The neuroprotective effect of immune serum of adeno-associated virus vaccine containing Aβ1-15 gene on amyloid toxicity

Ling-Yun Liu, Yuan-Yuan Ma¹, Tao Yang², Xin Li, Wen Li

Department of Neurology, Yangpu Central Hospital, Shanghai, People’s Republic of China; ¹State Key Laboratory of Medical Neurobiology, Department of Neurobiology, Shanghai Medical College, Fudan University, Shanghai, People’s Republic of China; ²Cancer Stem Cell Institute, Research Center for Translational Medicine, East Hospital, Tongji University School of Medicine, Shanghai, People’s Republic of China

Abstract

Objective: The aim of this study was to explore the effect of adeno-associated virus (AAV) serotype 2 vector vaccine containing amyloid-β peptide (Aβ) 1-15 fragment (AAV-Aβ15) immunized mice sera on counteracting Aβ1-42 peptide toxicity towards a primary culture cortical neurons. Materials and Methods: BALB/c mice were vaccinated via the intramuscular immunization route with AAV-Aβ15. The anti-Aβ antibody titer of immunized mice sera was quantified by sandwich Enzyme-Linked ImmunoSorbent Assay. The toxicity of Aβ1-42 peptide on neurons was assessed by morphology with an inverse microscopy and cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Results: AAV-Aβ15 could induce an Aβ-specific immunoglobulin G (IgG) humoral immune response in /c mice the anti-Aβ antibodies were detectable at 1 month after immunization, significantly increased at 2 and 4 months after immunization, and the immunized sera could attenuate cytotoxicity of Aβ1-42 peptide on primary culture cortical neurons. Conclusions: The immune serum of AAV-Aβ15 could play a neuroprotective effect against Aβ1-42 peptide toxicity, which would be beneficial for Alzheimer’s disease patients.

Key Words

Alzheimer’s disease, amyloid-beta, immunotherapy, vaccine

For correspondence:

Ling-Yun Liu, Ph.D., Department of Neurology, Yangpu Central Hospital, 450 Teng-Yue Road, Shanghai, 200090, People’s Republic of China. E-mail: liulynh@126.com

Ann Indian Acad Neurol 2013;16:603-8

Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized clinically by loss of memory and the cognitive functions. Symptoms of AD initially manifest as mild forgetfulness; however, lapse into mental confusion and agitation, followed by severe dementia, and death, which severely affected the life quality of aged people. The neuropathological hallmarks of AD are intracellular neurofibrillary tangles made of hyperphosphorylated microtubule associated protein, extracellular senile plaques composed of the amyloid-β peptide (Aβ) surrounded by dystrophic neurites with different numbers of activated microglia and reactive astrocytes. However, the primary cause of AD has not been elucidated clearly and no satisfied therapy for the AD to date. There are many theories about the pathomechanism of AD. For example, the “cholinergic hypothesis” has been postulated to explain the molecular mechanism of AD, which states loss of cholinergic markers and decline in acetylcholine neurotransmitter leading to impairment of cognitive and memory functions. Cholinesterase inhibitors, such as donepezil, rivastigmine, and galantamine, provide only symptomatic relief from the cognitive impairment but do not stop the progressive mental and behavioral decline.

In the past years, significant progress has been made in the understanding of the pathogenic mechanism of AD, and new therapeutic targets have become available that should allow the underlying disease process to be tackled directly. In this respect, the “amyloid cascade hypothesis” has become the dominant theory in the field. It is believed that Aβ accumulation in senile plaques plays a primary role in progression of AD, which initiates pathological processes including oxidative stress, disrupted calcium homeostasis, chronic inflammation, generation of
radicals, induction of apoptosis leads to cognitive impairment, and dementia in the end of life. Thus, therapies targeted at the clearance of Aβ or the prevention of its deposition and/or aggregation may be beneficial for treatment of AD. Various approaches to prevent aggregation of Aβ have been investigated. These methods include inhibition of secretase functions to reduce the amount of Aβ, inhibit amyloid fibrillation, and enhance the clearance of Aβ by small molecules, but none of the aforementioned treatments has produced satisfactory curative effects.[5] Immunotherapy may be one of the most promising strategies for prevention of Aβ deposition.[6] Aβ1-42 peptide active immunotherapy has been shown to elicit the high titer of anti-Aβ antibody, decrease amyloid burden in the brain and improve cognitive function in the transgenic mouse models.[7,8] These impressive results prompted the initiation of phase I/II clinical trials to evaluate the safety and tolerability of Aβ vaccination (AN1792). However, The phase IIA clinical trial (AN1792) was halted due to aseptic T-lymphocyte meningoencephalitis found in 6% of AD patients.[9] Thus, safer immunization modalities should focus on minimizing T-cell mediated inflammatory responses in efforts to prevent central nervous system (CNS) invasion of auto aggressive T-cells, at the same time promoting Aβ antibody-mediated clearance mechanisms.

Previous clinical investigation has revealed that B-cell-activating epitopes that elicit antibodies responses in humans and mice are located within the Aβ1-15 region while T-cell epitopes present in the C-terminus of Aβ are thought to be responsible for the adverse autoimmune inflammatory response. This segregation of T- and B-cell epitopes within the Aβ molecule allows the opportunity to induce anti-Aβ antibodies in the absence of Aβ-specific T-cell response. Moreover, some data also indicated that alternative immunogens encompassing the N-terminal epitope of Aβ (B-cell epitopes), generated specific antibodies against Aβ and elicited B-cell-mediated humoral immune responses without a significant T-cell-mediated immune response in mice.[10] At the same time, it is particularly crucial to adopt an immunization delivery system for preventing the AD besides selecting suitable Aβ fragment as antigen.

Recently, due to its inherent versatility, gene-based vaccination technology may allow for greater precision in antigen presentation and immunomodulation that could lead to safer and more efficacious vaccines for AD.[11] Among many viruses being developed as vectors for the human gene therapy currently, adeno-associated virus (AAV) is one of the most promising tools because AAV is non-pathogenic, non-toxic with low immunogenicity, and allows long-term gene expression in many tissues, including non-dividing cells like neurons. AAV has been successfully used in a number of animal models of neurological disorders. It is thought that AAV represents a well-positioned platform on which to build an Aβ-directed AD vaccine.[12]

Based on the above background, in the current study, we constructed an AAV serotype 2 vector vaccine containing Aβ1-15 gene fragment (AAV-Aβ15) and determined if immunization with the AAV vaccine would result in an adequate humoral immune response in mice. Furthermore, we explored the effect of immunized sera on counteracting cytotoxicity of Aβ1-42 in the primary culture cortical neurons.

### Materials and Methods

#### Construction of AAV plasmid pSNAV-Aβ15

The open reading frame of the human Aβ1-15 gene was chemically synthesized with the codons optimized for expression in mammalian cells (Bioyong Technologies Inc.), and then cloned into an immunization vector pSNAV2.0 between the strong enhancer/promoter of the cytomegalovirus (CMV) immediate early genes and the bovine growth hormone polyadenylation signals (BGH polyA). The constructed plasmid pSNAV-Aβ15 also contains two packaging signal inverted terminal repeats of AAV and a neomycin resistant gene (NEO). The plasmid pSNAV-Aβ15 was amplified in DH5α-cells and purified using a plasmid preparation kit (Qiagen Company). The presence of the Aβ1-15 gene was confirmed by sequencing. The diagrams of the base plasmid pSNAV2.0 and the constructed plasmid pSNAV-Aβ15 are shown in Figure 1.

#### Production of AAV vaccine AAV-Aβ15

AAV serotype 2 vector vaccine containing Aβ1-15 gene fragment (AAV-Aβ15) was prepared by the standard methods. Briefly, AAV-Aβ15 was generated by pSNAV-Aβ15 plasmid transfection with helper plasmids in human embryo kidney (HEK) 293 T-cells. Forty-eight hours after transfection, the cells were harvested and lysed in phosphate-buffered saline (PBS) by freeze thawing repeat 3 times. The titer of AAV-Aβ15 was determined by dot-blot with digoxin labeled CMV probe. The purity of AAV-Aβ15 was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

#### Animal immunization

BALB/c mice were obtained from the SLAC laboratory animal limited liability company (Shanghai, China) at the age of 2 months. BALB/c mice were vaccinated via the intramuscular immunization route with PBS vehicle, AAV-lac and AAV-Aβ15. Each group consisted of 8 mice, 50 µl of AAV vector containing 5 × 10⁷ particles was injected into the right gastrocnemius muscle of each mouse. Blood was collected from the lateral tail vein at 1, 2, and 4 months after intramuscular injection. The blood was allowed to clot, and then placed at 4 overnight to facilitate separation of the serum from the clot. The clots were removed, and the serum centrifuged at 10,000 × g for 10 min to pellet any remaining

Based on the above background, in the current study, we constructed an AAV serotype 2 vector vaccine containing Aβ1-15 gene fragment (AAV-Aβ15) and determined if immunization with the AAV vaccine would result in an adequate humoral immune response in mice. Furthermore, we explored the effect of immunized sera on counteracting cytotoxicity of Aβ1-42 in the primary culture cortical neurons.

---

**Figure 1:** Schematic representation of newly constructed vector pSNAV-amylloid-β peptide 15. The plasmid pSNAV2.0 represented an empty vector control contained the cytomegalovirus immediate early genes and the bovine growth hormone polyadenylation signals.

**Aβ1-15 peptide:** DAEFRHDSGYEVHHQ

**Aβ1-15 gene:** gatgccagattcggcagataggaagtcattcaca

---

604 Liu, et al.: The neuroprotective effect of AAV-Aβ15 on amyloid toxicity

Annals of Indian Academy of Neurology, October-December 2013, Vol 16, Issue 4
blood cells and debris. The clarified serum was transferred to a fresh tube and stored at 20°C until analyzed by Enzyme-Linked ImmunoSorbent Assay (ELISA). All animal housing and experimental procedures were performed in compliance with the guidelines of the Medical Experimental Animal Administrative Committee of Shanghai.

ELISA analysis for the anti-αβ antibody in serum
Anti-αβ antibody titer was quantified by sandwich ELISA. Ninety-six-well microtiter plates were coated with 100 µl of 2 µg/ml synthetic human Aβ1-42 peptide (Genemed Synthesis, Inc.) in 50 mM carbonate buffer (pH 9.6) overnight at 4°C. The wells were washed four times with the PBS containing 0.05% Tween 20 (PBS-T), and blocked with 200 µl of blocking buffer (5% goat serum and 1% bovine serum albumin in PBS-T) for 4 h at room temperature. Mouse serum was prepared in the PBS at initial dilution of 1:16 and subsequent two-fold dilutions were made. All samples were run in duplicate and simultaneously incubated at 37°C for 1 h followed by washing 6 times with PBS-T. Plates were blocked a second time with blocking buffer for 30 min at 37°C followed by washing 5 times, 100 µl of HRP-conjugated goat anti-mouse IgG diluted to 1:10,000 in PBS were added to the wells and incubated for 1 h at 37°C. Plates were then washed 6 times with PBS-T and developed with 100 µl of 2 M sulphuric acid. Plates were read spectrophotometrically at 450 nm in a microplate reader. Antibody concentrations were calculated using a calibration curve generated with known concentrations of an anti-Aβ1-42 monoclonal antibody, 8G7 (Enzo Life Sciences International, Inc.).

Primary cortical neuronal culture and neutralization test by serum anti-αβ antibody
Cortical neurons were obtained from the cortex of embryonic day 15 mouse according to the procedures described previously with modifications. In brief, after removal of the blood vessels and pia mater, freshly dissected cortical tissues were sectioned into approximately 1-2 mm² fragments and digested with 0.125% trypsin for 20 min at 37°C. The digestion was terminated by the addition of Dulbecco’s modified Eagle’s medium (DMEM) (Gibco, Invitrogen Corporation) containing 10% fetal calf serum. The cells were seeded at a density of 8 × 10⁵ cells/ml on poly-l-lysine (0.1 mg/ml)-coated dishes in DMEM containing 2% B 27 supplement (Gibco, Invitrogen) and maintained in a humidified incubator (5% CO₂) at 37°C. After 24 h in vitro, the culture medium was replaced with Neurobasal medium containing 2% B 27 supplement (Gibco, Invitrogen Corporation). All experiments were performed using the 9-10 day old cultures. The prevention of Aβ neurotoxicity was measured as follows: 2 µM Aβ1-42 peptide was incubated for a week at 37°C to produce fibrils, and then incubated with the sera of immunized animals 1 month after the intramuscular injection with AAV-αβ15 at dilutions of 1:10 or 1:100 for 24 h. The reaction mixtures were added to the wells containing primary cultures of neurons and incubated at 37°C for 2 days. Cell viability was assessed by measuring cellular redox activity with 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT).

Statistical analysis
All results are expressed as mean ± SD. The significance of the differences among multiple groups was analyzed using one-way ANOVA. The difference was considered statistically significant when P < 0.05.

Results

Detection of purity and titer of AAV-αβ15
Capsid of AAV-2 is composed of three kinds of proteins: VP1, VP2, and VP3, with a molecular weight of 87, 72, and 62 kD, respectively. The ratio of VP1, VP2, and VP3 in AAV-2 capsid is 1:1:10. Therefore, three bands with specific patterns could be seen when AAV-2 virus was analyzed by SDS-PAGE. Analysis of the AAV-αβ15 purity by coomassie brilliant blue stained SDS acrylamide gel electrophoresis is shown in Figure 2. Three clear bands representing AAV capsid proteins VP1, VP2, and VP3 could be seen on lane 2, and extremely low background was seen on lane 2, which demonstrated that contaminants had almost been removed from AAV-αβ15. The purity of AAV-αβ15 was estimated to be more than 95%. AAV genome containing particles was determined by the dot-blot method using digoxigenin-labeled CMV probe. The physical titer of the purified AAV-αβ15 is estimated to be about 1.5 × 10⁹ particles/ml.

Generation of anti-αβ antibody response in BALB/c mice
Sera samples from the vaccinated mice were analyzed for the titer of anti-αβ antibody by ELISA using synthesized Aβ1-42 peptide. The anti-αβ antibodies were detectable in the AAV-αβ15 vaccinated mice 1 month after immunization, the antibody titer further increased significantly in the following 2 months after immunization, and kept elevated at least 4 months after immunization as shown in Figure 3. No anti-αβ antibody was detected in either the AAV-lac or the PBS vehicle groups.

Partial neutralization of serum anti-αβ antibody
Sera from AAV-αβ15 vaccinated mice exhibited a partial protective effect in preventing the Aβ1-42 mediated neurotoxicity toward primary cultures of cortical neurons. The morphology of the neurons was evaluated under inverse microscopy. The cellular profile of untreated control neurons is smooth and with extensive neurite processes [Figure 4a].

Figure 2: Detection of the purity of adeno-associated virus-amyloid-β peptide 15 by SDS-PAGE. Lane 1: Low molecular weight protein marker; Lane 2: Sample
Neurons that were treated with the Aβ1-42 alone were shrunken perikaryon with the loss of neurite processes, even though some of the cells had a cytolytic death [Figure 4b]. The neurons of diluted serum (1:10) from AAV-Aβ15 group retained a phenotype similar to untreated control cells [Figure 4c]. The neurons of diluted serum (1:100) from the AAV-Aβ15 group also displayed shrinkage, loss of neurites [Figure 4d]. The viability of cortical neurons was evaluated by MTT assay. The survival rate of neurons decreased to 69.5 ± 9.2% after Aβ1-42 treatment alone. The neuronal survival rate that was treated by diluted serum (1:10) from AAV-Aβ15 significantly increased to 80.4% ± 9.9%, which showed that the toxicity of Aβ1-42 on neurons was decreased. However, the diluted serum (1:100) group did not show any significant effect [Figure 5]. These results indicated that AAV-Aβ15 vaccination could elicit "therapeutic" antibody titer within 1 month.

Discussion

Amyloid-beta immunotherapy has received considerable attention as a promising approach for reducing the level of Aβ in the central nervous system of AD patients. Based on the results obtained in animal and the human clinical studies, Aβ vaccine therapy is a principal strategy for AD treatment, but yet active peptide immunotherapy or passive immunotherapy has some problems to be solved before clinical application.[13] Newly developed gene-based vaccination was effective in reducing Aβ plaque on the model of mice and aged monkeys,[14,15] gene-based vaccination will be a promising therapy for AD in the future. A successful gene-based vaccine for AD depends on a number of key factors, such as the dose, route, delivery method, and vaccination schedule employed. As for Aβ gene-based vaccination is concerned, the same crucial problem is to select optimal immunogens and gene vector.

In order to avoid the adverse events observed in the human AN1792 clinical trial, the novel immunogens should be designed to target Aβ-specific B-cell epitopes and avoid Aβ-specific T-cell epitopes. Numerous studies have reported that the T-cell epitope is mapped within the Aβ15-42 fragment while the B-cell epitope is located within the Aβ1-15 region in humans, monkeys, and mice, so we selected the Aβ1-15 as immunogen.[16] What’s more, almost all shorter Aβ N-terminal fragments have been shown to elicit appropriate humor immune antibodies. These antibodies could recognize and remove the toxic soluble or the aggregated forms of the Aβ1-42, clean senile plaques, improve the ethology of APP mice.[17] Meanwhile immunizing with Aβ1-15 may be less likely to be recognized as a self-antigen and therefore, safer than full-length Aβ as an immunogen. In summary, the experimental data obtained with the Aβ1-15 fragment showed that this sequence could be an ideal immunogenic peptide of vaccine against AD.

Recently viral vector has been extensively applied in gene vaccines. AAV, a member of the genus Dependovirus of the subfamily Parvovirinae within the Parvoviridae family is being developed as a vector for the gene therapy in the central nervous system. AAV has several features that make it useful as a vehicle for immunotherapy. First of all, AAV particles are icosahedral in symmetry and are relatively resistant to heat, low pH, some detergents, and solvents, which makes them particularly stable compared with the most other virus vectors.[18] Secondly, AAV is safe since wild AAV is not pathogenic to human or other species and AAV DNA normally does not integrate into the cellular genome, rather, it remains in the episome.[19] About 50-90% of the population is seropositive for AAV and presence of the virus has been detected in the human genital tract.[20,21] However, there has been no conclusive evidence of any association of AAV with disease or pathology so far. Furthermore, AAV vector could sustain long-term expression of a protein in large animal models after a single administration in skeletal muscle. It is reported that after intramuscular injection of AAV vectors in non-human primates, a rise in the level of vector-expressed secreted proteins is seen in the serum during the first 2-3 months the protein is still highly expressed 6-8 months post-injection.[22] After intramuscular injection of AAV vectors in mouse, a gradually increase in transgene expression is also detected at the first 60 days, and the expression sustained without a significant diminution.

Figure 3: Serum anti-amyloid-β peptide antibody levels determined by Enzyme-Linked ImmunoSorbent Assay in immunized mice. Values given are mean ± SD. *P < 0.05 versus 1 month group.

Figure 4: Photomicrographs showing the neurotoxic effects of amyloid-β peptide (Aβ1-42) on the morphology of neurons and the protection afforded by serum anti-Aβ antibody. Scale bar = 50 μm [Figure (a) Control; (b) Aβ1-42; (c) Aβ1-42 + anti-Aβ antibody (1:10); (d) Aβ1-42 + anti-Aβ antibody (1:100)].
In the present study, we took advantage of the merits of short \( \beta \) immunogen and AAV vector, constructed a new AAV-\( \beta \) gene vaccine. Our findings revealed that intramuscular injection with the AAV-\( \beta \) could induce the generation of the IgG antibodies against \( \beta \)-peptide in BALB/c mice the IgG antibodies could be detected at 1 month post-injection, gradually increased in the first 2 months, and then remained constant without significant diminution until 4 months. Furthermore, the IgG antibodies could recognize the full-length \( \beta \)-peptide I-42 and play an anti-aggregating role as antibody raised against \( \beta \)-peptide peptide, thereby protected the neuronal viability against \( \beta \) induced damage. The above data provide preliminary experimental basis for the protective effect of the vaccine AAV-\( \beta \), in our further research, we will clarify the type of protective antibodies, observe the immunization effect of the AD transgenic mice and take measures to avoid adverse effects as much as possible.

Acknowledgment

We thank Che-jiang Wang for his efforts in data management and his technical support. Ling-Yun Liu, Yuan-Yuan Ma, these authors contributed equally to the work.

References

1. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer’s disease, Lancet 2011;377:1019-31.
2. Ittner LM, Götz J. Amyloid-\( \beta \) and tau: A toxic pas de deux in Alzheimer’s disease. Nat Rev Neurosci 2011;12:65-72.
3. Contestabile A, Ciani E, Contestabile A. The place of choline acetyltransferase activity measurement in the “cholinergic hypothesis” of neurodegenerative diseases. Neurochem Res 2008;33:318-27.
4. Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, et al. Decreased clearance of CNS beta-amyloid in Alzheimer’s disease. Science 2010;330:1774.
5. Sheng B, Gong K, Niu Y, Liu L, Yan Y, Lu G, et al. Inhibition of gamma-secretase activity reduces Abeta production, reduces oxidative stress, increases mitochondrial activity and leads to reduced vulnerability to apoptosis: Implications for the treatment of Alzheimer’s disease. Free Radic Biol Med 2009;46:1362-75.
6. Winblad B, Andreasen N, Minthon L, Floresser A, Imbert G, Dumortier T, et al. Safety, tolerability, and antibody response of active A\( \beta \) immunotherapy with CAD106 in patients with Alzheimer’s disease: Randomised, double-blind, placebo-controlled, first-in-human study, Lancet Neurol 2012;11:597-604.
7. Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid-\( \beta \)-beta attenuates Alzheimer-disease-like pathology in the PDDPP mouse. Nature 1999;400:173-7.
8. Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, et al. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer’s disease. Nature 2000;408:979-82.
9. Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: A case report. Nat Med 2003;9:448-52.
10. Maier M, Seabrook TJ, Lazo ND, Jiang L, Das P, Janus C, et al. Short amyloid-beta (Abeta) immunogens reduce cerebral Abeta load and learning deficits in an Alzheimer’s disease mouse model in the absence of an Abeta-specific cellular immune response. J Neurosci 2006;26:4717-28.
11. Cribs DH. Abeta DNA vaccination for Alzheimer’s disease: Focus on disease prevention. CNS Neurol Disord Drug Targets 2010;9:207-16.
12. Mouru A, Noda Y, Hara H, Mizoguchi H, Tabira T, Nabeshima T. Oral vaccination with a viral vector containing Abeta cDNA attenuates age-related Abeta accumulation and memory deficits without causing inflammation in a mouse Alzheimer model. PASEB J 2007;21:2135-48.
13. Monsonego A, Weiner HL. Immunotherapeutic approaches to Alzheimer’s disease, Science 2003;302:834-8.
14. Qu BX, Lambracht-Washington D, Fu M, Eager TN, Stüve O, Rosenberg RN. Analysis of three plasmid systems for use in DNA A beta 42 immunization as therapy for Alzheimer’s disease. Vaccine 2010;28:5280-7.
15. Toktak Y, Kajki L, Lu J, Okura Y, Kohyama K, Matsumoto Y. Assessment of non-viral amyloid-\( \beta \) DNA vaccines on amyloid-[reduction and safety in rhesus monkeys, J Alzheimer’s Dis 2010;22:1351-61.
16. Geylis V, Kourilov V, Meiner Z, Nennesimo I, Bogdanovic N, Steinitz M. Human monoclonal antibodies against amyloid-beta from healthy adults. Neurobiol Aging 2005;26:597-606.
17. Zhou J, Yao Z, Zhang G, Wang H, Xu J, Yew DT, et al. Vaccination of Alzheimer’s model mice with adenosine vector containing quadrivalent foldable Abeta (1-15) reduces Abeta burden and behavioral impairment without Abeta-specific T cell response. J Neurol Sci 2008;272:87-98.
18. Lai CM, Lai YK, Rakoczy PE. Adeno-virus and adeno-associated virus vectors. DNA Cell Biol 2002;21:895-913.
19. Carty NC, Nash K, Lee D, Mercer M, Gottschall PE, Meyers C, et al. Adeno-associated viral (AAV) serotype 5 vector mediated gene delivery of endothelin-converting enzyme reduces Abeta deposits in APP+PS1 transgenic mice. Mol Ther 2008;16:1580-6.
20. Chirmule N, Propert K, Magosin S, Qian Y, Qian R, Wilson J. Immune responses to adenovirus and adeno-associated virus in humans. Gene Ther 1999;6:1574-83.
21. Hermonat PL, Plotl RT, Santin AD, Parham GP, Flick JT. Adeno-associated viral DNA vaccines inhibit oncogenic transformation of primary human keratinocytes by a human papillomavirus type 16-ras chimeric. Gynecol Oncol 1997;66:487-94.
22. Tomornhoff A, Chérel Y, Guibaud M, Penaud-Budloo M, Snyder RO, Haskins ME, et al. Safety and efficacy of regional intravenous (i.r.) versus intramuscular (i.m.) delivery of rAAV1 and rAAV8 to nonhuman primate skeletal muscle, Mol Ther 2008;16:1291-9.
23. Lu YY, Wang LJ, Muramatsu S, Ikeguchi K, Fujimoto K, Okada T, et al. Intramuscular injection of AAV-GDNF results in sustained expression of transgenic GDNF, and its delivery to spinal...
motoneurons by retrograde transport. Neurosci Res 2003;45:33-40.

24. Burger C, Gorbatyuk OS, Velardo MJ, Peden CS, Williams P, Zolotukhin S, et al. Recombinant AAV viral vectors pseudotyped with viral capsids from serotypes 1, 2, and 5 display differential efficiency and cell tropism after delivery to different regions of the central nervous system. Mol Ther 2004;10:302-17.

How to cite this article: Liu L, Ma Y, Yang T, Li X, Li W. The neuroprotective effect of immune serum of adeno-associated virus vaccine containing Ab1-15 gene on amyloid toxicity. Ann Indian Acad Neurol 2013;16:603-8.

Received: 28-09-12, Revised: 22-02-13, Accepted: 11-03-13

Source of Support: This research was supported by foundation of Shanghai municipal health bureau (2008/048), Conflict of Interest: Nil