[14:10 ~ 14:50]

The Current Status and Perspective on the Development of Therapeutic Drugs for Alzheimer's Disease

Yoshiharu Yamanishi*, Nobuyuki Mori and Naoki Nagakura
Tsukuba Research Laboratories, Eisai Co., ltd.
5-1-3 Tokodai, Tsukuba-shi, Ibaraki 300-2635 Japan

Introduction
Alzheimer's disease (AD) is the major cause of dementia in middle-to-old-age individuals and is now currently the fourth leading cause of death among adults in the United States alone. It is characterized by a progressive neurodegeneration affecting memory and by the presence of senile plaques, composed mainly of amyloid beta peptides and also by the presence of neurofibrillary tangles.
The recent success of donepezil and tacrine, two acetylcholinesterase inhibitors that enhance memory by elevating the level of acetylcholine in the brain of Alzheimer patients has stir some interests into the discovery of compounds that affect the cholinergic system. But because this class of drugs may be a symptomatic therapy for AD, research interests are shifting more recently toward the development of drugs that would affect amyloid- β peptide (Aβ) metabolism, which is now thought to be closely associated with the progression of AD.
At present, current AD therapies or the ones in development can be divided into 3 categories: cholinergic drugs, neuroprotective drugs, and drugs that affect Aβ metabolism either via enzymatic inhibition or immunotherapy (1).

Cholinergic and other symptomatic drug
A series of current cholinergic, neuroprotective and symptomatic drugs in development is shown in table I and II. There are two kinds of cholinergic drugs. The first category comprises acetylcholine agonists that directly stimulate the acetylcholine receptors (muscarinic or nicotinic). A second category includes cholinesterase inhibitors that increase endogenous acetylcholine concentration by inhibiting acetylcholinesterase and therefore indirectly stimulate the acetylcholine receptors.
The development of cholinergic agonists have currently been declined, because it is very difficult for muscarinic and nicotinic agonists to escape the peripheral cholinergic side effects such as diarrhea, nausea, vomiting, and abdominal pain.
The only currently approved AD drug that tackle into the cholinergic systems include the following cholinesterase inhibitors: tacrine, donepezil, rivastigmine and galantamine (2). Phenserine and huperzineA, two other acetylcholinesterase inhibitors are in clinical stage. Phenserine is distinct because it can also inhibit Aβ production in addition to its anti-cholinesterase effect. HuperzineA is the most potent cholinesterase inhibitor in development, and a once-a-week patch formulation is being developed in the USA (3). MKC-231, a compound that is having an accelerating effect on high affinity choline uptake is under PhII study (4).

Other symptomatic drugs in clinical phase II study include NS2330, a monoamine reuptake inhibitor such as norepinephrine, dopamine and serotonin, AC3993, a benzodiazepine inverse agonist and S-8510, a benzodiazepine partial inverse agonist.

Neuroprotective drug

Neuroprotective drugs are expected to slow down AD progression. In this category, memantine, an weak NMDA receptor antagonist was approved to treat moderate-to severe AD in Europe and America and is also under testing for its use to treat mild AD (5). Galantamine, a cholinesterase inhibitor, was also reported to have neuroprotective effect due to its modulation of the nicotinic receptor. However, clinical studies have not yet been able to clearly demonstrate that memantine and galantamine worked via a neuroprotective effect in patients. Another neuroprotective agent, ONO-2506PO is reported to affect astrocytes function and is now under clinical phase II in Europe (6). Icosapentaenoic acid ethyl (epadale capsule 300TM) now being used to treat hyperlipidemia and arteriosclerosis is now being tested for its effectiveness in AD. Finally, T-588 that reportedly shows neuroprotective effect to brain ischemia   and Aβ-induced neurotoxicity is under clinical testing (7).

Drug discovery target molecule for AD based on Aβ theory

Because it is assumed that Aβ may play a main role in the development of AD, major drug discovery efforts are now focusing on Aβ metabolism (Figure 1).
Current drug under investigation can be divided into 4 categories: (1) drugs that inhibit the production of Aβ coming from two distinct proteolytic activities by the β- and γ-secretases, (2) drugs that enhance the degradation of Aβ by two endopeptidases (neprylisine and IDE (insuline degrading enzyme)), (3) drugs that inhibit the polymerization of Aβ and, (4) drug that enhances Aβ disposition from brain by via an immunotherapy approach.

Aβ production inhibition therapy

The production of Aβ comes from the proteolytic action of two enzymes, β-secretase and γ-secretase. Inhibitors of these enzymes are now under development. It is now known that two single soluble proteins called BACE1 (8) and BACE2 (9) possesses β-secretase activity and that the γ-secretase activity is carried out by a high molecular weight multi-proteins complex, comprised of preseniline, pen-2, aph1 and nicastrin (10).
Configuration analysis research has revealed that the β-secretase catalytic pocket is quite big and similar to other aspartyl protease therefore the discovery of a small molecular weight β-secretase inhibitor has not been reported yet, although peptidomimetic β-secretase inhibitors were discovered (11). The development of other more selective allosteric inhibitors is now actively being pursued.
On the other hand, several low molecular γ-secretase inhibitors that are active orally have been reported. The results of a PhI study with Eli Lilly's LY450139 was presented at the 9th International Conference for Alzheimer's disease and related diseases (ICAD) in Philadelphia (12). Although these gamma-secretase inhibitors can potently reduce Aβ production (13), they are likely to cause side effects since the gamma-secretase complex is important for the processing of several transmembrane signaling molecules including NOTCH which is important to regulate cell differentiation (14).
.
In recent years, some non-steroidal anti-inflammatory drug (like sulindac, flurbiprofen) have been shown to modulate the γ-secretase activity by shifting the production of the

most toxic abeta species ($A\beta_{1-42}$) to shorter less toxic forms, mainly $A\beta_{1-38}$ (15). In addition, it became clear that this activity does not depend on cyclooxygenase inhibition, but rather is related with the chemical structure characteristic of certain non-steroidal anti-inflammatory drug. This observation is offering new clues on how to develop a gamma-secretase inhibitor with limited side effects.

$A\beta$ degradation
Dr. Saido and colleagues at RIKEN Brain Science Institute reported that some neuropeptides, including somatostatin could enhance the degradation of abeta peptides via the stimulation of neprylisine, a protease involved in brain $A\beta$ clearance. New approaches targeting $A\beta$ degradation are therefore expected to see the day in the near future (16).

.

$A\beta$ polymerization inhibitor
Neurochem reported that Alzhemed^{TM} binds $A\beta$ and this inhibits the polymerization of $A\beta$, which is a key process leading senile plaques formation. Alzhemed^{TM} was shown to reduce plaque formation in a transgenic Alzheimer mouse model. This compound was confirmed to be safe and tolerable in a PhII study and is now undergoing PhIII stage (17). It is expected to slow the progression of AD.

Clioquinol (Chinoform) inhibits the formation of senile plaque by binding to zinc and cupper ions that are closely related to the polymerization of $A\beta$ (18). However, this compound caused SMON in Japan several years ago when it was used as an antibiotic. Prolonged use of clioquinol caused depletion of vitamin B12, so co-administration with vitamin B12 is expected to prevent SMON to develop.
Clioquinol was co-administered with vitamin B12 supplements in a PhII study and is now under PhIII study. Prana Biotechnology, who is conducting the clioquinol study is also pushing PBT-1, another drug with a similar mechanism, in PhII.

Immunotherapy
AN1792, an AD vaccine using $A\beta_{42}$ peptide, was the first attempt developed to use immunotherapy to cure AD patients. After promising pre-clinical results in several species (mice, rabbits, guinea pigs, and monkeys) (19), clinical trials were initiated with adjuvant QS-21. Although phase I trials showed good tolerability, Ph IIa trials were halted when 18 of 298 patients immunized with AN-1792 presented symptoms consistent with meningioencephalitis (20). Importantly, pathological examination of the brain at the autopsy revealed there was a reduction of senile plaque burden after vaccine inoculation suggesting that the immunotherapy approach was working.

In addition, additional results of the Ph IIa study were reported at ICAD. A rise of antibody titer was observed in 59 cases out of 300 in the patients that received the vaccine. Data showed a trend in improved memory in vaccine responders compared with placebo. However, other cognitive analysis, such as Mini-Mental State Examination, showed no improvement (21).
Brain imaging analysis also revealed a reduction of the brain volume in vaccine responders. It is speculated that the loss in brain volume could be due to the clearance of

amyloid plaques and/or reduction of brain edema. In four autopsy cases, a depletion of brain amyloid plaques was seen and this indicates that amyloid immunotherapy can elicit an immune response that, in some cases, could lead to amyloid clearance.

Recently, passive immunization, where an antibody is directly injected into the blood is getting a lot of interest and is being pursued by several companies. It is believed that this approach will be safer because it is likely to avoid the triggering of a full-scale humoral immunity, which may have cause the death of patients in the initial AN1792 trial.

Dr. Tabira and colleagues (22) at NLS prepared an orally administrable $A\beta_{43}$ or $A\beta_{21}$ DNA vaccine using the Adeno-associated virus vector to induce selective humoral immunity by targeting the gut-associated immune system. In tg2576 mice, the DNA vaccine potentially activated the gut immune system and induced a selective humoral immune response that significantly reduced amyloid deposits in mouse brain.

Dr. Zhang and colleagues (23) at Peking Union Medical College also took a similar approach, but added the incorporation of cholera toxin B DNA vaccine using the naked plasmid. The DNA vaccine was administered intramuscularly and selectively elucidated a humoral immunoresponse that reduced the amyloid deposits in APP Tg mice brain. Finally, another pursued alternative approach to reduce $A\beta$ deposit is passive immunization using anti-$A\beta$ monoclonal antibody (24). This is the approach being developed now by Elan/Wyeth using humanized anti-$A\beta$ monoclonal antibody. A Ph I study has started after the fourth quarter in 2003 and is ongoing. In addition, Elan/Wyeth is trying to develop a conjugated vaccine that is designed to initiate a response that will allow escaping the full activation of the cellular immunity system.

There are two possible explanations for the plaque reduction seen with the immunotherapy approaches (). One explanation is that anti-$A\beta$ antibodies pass into the brain and activate microglial cells that in turn reduce amyloid deposit by phagocytosis (25). A second explanation is that anti-$A\beta$ antibodies present in the blood bind $A\beta$ peptides in the blood and this stimulates $A\beta$ transport from brain to blood by a concentration gradient. This is referred in the literature as the sink theory (26).

Conclusion

For many years, AD has been thought to be non-treatable. However, AChE inhibitors, like donepezil, and some other symptomatic drugs offered a first hope that that this disease could be treatable. These drugs are and will still be helpful but there is no doubt that the cost of this disease both in terms of human suffering and economics will be enormous unless therapeutical treatments that can prevent or stop its progression are found. At the present time, given the probable pivotal role of $A\beta$ in AD, drugs that affect $A\beta$ metabolism or its clearance from the brain are the ones expected to fill up this role.

In addition, because it is believed that patients affected by mild cognitive impairment (MCI) are often likely to develop AD (27), as it was well illustrated at the last 9th ICAD conference, it may be warranted in the future to apply this category of drugs on MCI patients.

Ferences

1. Scarpini, E., Scheltens, P. and Felsman, H. (2003). Treatment of Alzheimer's disease: current status and new perspectives. *Lancet Neurol.* **2**, 539-547

2. Lanctot, K., L, Herman, N. et al. (2003) Efficacy and safety of cholinesterase inhinitors in Alzheimer's disease: a meta-analysis. *CMAJ.* **169**, 557-564

3. Quan, D. and Wang, Y, (2004) A novel treatment for Alzheimer's disease- Huperzine a transdermal deliverly system. *ICAD* S196 (P1-348)

4. Akaike, A., Maeda, T., et al. (1998) Protective effect of MKC-231, a novel high affinity choline uptake enhancer, on glutamate cytotoxicity in cultured cortical neurons. *Jpn. J. Pharmacol.* **76**. 219-222

5. Tariot., P. N., Farlow M. R., et al. (2004) Memantine treatment in patients with with moderate to severe Alzheimer disease already receiving donepezil: a randomized control trial. *JAMA* **291**, 317-324

6. Tateishi, N., Mori, T., et al. (2002) Astrocytic activation and delayed infarct expansion after permanent focal ischemia in rats. Part II: suppression of asrocytic activation by a novel agent ®-2- propyloctanoic acid (ONO-2506) leads to mitigation of delayed infarct expansion and early improvement of neurologic deficits. *J. Cereb. Blood Flow Metab.* **22**, 723-734

7. Yamamoto, A., Ago, Y. et al. (2003) Protective effect of T-588 on toxic damage by serum deprivation and amyloid-β protein in culture neurons. *J. Pharmacol. Sci.* **92**, 153-156

8. Yan, R., Han, P. et al. (2001) The transmembrane domain of the Alzheimer's beta-secretase (BACE1) determines its late Golgi Localization and access to beta-amyloid precursor protein (APP) substrate. *J. Biol. Chem.* **276**, 36788-96

9. Farzan, M, Schnitzer, C., E. et al. (2000) BACE2, a beta secrerase homolog, cleaves at the beta site and within the amyloid-beta region of the amyloid-beta precursor protein. *Proc. Natl. Acad. Sci. USA* **97**, 9712-17

10. Takasugi, N., Tomita, T. et al (2003) The role of presenilin cofactors in the γ-secretase complex. *Nature* **422**, 438-441

11. Ghosh, A., K., Bilcer, G, et al (2001) Structure based design: potent inhibitors of human brain mepasin 2 (beta-secretase). *J. Med. Chem.* **44**, 2865-68

12. Gitter, B., D., Czilli, D., L, et al. (2004) Stereoselective inhibition of amyloid beta peptide secretion by LY450139, a novel functional gamma secretase inhibitor. *ICAD*, S-571 (P4-339)

13. Lanz, T., A., Himes, C., S., et al. (2003) The γ-secretase inhibitor *N*-[*N*-(3,5-difluoro-phenacetyl)-L-alanyl]-*S*- phenylglycine *t*-butylester reduces Aβ levels in vivo in plasma and cerebral fluid in young (plaque-free) and aged (plaque-bearing) Tg2576 mice. *J. Pharmacol. Exp. Ther.* **305**, 864-871

14. Petit, A., Pasini, A, et al. (2003) JLK isocoumarin inhibitors: selective γ-secretase inhibitors that do not interfere with Notch pathway in vitro or in vivo. *J. Neurosci. Res.* **74**, 370-377

15. Takahashi, Y., Hayashi, I., et al. (2003) Sulindac sulfide is a noncompetitive γ-secretase inhibitor that preferentially reduces Ab_{42} generation. *J. Biol. Chem.* **278**, 18664-18670

16. Iwata, N., Mizukami, H., et al. (2004) A possibility of gene therapy for Alzheimer's disease by neprilysin gene transfer. *ICAD* S590 (P4-406)

17. Aisen, P., A., Mehran, M., et al. (2004) Clinical data on Alzhemed^{TM} after 12 months of treatment in patients with mild to moderate Alzheimer's diseased. ICAD S20 (O1-05-06)
18. Bush, A. (2004) Copper and zinc in Alzheimer's disease: from bench to clinic. ICAD S69 (S4-02-06)
19. Schenk, D., Barbour, R., et al. (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* **400**, 173-177
20. Orgogozo, J., M., Gilman, S., et al. (2003) *Neurology* **61**, 46-54
21. Hock, C., Konietzko, U., ea al (2003) *Neuron* **38**, 547-554
22. Tabira, T., and Hara, H. (2004) Oral a beta vaccine is safe and effective. *ICAD* S64 (O3-06-04)
23. Zhang, J., Xu, S., et al (2003) *Neurobiol. Dis.* **14**, 365-379
24. Solomon, B., Koppel, R., et al (1997) Disaggregation of Alzheimer beta-amyloid by site-directed mAβ. *Proc. Natl. Acad. Sci. USA* **94**, 4109-4112
25. Bard, F., Cannon, C., et al. (2000) Peripherally administered antibodies against amyloid beta peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat. Med.* **6**, 916-919
26. Demattos, R., B., Bales, K., R., (2001) Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* **98**, 8850-8855
27. Petersen, R., C., Smith, G., E., et al. (1999) Mild cognitive impairment : clinical characterization and outcome. *Arch. Neurol.* **56**, 303-38

Drugs for Alzheimer's Disease in Clinical Research (I): Cholinergic, Other Symptomatic, and Neuroprotectants

Compound	Company	Stage	Action Mechanism
Cholinergic			
Galantamine	Jansen	Jap. : PhaseⅢ	AChE Inh.、 Nicotinic Modulation
Phenserine	Axonyx	West. : PhaseⅢ	AChE Inh.、 Aβ産生抑制
Huperzine A	Debiopharm./	West. : Phase Ⅱ	AChE Inh.: Once a Day Prodrug
	Xel Herbaceutical	Chaina : Phase Ⅰ	AChE Inh.: Once a Week Patch
MKC-231	Mitsubishi	West. : Phase Ⅱ	High Affinity Choline Uptake Enhancer
Other Symptomatic			
NS 2330	Nippon ＢＩ	Jap. : Phase Ⅰ	Monoamine Reuptake Inhibitor
	ＢＩ	West. : Phase Ⅱ	
AC 3933	Dainippon	Jap. : Phase Ⅰ	Benzodiazepine Inverse Agonist
	(Licensee Aventis)	West. : Phase Ⅱ	
S-8510	Shionogi	Jap. : Phase Ⅱ	Benzodiazepine Partial Inverse Agonist
	(Licensee GSK)	West. : Phase Ⅰ	
Neuroprotectants			
Memantine	Daiichi Suntory	Jap. : Phase Ⅱ	ＮＭＤＡ Antagonist
ONO-2506PO	Ono	Jap. : Phase Ⅰ	Astrocyte Modulator
		West. : Phase Ⅱ	
Ethyl Icosapentate	Mochida	Jap. : Phase Ⅱ	Anti-Hyperlipidemia (Neuroprotection)
T-588			
	Toyama Chemical	Jap. : Phase Ⅱ	Neuroprotection
Rasagiline		West. : Phase Ⅱ	
	Teva／Eisai	West. : Phase Ⅱ	ＭＡＯ－Ｂ Inhibitor (Neuroprotection)

Drugs for Alzheimer's Disease in Clinical Research (II): Amyloid β Related Therapy

Compound	Company	Stage	Action Mechanism
A β - Formation Inhibitor 　LY450139	Eli Lilly	West. : Phase I	γ -Secretase Inhibitor
A β - Polymerization Inhibitor 　Alzhemed™ 　Clioquinol	Neurochem Prana	West. : Phase III West. : Phase II	A β - Polymerization Inhibitor Zinc, Copper Chelator
Immuno-therapy 　ABB-001	Elan/Wyeth	West. : Phase I	Human Anti-A β Monoclonal Antibody

Drug Discovery Approach for Alzheimer's Disease
- Targeting Aβ processing -