Review

Plant-based vaccines for Alzheimer’s disease

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(Communicated by Masanori OTSUKA, M.J.A.)

Abstract: Alzheimer’s disease (AD) is one of the major causes of chronic and progressive cognitive decline, with the pathological hallmarks of senile plaques and neurofibrillary tangles. Amyloid β peptide (Aβ) is the main component of senile plaques, and the pathological load of Aβ in the brain has been shown to be a marker of the severity of AD. To prevent the accumulation of plaques, novel and safer plant-based vaccine strategies have been suggested. In this review, we summarize the results of plant vaccines against Aβ.

Keywords: amyloid β protein, oral vaccine, plant-based vaccine, Alzheimer’s disease

Introduction

In Japan, Alzheimer’s disease (AD) is the most common type of dementia, accounting for 63% of dementia cases (Fig. 1). The increase in the proportion of elderly people aged 65 or older is expected to exceed 40% of the population. It is also estimated that 81.1 million people will eventually suffer from AD in the World Health Organization (WHO) regions. The WHO has emphasized that finding new treatments or preventive measures for AD are vitally important.

AD is one of the major causes of chronic and progressive age-associated cognitive decline. Its characteristic pathological hallmarks are extracellular senile plaques and intracellular hyperphosphorylated tau protein in neurofibrillary tangles, and brain weight loss.

To date, three genes have been found to be mutated in early onset AD: presenilin 1 (PS1), presenilin 2 (PS2), and amyloid precursor protein (APP). PS1 and PS2 are subunits of γ-secretase, which produces Aβ from the substrate APP. The results indicate that mutations in the Aβ production pathway are the major cause of juvenile AD, whereas apolipoprotein E (ApoE) was identified as the gene responsible for late-onset AD in people around the age of 70 years. The E4 isoform of ApoE increased the risk of developing late-onset AD 11-fold, and it also increased the risk of dementia due to sports-induced brain injury 4-fold.

Clinical trials based on the amyloid cascade hypothesis have been conducted, many of which have failed in phase III. These include solanezumab (monoclonal antibody against soluble Aβ), bapineuzumab (monoclonal antibody against the N-terminal portion of Aβ), verubecestat (a BACE1 inhibitor), and semagastat (a γ-secretase inhibitor) among others. For example, doses of verubecestat of 12 and 40 mg per day for 78 weeks did not reduce the cognitive or functional decline in patients with mild-to-moderate AD, although PET analysis showed that it reduced the concentration of Aβ 40 and Aβ 42 in the cerebrospinal fluid and the total brain amyloid load.

It is very difficult to cure patients in the later stages of AD. To improve the outcomes of clinical trials, therapies should be initiated prior to the accumulation of Aβ in the pre-symptomatic stages. After the onset of the disease, drugs have limited effects on preexisting Aβ plaques. In addition, effective biomarkers, such as Aβ imaging and CSF- or serum-based biomarkers are needed, because approximately 20–30% of individuals enrolled in trials with a clinical AD diagnosis did not suffer from a pure form of AD.

Active vaccines

Aβ is the major constituent of the senile plaques in patients with AD. It consists of 40–42 amino acids,
and the longer Aβ42 has a greater propensity to aggregate than the shorter Aβ340. Aβ is produced from APP by β- and γ-secretases and is then secreted into the extracellular space. Less Aβ342 is secreted than Aβ340, but it can form neurotoxic oligomers.

Because the major peptide constituent of amyloid plaques is Aβ42, the first active vaccine, AN1792, consisted of human aggregated Aβ42 with QS21 saponin adjuvant. Although a phase I study indicated a positive antibody response by muscle injection and a reduced decline in Disability Assessment for Dementia score, the adjuvant triggered a severe inflammatory response, resulting in meningoencephalitis in 18 out of 298 subjects in a phase II trial. However, none of the 74 patients in the control group developed meningoencephalitis. At 1 year after vaccination, 19 antibody-generating responders remained unchanged on the Mini-Mental State Examination (MMSE) and their Disability Assessment for Dementia scores declined compared with the controls. Although the AN1792 trials were terminated due to the meningoencephalitis, it showed a promising antibody response and slowed functional decline.

After the AN1792 trial, several Aβ vaccines underwent clinical trials. CAD106 targeted antibody production against Aβ1–6 to serve as a B-cell epitope without generating a T-cell response. ACC-001 was an Aβ1–7 fragment coupled to a nontoxic diphtheria toxin, and AD02 used the N-terminal 6 amino acids of Aβ.

Phase II trials of ACC-001 were terminated in 2014 because of adverse effects associated with autoimmune responses. The AD02 study was terminated by the sponsor based on the results of a follow-up extension study. However, following on from these trials, the next generation of active vaccines should target more specific epitopes to induce more favorable immune responses.

Oral vaccines

During the course of the experiments to find a vaccine, it was noted that oral vaccination was safer than subcutaneous injection. In the human body, immunoreactivity against the self is usually suppressed. This phenomenon is known as immunotolerance, which is affected by the balance between Th1 and Th2 helper T cells. When Th1 cells are activated, cellular responses against the self increase, and this leads to the development of autoimmune diseases such as meningitis. When Th2 cells are activated, antibody production is induced. Safer vaccine therapies using the Th2 reaction via mucosal immunization are more desirable. In mucosal immunization, immunotolerance can be induced, and adjuvants delivered via the antigen must be taken into consideration.

Previous results using Aβ as an antigen suggested that the inflammatory Th1 response was significantly reduced by oral immunization. In most clinical cases, however, vaccinations are usually delivered by subcutaneous injection. Although this method resulted in a high level of immunization, adverse effects should be avoided. These findings may help to develop a safe and cost-effective immunotherapy protocol.

Plant-based vaccines against AD

The first genetically modified staple crop was Golden Rice. GM technology was used to engineer the biochemical pathway for the synthesis of provitamin A into the starch-storing tissue of the rice seed. Although there have not been large-scale trials to study the safety of GM-food, Golden Rice is not marketed. Recently, however, this technology has provoked opposition by anti-GM-advocates but, despite this, the medical use of GM-crops has gradually gained public support.

The first trial to express Aβ in plants was undertaken by Kim et al. They expressed human Aβ42 in potato. A transgenic potato carrying 5 tandem repeats of a mutated version of Aβ expressed Aβ and the immunogenic properties of the chimeric protein (5Aβ42) were reported to be elevated. In 2004, a GFP-fused Aβ42 protein was expressed in the leaves of green pepper Capsicum annuum. An oral vaccination was tested in the Tg2576 transgenic mouse model of AD resulting in anti-Aβ antibodies being effectively induced after oral immunization. A detailed study suggested no inflammatory reactions after oral administration and a reduction of Aβ in the immunized Tg2576 mice. In contrast, subcutaneous injection of the same antigen also reduced Aβ accumulation in spite of a severe inflammatory Th1 response.

Following these experiments, several laboratories attempted to express Aβ in plants, including tomato, tobacco, and rice. Rice lines were developed to express a GFP-Aβ fusion protein (Fig. 2). This was reviewed as “the most advanced plant-based Alzheimer’s vaccination model” by Rosales-Mendoza et al. The results are summarized in Table 1.

Vaccine therapy should have no side effects. Oral or mucosal vaccinations have fewer side effects.
than those induced by injection. Therefore, to express Aβ in an edible plant is important for new-generation therapies. Edible vaccines could also be produced in GM-crops such as rice or soybeans because Aβ accumulated in seeds could be stored for long periods and transported without refrigeration. Aβ42 fused with GFP was introduced into rice using an Agrobacterium-based method. Fluorescence was localized mainly in the aleurone layer of brown rice, whereas there was little fluorescence in polished rice. Therefore, brown rice should be eaten for an AD vaccination to succeed.

Next, Tg2576 mice were orally immunized. Mice were fed Aβ rice mixed with the non-toxic cholera toxin B subunit to raise the antibody titer. Immunization induced a non-inflammatory Th2 reaction as was seen in the green pepper study. Intracerebral Aβ decreased and the vaccine improved memory, as assessed using a Y-maze test. Taken together, these results suggested that the new edible rice vaccine may have therapeutic effects in AD.

Problems with oral vaccination

Oral vaccination against AD is an ideal preventive measure, because it is safe and cost-effective. However, to increase the quantity of the antibody titer in oral vaccines is very difficult and it is usually necessary to utilize an oral adjuvant. The first clinical trial of AN1792 used saponin QS21 as an adjuvant but it triggered severe side effects by intramuscular injection. Therefore, a suitable adjuvant should be employed even in oral vaccination. The cholera toxin B subunit has been used in animal experiments but not in human experiments.

Medicinal herbs are a potential choice as adjuvants because they may contain natural saponins and they have inherent beneficial effects, such as anti-oxidant, detoxification, and sedation activities. We are currently trialing the expression of Aβ in medicinal herbs.

Conclusions

Two types of immunization, active and passive, have been employed in humans. The former uses antigens and the latter uses antibodies. Passive immunity has been trialed, and positive results have been obtained in cancer treatments using humanized monoclonal antibodies. However, passive immunity has side-effects such as nausea and hemorrhagia, as well as having a high cost. From this perspective, active immunity is more suitable for the treatment of chronic diseases due to its low cost and safety history. Active immunity could prevent the onset of chronic diseases.
A recent report indicated that aducanumab, a monoclonal antibody being tested in symptomatic AD, appears to be capable of reducing Aβ aggregation and, in some cases, it can eliminate the Aβ signal in a dose-dependent manner. Although it is possible to get rid of the Aβ, it is not clear whether it is possible to restore the cognitive function of the patients. In addition, it is unlikely that intravenous injection of antibodies will be of practical use. The patients have to be injected periodically over a prolonged period of time. Other lifestyle activities, such as physical exercise, nutrition and educational level, as well as combination therapies with other standard AD drugs are also important in preventing the disease.

References
1) Intiaz, B., Tolppanen, A.M., Kivipelto, M. and Soininen, H. (2014) Future direction in Alzheimer’s disease from risk factors to prevention. Biochem. Pharmacol. 88, 661–670.
2) Selkoe, D.J. and Schenk, D. (2003) Alzheimer’s disease: Molecular understanding predicts amyloid-based therapeutics. Annu. Rev. Pharmacol. Toxicol. 43, 545–584.
3) Querfurth, H.W. and LaFerla, F.M. (2014) Alzheimer’s disease. N. Engl. J. Med. 362, 329–344.
4) Solomon, S., Sperling, R.A., Blennow, K., Klunk, W., Raskind, M. et al. (2014) Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer’s disease. N. Engl. J. Med. 370, 322–333.
5) Doody, R.S., Thomas, R.G., Farlow, M., Ivatsuboto, T., Vellas, B., Joffe, S. et al. (2014) Phase 3 trials of solanezumab in mild-to-moderate Alzheimer’s disease. N. Engl. J. Med. 370, 311–321.
6) Egan, M.F., Kost, J., Tariot, P.N., Aisen, P.S., Cummings, J.L., Vellas, B. et al. (2018) Randomized trials of Verubecestat for mild-to-moderate Alzheimer Disease. N. Engl. J. Med. 378, 1691–1703.
7) Vinals, J. and Sanz-Ros, J. (2018) Alzheimer’s disease: Only prevention makes sense. Eur. J. Clin. Investig. 48, e13005.
8) Golde, T.E., DeKosky, S.T. and Galasko, D. (2018) Alzheimer’s disease: The right drug, the right time. Science 362, 1250–1251.
9) Vinals, J. and Sanz-Ros, J. (2018) Alzheimer’s disease: The right drug, the right time. Science 362, 1250–1251.
10) Beyer, A.J., Bullock, R., Jones, R.W., Wilkinson, D., Paterson, K.R., Jenkins, L. et al. (2005) Evaluation of the safety and immunogenicity of synthetic Aβ342 (AN1792) in patients with AD. Neurology 64, 94–101.
11) Orgogozo, J.M., Gilman, S., Dartigues, J.F., Laurent, B., Puel, M., Kirby, L.C. et al. (2003) Subacute meningoencephalitis in a subset of patients with AD after Aβ42 immunization. Neurology 61, 46–54.
12) Hock, C., Konietzko, U., Streffer, J.R., Tracy, J., Signorell, A., Mueller-Tillmans, B. et al. (2003) Antibodies against β-amyloid slow cognitive decline in Alzheimer’s disease. Neurology 63, 547–554.
13) Vellas, B., Black, R., Thal, L.J., Fox, N.C., Daniels, M., Tompkins, C. et al. (2009) Long-term follow-up of patients immunized with AN1792: Reduced functional decline in antibody responders. Curr. Alzheimer Res. 6, 144–151.
14) Sterner, R.M., Takahashi, P.Y. and Yu Ballard, A.C. (2016) Active vaccines for Alzheimer disease treatment. J. Am. Med. Dir. Assoc. 17, 862.e11–862.e15.
15) Potrykus, I. (2010) Lessons from the “humanitarian golden rice” project: Regulation prevents development of public good genetically engineered crop product. N. Biotechnol. 27, 466–472.
16) Kim, H.S., Eunym, J.W., Kim, M.S., Lee, B.C., Mook-Jung, I., Jeon, J.H. et al. (2003) Expression of human β-amyloid peptide in transgenic potato. Plant Sci. 165, 1445–1451.
17) Youm, J.W., Kim, H., Han, J.H., Jang, C.H., Ha, H.J., Mook-Jung, I. et al. (2005) Transgenic potato expressing Aβ reduce Aβ burden in Alzheimer’s disease mouse model. FEBS Lett. 579, 6737–6744.
18) Szabó, B., Hori, K., Nakajima, A., Sasagawa, N., Watanabe, Y. and Ishiura, S. (2004) Expression of amyloid-βI–40 and 1–42 peptides in Capsicum annuum var. angulosum for oral immunization. Assay Drug Dev. Technol. 2, 383–388.
19) Ishii-Katsuno, R., Nakajima, A., Katsuno, T., Nojima, J., Futai, E., Sasagawa, N. et al. (2010) Reduction of amyloid β-peptide accumulation in Tg2576 transgenic mice by oral vaccination. Biochem. Biophys. Res. Commun. 399, 593–599.
20) Youm, J.W., Jeon, J.H., Kim, H., Kim, Y.H., Ko, K., Joung, H. et al. (2008) Transgenic tomatoes expressing human β-amyloid for use as a vaccine against Alzheimer’s disease. Biotechnol. Lett. 30, 1839–1845.
21) Yoshida, T., Kimura, E., Koike, S., Nojima, J., Futai, E., Sasagawa, N. et al. (2011) Transgenic rice expressing amyloid β-peptide for oral immunization. Int. J. Biol. Sci. 7, 301–317.
22) Nojima, J., Ishii-Katsuno, R., Futai, E., Sasagawa, N., Watanabe, Y., Yoshida, T. et al. (2011) Production of anti-amyloid β antibodies in mice fed rice expressing amyloid β. Biosci. Biotechnol. Biochem. 75, 396–400.
23) Nojima, J., Maeda, A., Aoki, S., Suo, S., Yanagihara, D., Watanabe, Y. et al. (2011) Effect of rice-expressed amyloid β in the Tg2576 Alzheimer’s disease transgenic mouse model. Vaccine 29, 6252–6258.
24) Rosales-Mendoza, S., Rubio-Infante, N., Zarazua, S., Govea-Alonso, D.O., Martel-Gallegos, G. and Moreno-Fierros, L. (2015) Plant-based vaccines for Alzheimer’s disease: An overview. Expert Rev. Vaccines 13, 429–441.
25) Sevigny, J., Chiao, P., Bussiere, T., Weinreb, P.H., Williams, L., Maier, M. et al. (2016) The antibody aducanumab reduces Aβ plaques in Alzheimer’s disease. Nature 537, 50–56.

26) Cummings, J.L., Tomg, G. and Ballard, C. (2019) Treatment combinations for Alzheimer’s disease: Current and future pharmacotherapy options. J. Alzheimers Dis. 67, 779–794.

(Received Jan. 23, 2019; accepted Mar. 22, 2019)

Profile

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