ALZHEIMER'S DISEASE: AGING, INSOMNIA AND EPIGENETICS

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SUMMARY

Alzheimer's disease (AD) is the most common form of dementia. Severe memory loss, confusion, and impaired cognitive abilities characterize AD. It was only a century after Alzheimer's discovery that scientists were able to shed light on the mystery of its cause, but AD has also become a globally important health issue and the treatment of AD is a challenge for modern medicine. At present, there are five drugs approved in the United States for the treatment of AD, namely, donepezil, galantamine, rivastigmine, and tacrine (which are all cholinesterase inhibitors); and memantine (which is a glutamate receptor antagonist). However, these drugs show only modest effects on AD patients. Thus, new investigations are necessary for pharmacological development in AD. This brief review focuses on new studies that demonstrate the link between epigenetics and AD, and explores the possibility that insomnia may be one factor that effects AD. [*Taiwan J Obstet Gynecol* 2010;49(4):468–472]

Key Words: Alzheimer's disease, epigenetics, insomnia, orexin

Introduction

A paradox of neurodegenerative diseases is that they are easy to diagnose as a result of abnormal behavior, but are hard to treat. Alzheimer's disease (AD) is an example of such a disease. A typical symptom of AD in its initial phase is episodic or severe memory loss/impairment, which can be easily characterized. However, AD remains an incurable disorder to this day and senile dementia caused by AD affects more than 20 million people worldwide [1,2]. With the dramatic increase in the aged population (i.e. the number of people over 60 years of age is expected to triple by 2050 in China) [3], AD is considered a crucial global health issue, and finding a suitable treatment for AD has become a challenge for modern medicine.

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The first case of AD was described by Alois Alzheimer on November 3, 1906, at the 37th meeting of the Society of Southwest German Psychiatrists in Tübingen, Germany [2]. The brain anatomy of patients with AD has helped identify two hallmarks that define the neuropathological characteristics of AD, namely, the neuritic plaques and the neurofibrillary tangles. In the 1960s, abnormal amyloid-like filaments in the plaques and tangles were observed under the electron microscope [4,5]. It has also been established that the localization of plaques and tangles are different: plaque filaments are deposited extracellularly, while most of the tangle filaments are present intracellularly, deposited in nerve cell bodies as well as in the neuropil in neuritis. The presence of these two unique deposits has greatly helped in characterizing AD [6]. In addition, plaques appear as filaments 10 nm in diameter under electron microscope, exhibiting a cross-β structure as well as characteristic dye-binding properties [2]. The next breakthrough to clarify the mechanism of AD took another 20 years, and revealed that the major molecular compositions of the plaques and tangles were different: amyloid-β

(Aβ) peptide is the major plaque component [7–10], while hyperphosphorylated tau protein is the major tangle component [11–15]. The 40–42-amino acid Aβ peptide can be derived from the sequential cleavage of the amyloid precursor protein (APP), a type 1 transmembrane protein, by two proteases, β- and γ-secretase [1,2]. Tau is a protein involved in microtubule assembly and stabilization. There are six tau isoforms produced from a single gene through alternative RNA splicing in the human brain [16]. Consistent with these biochemical studies of the abnormal filaments in AD, transgenic mouse models of AD that target both the APP and tau genes also confirm their genetic pathogenicity. Thus, the expression of APP and tau proteins became the major targets of drugs currently being tested for AD [1]. An understanding of how to regulate the expression of APP and tau in the brain will be critical to treatments for AD.

Because AD is a complicated neurodegenerative disease to cure, most AD patients also receive antipsychotic or antidepressant drugs aside from the actual AD drug to manage neuropsychiatric and behavioral symptoms, or take over-the-counter medications with uncertain therapeutic value such as ginkgo biloba and vitamins C and E [17–22]. Recently, dimebon failed in a Phase III clinical trial for AD, although this antihistamine drug was once thought to be the "dark-horse" of Alzheimer's medications [23]. At present, there are five drugs used in the treatment of AD: (1) the cholinesterase inhibitors: donepezil, galantamine, rivastigmine and tacrine; and (2) the glutamate receptor antagonist: memantine. All have been approved in the United States for treatment of AD [17,22], although tacrine is now rarely used because of hepatotoxicity. Cholinesterase inhibitors can combat the impairment of cholinergic neurons in AD patients by slowing the degradation of acetylcholine released from synapses during neurotransmission. Memantine prevents the overstimulation of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors to prevent calcium-induced excitotoxicity, which may contribute to the pathogenesis of AD and other neurodegenerative conditions [24]. Recent studies have suggested that memantine, an analogue of amantadine that inhibits the internal ribosome entry site (IRES) of picornavirus-mediated translation [25], can also prevent expression of APP and tau via the inhibition of a translation initiation mechanism that is mediated by the IRES [26]. In clinical trials, both cholinesterase inhibitors and memantine have proven beneficial to AD patients [17–21]. However, these drugs only show modest effects on AD patients' cognitive test scores, behavioral measures, and functional outcomes. In this review, we describe some recent interesting findings that point to new concepts about AD.

AD and Insomnia

Sleep is a complex physiological state, and its ultimate function remains to be elucidated. A characteristic of sleep is the sleep-wake cycle. One interesting study indicated that Aβ levels of brain interstitial fluid (ISF), when analyzed by *in vivo* microdialysis, were significantly increased while awake as compared with the sleeping state both in C57BL6 mice and human APP transgenic mice, Tg2576 [27]. Cerebrospinal fluid (CSF) levels of Aβ also fluctuate during the sleep-wake cycle in humans; mean peak CSF Aβ levels from 7 pm to 9 pm were found to be 27.6% higher than mean trough CSF Aβ levels from 9 am to 11 am [27]. These results raise interesting questions: (1) Does insomnia have a link with AD? (2) Do factors that are involved in sleep contribute to the pathogenesis of AD? Since it was established that the neuropeptide orexin can regulate wakefulness and can be strongly implicated in sleeping and waking disorders [28], it would be fascinating to know whether the concentration fluctuation of orexin can affect the concentration of Aβ in the brain. Orexin is released from hypothalamic neurons and fluctuates diurnally similar to that of ISF Aβ [29]. The function of orexin is mediated through orexin receptors. The orexin family (orexin-A and orexin-B) has two receptor subtypes: orexin receptor 1 and orexin receptor 2 [30]. Interestingly, when a dual orexin receptor antagonist, almorexant, was infused intracerebroventricularly (icv) during *in vivo* microdialysis for an ISF Aβ experiment, Kang et al discovered that the ISF Aβ levels were suppressed and the natural diurnal variation of Aβ was also abolished [27]. Furthermore, they also revealed that ISF Aβ levels were significantly increased upon icv infusion of orexin-A (1.5 pmol/hr, a dose that could induce wakefulness in rodents). The physiological function of APP is not well established, although some reports indicated that APP proteins were involved in regulation of NMDA receptors [31,32]. These results implied that Aβ might be involved in the physiological regulation of sleep. More importantly, these findings suggested that sleep disturbances might correlate with the pathogenesis of AD. Kang et al also demonstrated significantly greater Aβ plaque deposition in multiple sub-regions of the cortex when chronic sleep restriction was implemented 20 hours daily in APP transgenic mice (APPswe/PS1dE9 genotype) for 21 days [27]. Consistent with this remarkable observation was the fact that systemic treatment with almorexant once daily for 8 weeks could significantly decrease Aβ plaque formation in aged APP transgenic mice. Hence these data offered a new concept about how sleep and the optimization of sleep time could possibly prevent aggregation of Aβ and slow the progression of AD. Further

study, however, is needed to confirm the hypothesis that almorexant, developed by a pharmaceutical company for the treatment of primary insomnia, is a potential drug that can inhibit the deposition of Aβ plaque and thus prevent AD pathogenesis.

AD and Epigenetic Change with Aging

A striking feature of AD is that most cases are sporadic, with dominantly inherited forms accounting for less than 1% of the total [2]. Although some genes associated with early-onset AD have already been identified, genetic causes cannot explain an overwhelming proportion of diagnosed AD cases. Environment or nurture, like the aforementioned insomnia, may also be critical for the pathogenesis of AD. Thus, the question is how environmental factors rather than the genetic factors correlate with AD. Besides, recent studies concerning the chemical modification of chromatin may have highlighted a new direction in the study of the etiology and drug development of AD. These studies suggested that DNA methylation and histone acetylation and methylation, collectively known as epigenetics, in the neuron may change with the aging process and can probably explain the observed anomalies in AD, as aberrant epigenetic patterns may be acquired during developmental stages [33].

Epigenetic mechanisms of gene regulation have long been familiar to developmental and cancer biology. These molecular mechanisms alter the activity of genes without changing their DNA sequence. Acetylation of histones tends to enhance gene expression, whereas methylation of histones and DNA tends to suppress gene expression [34]. Although during the early 1990s a study from one post-mortem brain sample of an unaffected patient suggested that the APP promoter is always unmethylated in the brain and hence may not be regulated by DNA methylation in the brain of healthy individuals [35], this study did not compare statistical relevant quantities of samples; nor did the study reveal whether DNA methylation of the investigated sites was present in AD patients [33]. In the late 1990s, several studies demonstrated that abnormalities in DNA methylation were involved in brain disorders that caused intellectual impairment, including Angelman syndrome and Rett syndrome [33]. More importantly, epigenetic studies in neurons also found that epigenetic mechanisms played an important role in learning and memory and could help to explain why memory declined with age [36], while also highlighting a new drug target for AD. One study found that training mice to associate a certain location with a mild electric shock could reduce DNA methylation on the brain-derived neurotrophic factor (*bndf*) gene in the hippocampus. Demethylation of the *bndf* gene boosted its activity and could promote new synaptic connections between neurons, as well as allowing more efficient memory retention [37]. Furthermore, infusions of a DNA methyltransferase inhibitor, zebularine, significantly induced *bdnf* DNA demethylation and was sufficient to increase mRNA levels of *bdnf* in the hippocampus [37]. Consistent with this finding was the discovery that when long-term memory formation was blocked by the NMDA receptor use-dependent antagonist MK801, *bdnf* DNA methylation as well as *bdnf* gene expression was altered in the hippocampus, leading to a deficiency in memory formation [37]. These results suggested that epigenetic modification of the *bdnf* gene may be a pivotal mechanism for memory consolidation. This not only demonstrated how epigenetics affected the formation and maintenance of memory, but also implied that epigenetic modification of chromatin in neurons might be the molecular basis for memory decline experienced during aging and provides a new clue in finding drugs that can treat AD.

Recently, an interesting study found that age-dependent memory impairment was associated with altered histone acetylation in mice. Peleg et al [38] employed quantitative immunoblot analysis (using fluorescent secondary antibodies), high-density oligonucleotide microarray, quantitative real time polymerase chain reaction, chromatin-immunoprecipitation (ChIP) and ChIP-sequencing to investigate the chromatin remodeling that is associated with age and fear conditioning, a commonly used model for hippocampus-dependent associative learning in mice. First, they subjected 3-, 8-, and 16-month-old C57BL/6 mice (mean life span about 26–28 months) to contextual fear conditioning and found that 16-month-old mice exhibited impaired associative learning. They also showed that these 16-month-old mice, but not 3-month-old mice, failed to upregulate histone H4 acetylation on lysine residues (K) 12 (H4K12) specifically, although the levels and activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs) were similar in 3- and 16-month-old mice [38]. Importantly, this deregulated H4K12 acetylation in aged mice correlated with the loss of almost all memory-associated hippocampal transcriptome in 3-month-old mice [38]. More specifically, the transcriptional regulation of an actin regulatory protein, Form 2, which is necessary for normal memory formation, was disrupted by the deregulated H4K12 acetylation upon aging. Hence this study illustrated that learning and memory could be recovered through the epigenetic remodeling of chromatin. Consistent with these findings, Fisher et al

demonstrated that sodium butyrate, a drug that inhibits HDACs and promotes histone acetylation, improved learning and memory in CK-p25 Tg mice, a mouse model of AD [39]. Thus, drugs that either enhance histone acetylation through HATs or inhibit histone deacetylation through HDACs may act as candidates for treating AD and other neurodegenerative diseases that impair learning and memory.

Conclusion

Since a small group of psychiatrists used the silver staining method developed by Max Bielschowsky in 1902 to describe abnormal neuritic plaques and neurofibrillary tangles in the brain of AD patients [2], the treatment of this neurodegenerative disease has been a challenge to modern medicine. However, advances in histology and protein chemistry in the 1960s has since allowed identification of the protein composition of the deposits found in abnormal neuritic plaques and neurofibrillary tangles; that is, the Aβ and tau proteins opened the first window to this therapeutic pursuit. When the enzymes that produce Aβ were finally discovered in the 1990s, inhibitors of secretases took the limelight as drugs which could conquer AD. Besides the "enzyme hunter", progress in receptor biochemistry and neuroscience has also led to the proposal of certain drugs which could be beneficial in AD. However, these drugs only showed modest effects on AD patients. It was only after the development of genetics and molecular biology studies on AD that mutations in the *APP* gene as well as inheritance of the ε4 allele of apolipoprotein E were recognized as contributory factors to the pathogenesis of AD. In spite of this, most cases of AD are sporadic, with dominantly inherited forms accounting for less than 1% of AD patients. Thus, both environmental factors and epigenetic changes may present a critical issue for the treatment of AD. These speculations are also consistent with the characteristics of AD, that is, the long pre-symptomatic phase of AD. New findings that epigenetic change can characterize the mouse model of AD and that optimization of sleep time could potentially slow the development of AD point to new avenues in the search for a cure for AD.

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