation, and glycosylation (Konsoula and Barile, 2012). Both histone acetylation and phosphorylation are related to transcriptional activation (Rao et al., 2012). Rao et al. detected that the postmortem frontal cortex of AD patients showed significantly increased levels of global DNA methylation and global histone phosphorylation compared to a control, but did not show increased global histone H3

acetylation (Rao et al., 2012). A previous study demonstrated that valproic acid (VPA) inhibits Aβ deposition and plaque in APP23 transgenic AD model mice (Qing et al., 2008). They detected that VPA inhibits γ-secretase cleavage of amyloid precursor protein (APP) and Aβ production in addition to glycogen synthesis kinase (GSK-3) activity (Qing et al., 2008).

The AD model Tg6799 transgenic mice overexpress mutant human APP695, which includes the Swedish (K670N, M671L), Florida (I716L), and London (V717I) mutations, and mutant human presenilin 1 (PS1) (M146L and L286V) (Lalonde et al., 2012). Tg6799 mice have abnormal hippocampus morphology, abnormal neuronal morphology, and brain inflammation (Oakley et al., 2006). This model shows Aβ42 deposition, microgliosis, and astrogliosis begin in the brain at 2 months of age and synapse degeneration begins at 4 months of age and there is abnormal spatial learning in Y-maze tests at 5 months of age.

In this study, we examined the changes of biochemical markers; of (1) NF-κB, IL-1β, and TLR4 to detect neuroinflammation, (2) sAPP, APP, p-tau, to detect pathological progression of AD, (3) the regulation of NGF and TrkA expression to detect neurotrophic protection mechanism in the plasma and hippocampus of Tg6799 mice with age. In addition, we determined the effects of VPA, HDAC inhibitor, on the levels of the specific biochemical markers as well as the number of choline acetyltransferase (ChAT)-positive neurons in medial septum area and the spatial memory by Morris water maze test at three different disease stages (pre-symptomatic, early-symptomatic, and late-symptomatic stages).

EXPERIMENTAL PROCEDURES

Animals and VPA administration

Tg6799 transgenic mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were housed under a 12-h light–dark cycle with free access to food and water. All animal studies were conducted in accordance with IACUC guidelines and were approved by the IACUC committee at the Hanyang University (HY-IACUC-09-017). Male mice (n = 16 in each group) were injected with VPA (Sigma, St. Louis, MO, 250 mg/kg i.p., modified from Dowdell et al. (2009) or 0.01 M phosphate-buffered saline (PBS) for 5 days at 1 month, 5 months, and 10 months of age. Mice in each group were sacrificed 24-h after last injection.

RNA isolation and quantitative reverse transcription polymerase chain reaction (RT-PCR)

RNA samples were isolated from blood of the mice using a Mouse RiboPure – Blood RNA Isolation Kit (Ambion, Austin, TX) or isolated from the hippocampus using TRI-Reagent (Sigma–Aldrich, St. Louis, MO). The SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA) was used to synthesize the first-strand cDNA from equal amounts (500 ng) of the RNA samples. The following primer sequences were used for RT-PCR: nuclear factor kappaB (NF-κB), 5′-GGC GGC ACG TTT TAC TCT TT-3′ (forward) and 5′-CCG TCT CCA GGA GGT TAA TGC-3′ (reverse); IL-1β, 5′-CTG GTG TGT GAC GTT CCC ATT A-3′ (forward) and 5′-CCG ACA GCA CGA GGC TTT-3′ (reverse); TLR4, 5′-TGT AAC GCA ACA GCT TCA GG-3′ (forward) and 5′-CTG CCA GAG ACA TTG CAG AA-3′ (reverse); NGF, 5′-AGC CTC CTG AAT GAG CAC AC-3′ (forward) and 5′-TCC ATC TCT CCT GCA CAC AG-3′ (reverse); TrkA, 5′-ATG CTG CGA GGC CAG CGG CA-3′ (forward) and 5′-CCT GAC AGG GTC AAG TCC TG-3′ (reverse) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), 5′-AGA ACA TCA TCC CTG CAT CC-3′ (forward) and 5′-TCC ACC ACC CTG TTG CCTG TA-3′ (reverse). The PCR were performed with 40 cycles of denaturation (94 °C for 1 min), annealing (58 °C for 1 min), and extension (72 °C for 2 min).

Protein preparation

Blood samples were collected in 0.1 M sodium citrate and homogenized with 300 μl of 0.09 M EDTA. Homogenates were centrifuged at 200g for 10 min at room temperature to remove erythrocytes and the buffy coat fraction. Plasma was then centrifuged at 1200g for 10 min at room temperature. Pellets were washed with 100 μl of 10 mM Tris–Cl, pH 7.4, and resuspended in lysis buffer that contained 10 mM Tris–Cl, pH 7.4, 1 mM ethylene glycol tetraacetic acid, 100 mM phenylmethanesulphonyl fluoride (PMSF), and protease inhibitors (10 μg/ml aprotinin, 25 μg/ml leupeptin, and 10 μg/ml of pepstatin).

Protein samples were isolated from the hippocampus in a homogenization buffer (50 mM Tris–Cl, pH 8.0, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100) that contained the following protease and phosphatase inhibitors: 10 μg/ml aprotinin, 25 μg/ml leupeptin, 10 μg/ml pepstatin, 10 μg/ml PMSF. Homogenates were centrifuged at 14,000g for 30 min at 4 °C and the supernatants were collected and stored at −80 °C before use.

Western blot

Equal amounts (10 μg) of protein sample were prepared from mouse brain tissue and blood plasma was used for western blot analysis (Seo and Isacson, 2005). Protein samples were loaded in 10% sodium dodecyl surfate (SDS)–polyacrylamide gels and transferred to polyvinylidene difluoride (PVDF) membrane (BIO-RAD, Hercules, CA). The following antibodies were used for primary antibody reaction: monoclonal anti-APP (1:5000; Chemicon, Billerica, MA), monoclonal anti-phospho-tau (Ser396) (1:3000, Cell Signaling, Danvers, MA), monoclonal anti-TrkA (1:5000, Abcam, Cambridge, MA), and GAPDH (1:2500, Millipore, Billerica, MA) and for secondary antibody reaction: horseradish peroxidase (HRP)-linked anti-rabbit (1:2500; Vector, Burlingame, CA) and anti-mouse (1:2500; Vector, Burlingame, CA) immunoglobulin G (IgG) antibodies. Visualization was performed using enhanced chemilumescent (ECL) solution (Amersham, Buckinghamshire, UK) according