Dual Action of Memantine in Alzheimer Disease: A Hypothesis

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SUMMARY

Objective: In this study, we proposed a hypothesis to explain the mechanisms of memantine action in treating Alzheimer disease (AD). Memantine may reduce the expression of amyloid precursor protein and tau protein, as well as acting as an antagonist of *N*-methyl-D-aspartate receptors in the brain.

Results: Two neuropathologic characteristics of AD are neuritic plaques and neurofibrillary tangles. The major molecular components of the plaques and tangles are amyloid-β peptide and tau, respectively. Drugs able to reduce the expression of amyloid-β and tau protein provide potential pharmaceutical treatments for AD. We found that memantine inhibited internal ribosome entry site-mediated translation initiation in COS-1 cells. This suggests that the memantine may not only inhibit neuronal excitotoxicity, but also act as an inhibitor of the internal ribosome entry site, to block the expression of amyloid precursor protein and tau in neurons.

Conclusion: Memantine may function not only as an antagonist of *N*-methyl-D-aspartate receptors, but also as an inhibitor of the internal ribosome entry site to block the expression of amyloid precursor protein and tau, and so ameliorate the symptoms of AD. [*Taiwan J Obstet Gynecol* 2009;48(3):273–277]

Key Words: Alzheimer disease, amyloid-β peptide, internal ribosome entry site, memantine, *N*-methyl-D-aspartate receptors

Introduction

Neurodegenerative diseases are easy to diagnose but hard to treat. Alzheimer disease (AD) is a typical example. AD is characterized by severe memory loss, with episodic memory being particularly impaired during the initial phases. However, the disorder is not currently curable [1]. The first case of AD was described by Alois Alzheimer at the 37th meeting of the Society of Southwest German Psychiatrists in Tubingen, Germany [2]. A hundred years later, it is the most common neurodegenerative disease in our modern but elderly society, and this senile dementia affects more than 20 million people worldwide [2]. The anatomy of the brain in AD patients shows two defining neuropathologic characteristics: neuritic plaques and neurofibrillary tangles. Under the electron microscope, abnormal amyloid-like filaments can be seen in the plaques and tangles [3,4]. The localization of plaques and tangles are different; plaque filaments are extracellular, but most of the tangle filaments are intracellular and are deposited in nerve cell bodies, as well as in neurites in the neuropil. The major molecular components of the plaques and tangles also differ; amyloid-β (Aβ) peptide is the major plaque component, while tau protein is the major tangle component. The 40–42-amino acid Aβ peptide is derived from sequential cleavage of amyloid precursor protein (APP), a type 1 transmembrane protein, by two proteases, β- and γ-secretase [1,2]. Transgenic mouse models of AD targeting the APP and tau genes have confirmed these pathogenic factors. Thus, inhibition of APP and tau expression may provide a strategy for combating AD [1].

Because AD is not yet curable, many AD patients are prescribed antipsychotics or antidepressants to manage

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their neuropsychiatric and behavioral symptoms, or take over-the-counter preparations with unknown therapeutic values, including *Ginkgo biloba* and vitamins C and E [5–9]. Five drugs are currently approved for the treatment of AD in the United States: (1) the cholinesterase inhibitors donepezil, galantamine, rivastigmine and tacrine, and (2) the glutamate receptor antagonist memantine [5,10]. Tacrine, however, is now rarely used because of its hepatotoxicity. Cholinesterase inhibitors can combat impairment of cholinergic neurons in AD patients by slowing the degradation of acetylcholine release at synapses during neurotransmission. Memantine prevents 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 [11]. In clinical trials, both cholinesterase inhibitors and memantine have shown benefit in AD patients [5–9], but have produced only modest effects on cognitive test scores, behavioral measures, and functional outcomes. It is interesting to note that none of these drugs target the hallmarks of AD, APP or tau, even though reduction of Aβ production and tauopathies by inhibition of APP and tau expression would be expected to ameliorate the symptoms of AD.

In this study, we proposed a hypothesis to explain the mechanism of memantine action. Memantine may function not only as an antagonist of NMDA receptors, but also as an inhibitor of a novel translation initiation mechanism, the internal ribosome entry site (IRES), so blocking the expression of APP and tau protein and thereby relieving the symptoms of AD.

Materials and Methods

Cell culture, plasmids, and transfection

COS-1 cells (African green monkey kidney fibroblast-like cell line) were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. pGS-EV71 plasmids were generated as described by Chen et al [12]. The EV71 IRES was flanked by the β-galactosidase and secreted alkaline phosphatase (SEAP) reporter genes. COS-1 cells were then transfected with the plasmids using Lipofectin reagent (Invitrogen, Carlsbad, CA, USA) and plated onto 24-well plates at a density of $0.5–2 \times 10^5$ cells/well. The cells were repeatedly washed with serum-free medium to remove all traces of serum prior to transfection. One microgram of plasmid was diluted in 200 μL of serum-free Dulbecco's modified Eagle's medium, and 1 μL of Lipofectin reagent was added. The DNA–Lipofectin mix was incubated for 15 minutes to allow DNA–Lipofectin complex formation, and then transferred to the cells at a final volume of 0.5 mL in serum-free medium. The transfected cells were then incubated at 37°C in 5% CO_2. After 12 hours, the transfection medium was replaced with medium containing fetal bovine serum and antibiotics, and the cells were cultured for a further 12 hours before being used in the following assays.

Measurements of SEAP and β-galactosidase activity

After transfection, supernatants were harvested and analyzed for SEAP activity using BD Great EscAPe SEAP detection kits (Clontech Laboratories, Mountain View, CA, USA). For the β-galactosidase activity assay, the transfected cells were lysed for 10 minutes in 300 μL of culture cell lysis reagent containing 100 mM potassium phosphate (pH 7.8), 1 mM EDTA, 10% Triton X-100, and 7 mM β-mercaptoethanol. After centrifugation at 15,200*g* for 30 minutes, the lysate supernatant was assayed for β-galactosidase activity using a Luminescent β-galactosidase Detection Kit II (BD Biosciences, San Jose, CA, USA). The chemiluminescent intensities reflecting relative SEAP activities and β-galactosidase activities were detected using a chemical luminescence counter (Mithras LB 940; Berthold Technologies, Bad Wildbad, Germany). Both SEAP and β-galactosidase activities were expressed as relative light units. The detection limit for SEAP was about 10^{-13} g. Thus, the sensitivity of our bicistronic assay was comparable with *Renilla* and firefly luciferase assays. Because SEAP was secreted into the culture medium, it was possible to measure the alkaline phosphatase activity without lysis of the cells.

Results and Discussion

IRES and translation initiation

Translation initiation for protein synthesis in eukaryotic cells proceeds in two ways: by a cap- and 5′ end-dependent mechanism and by a cap-independent mechanism that acts through an internal RNA element called the IRES. Most eukaryotic messenger RNAs (mRNAs) use a cap structure (m^7Gppp) at the 5′ end, which is recognized by translation initiation factor eIF4F (a trimeric complex containing the cap-binding protein eIF4E, a scaffold protein eIF4G and an RNA helicase eIF4A). This cap-bound eIF4F complex interacts with the 40S ribosome subunit bound to eIF3-eIF2-methionyl-tRNA and then scans the 5′ untranslated region of mRNA until it detects an AUG triplet in an appropriate context, allowing it to complete translation initiation [13]. By contrast, IRES-dependent translation initiation recruits the ribosome machinery through a stable RNA structure, and may be cap-binding protein eIF4E

independent. IRES elements were first identified in the RNAs of the Picornaviridae, which have highly structured 5′ untranslated regions but no cap structure at the 5′ end [14,15]. In these viruses, the IRESs can fold into a functional secondary RNA structure and mediate translation initiation, thereby functioning like some protein initiation factors and allowing cap-independent translation [16]. These results demonstrate why viruses-encoded proteases that cleave protein translation initiation factors like eIF4G can reduce the efficiency of the host cell's cap-dependent translation initiation and favor virus IRES-mediated translation.

Since their first discovery in picornaviruses in 1988, IRES elements have been shown not to be restricted to these small RNA viruses, but to also occur in the genomes of retroviruses and even DNA viruses, such as HIV and herpes simplex viruses [17]. IRES are also found in insect viruses, like *Rhopalosiphum padi* virus and *Perina nuda* virus [18–20]. More interestingly, IRES elements have also been found in several cellular mRNAs [21]. In eukaryotic cells, IRES-dependent translation of cellular mRNAs has been reported to occur when cap-dependent translation is impaired, for instance, under conditions of apoptosis, heat shock stress, viral infection, and in the G_2/M phase of the cell cycle [21–23]. Further studies have also suggested that the IRES may increase translation efficiency at postsynaptic sites following synaptic activation [24]. This implies that IRES may play an important function in the central nervous system.

Transcripts of APP and tau genes containing IRES

It is interesting to note that ribosomes and other components of the translation machinery are found in neurons within dendritic processes, though at lower levels than in the cell body [25–27]. In addition, a number of mRNAs have been shown to be transported into dendrites and translated locally [28,29]. Moreover, many dendritically localized mRNAs encode proteins that are critical for certain forms of synaptic plasticity [30]. The dendritic processes may not contain all the translational machinery required to support cap-dependent translation, and IRES may be responsible for translation of the dendritically localized mRNAs. It has been shown that IRES are present within the 5′ leader sequences of five dendritically localized mRNAs: those for the activity-regulated cytoskeletal protein, the α subunit of calcium-calmodulin-dependent kinase II, dendrin, microtubule-associated protein 2, and neurogranin [24]. In addition, the translation of fibroblast growth factor 2, which plays a fundamental role in brain functions, is also regulated by IRES [31]. Recent evidence also indicates that the hallmarks of AD, APP and tau mRNA are translated through IRES [32,33].

Qin and Sarnow [34] found that APP mRNA associated with polyribosomes during mitosis, when cap-dependent translation is greatly reduced. In addition, translation of a second cistron from a dicistronic DNA construct increased when the APP 5′ untranslated region was placed into its intercistronic region. These results support the fact that APP synthesis occurs through an alternate translation initiation mechanism. Interestingly, both elevated intracellular iron levels and ischemia individually appear to promote AD at the level of translation. Thus, translation initiation via IRES in the 5′ leader sequences of APP and tau also provides a potential new drug target for AD treatment.

Memantine and amantadine inhibit IRES-mediate translation initiation

Memantine was approved as a therapeutic drug for moderate-to-severe AD in 2002 by the European Agency for the Evaluation of Medicinal Products, followed in 2003 by the USA Food and Drug Administration [35]. Memantine is a low-to-moderate affinity antagonist of NMDA receptors. Despite its relatively recent approval for AD, memantine is not a new drug. Eli Lilly synthesized it in the early 1960s for the treatment of diabetes mellitus [35], and it was studied in the 1980s as a drug for various neurologic diseases (e.g. Parkinson disease, neurogenic bladder disorders, and coma). The first reported use of memantine (intravenous) in patients with AD was published in 1986 [35]. Acting as an NMDA receptor antagonist, memantine can block the excitotoxicity evoked by the pathogenesis of AD and other neurodegenerative processes [1]. However, NMDA receptors are not only involved in the excitotoxicity of neurons but are also critical glutamate receptors that mediate the learning and memory functions of the brain. Thus, it is likely that memantine does not act simply as an antagonist of NMDA receptors in AD. The structure of memantine is similar to that of the tricyclic symmetric amine compound, amantadine (Figure 1), which is able to block the IRES-mediated translation of enterovirus 71 or encephalomyocarditis virus [12]. Amantadine, which was developed in the 1960s, has diverse uses, ranging

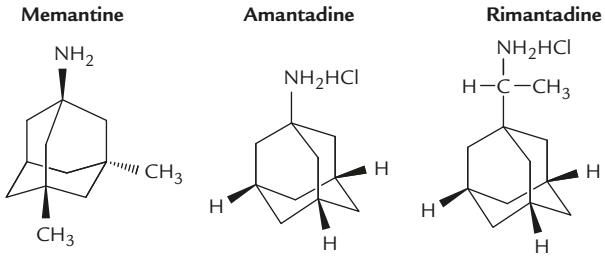

Figure 1. The chemical structures of memantine, amantadine and rimantadine.

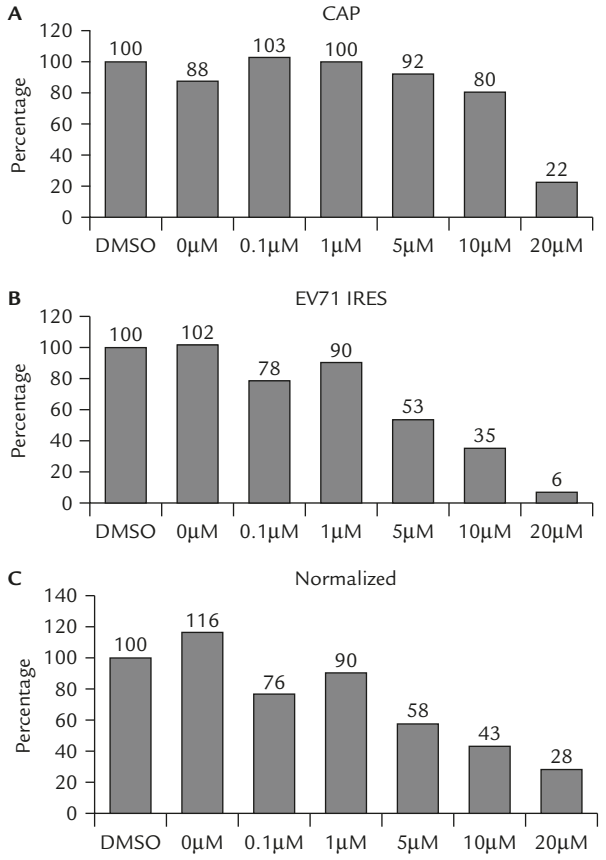

Figure 2. Effect of memantine on cap-dependent and EV71 IRES-mediated translation in COS-1 cells. pGS-EV71-transfected COS-1 cells were treated with various concentrations of memantine for 12 hours. Cells were harvested 24 hours after transfection, and lysates were analyzed by β-galactosidase and secreted alkaline phosphatase (SEAP) reporter assays. (A) Cap-dependent translation was measured as β-galactosidase activity, and (B) EV71 IRES-driven translation was measured as SEAP activity. (C) SEAP activity was normalized with β-galactosidase activity. Data are expressed as the mean of three independent experiments.

from prevention of influenza A infection to the treatment of Parkinson disease [36]. This result implies that memantine, like amantadine, may act as an inhibitor of IRES-mediated translation. Figure 2 shows that memantine can block EV71 IRES activity, but does not interfere with cap-dependent translation. This suggests that memantine can also act as an inhibitor of the IRES, controlling the translation of the APP and tau proteins. We, therefore, hypothesize that memantine exerts dual actions in the treatment of AD; it not only blocks the excitotoxicity of NMDA receptors, but also prevents the expression of APP and tau proteins through IRES. The inhibition of APP and tau expression by memantine may be responsible for the reduction of Aβ production and tauopathies in AD patients.

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