Review J. Trad. Med. 22(Suppl.1), 176－182, 2005

Scientific basis for the anti-dementia drugs of constituents from Ashwagandha (*Withania somnifera*)

Chihiro TOHDA,[*a)] Katsuko KOMATSU[b,c)] and Tomoharu KUBOYAMA[b,c)]

[a)]Research Center for Ethnomedicine, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan. [b)]Division of Pharmacognosy, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan. [c)]21st Century COE program, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan. *(Accepted April 18, 2005.)*

Although Ashwagandha (root of *Withania somnifera*) has been used for multi purposes but mainly as a tonic in the traditional Indian Ayurvedic medicine, this review focused on nootropic effects of Ashwagandha itself and isolated compounds from it. Several reports showed sitoindosides VII - X as active compounds for cognitive enhancer. However, our study revealed other active constituents, withanolide A, withanoside IV and withanoside VI, could improve Aß (25-35)-induced memory impairment, neuronal atrophy and synaptic loss in the cerebral cortex and the hippocampus. Treatment with Aß (25-35) induced axonal and dendritic atrophy, and pre-synaptic and post-synaptic losses also in cultured rat cortical neurons. Subsequent treatment with withanolide A, withanoside IV and withanoside VI induced significant reconstruction of pre-synapses and post-synapses, in addition to regeneration of both axons and dendrites in the neurons. Withanolide A, withanoside IV and withanoside VI are therefore important candidates for the therapeutic treatment of neurodegenerative diseases, as it is able to reconstruct neuronal networks.

Key words Ashwagandha, dementia, nootropic effect, synaptic regeneration.

I. Introduction

Ashwagandha (*Withania somnifera* Dunal ; Solanaceae, roots and leaves) is widely used as a multi-purpose medicinal plant in Ayurvedic medicine, the traditional medical system of India. It is popular as Indian ginseng and winter cherry, and has been used historically as a tonic to increase energy, improve overall health and longevity, prevent disease in athletes, and the elderly.[1,2] At present, at least 12 alkaloids, 35 withanolides, and several sitoindosides have been isolated from this plant. Clinical trials and animal researches support the use of Ashwagandha for cognitive and neurological disorders, anxiety, inflammation, cancer and so on using the extract or isolated compounds (Table 1). Concerning an activity for cognitive enhancement, sitoindosides were demonstrated as active principles in Ashwagandha. In our studies, however, withanolide A,

Table 1 Pharmacological effects of extracts and constituents of Ashwagandha

Effect	Constituents	References
cognitive enhancement	water-soluble fraction of 50% ethanol extract of roots	5
	extract of roots	6
	equimolar mixture of sitoindosides VII - X and withaferin A	4, 7
	sitoindoside IX, sitoindoside X	3
	witnanolide A, withanoside IV, withanoside VI	19, 23
anti-inflammation	powder of roots	29, 30, 31, 32
	methanol extract of roots	33
	sitoindoside IX, sitoindoside X	3
anti-tumor	aqueous-methanolic extract of leaves	34
	ethanol extract of roots	35
	withaferin A, withanolide D	26, 27, 36
	sitoindoside IX, sitoindoside X	3
anti-stress	powder of roots	37
	sitoindoside VII, sitoindoside VIII	28
anti-oxidant	powder of roots	38
	authenticated extract of roots	39
adaptogenic	water-soluble fraction of 50% ethanol extract of roots	5
	authenticated extract of roots	40
hypothyroidism	aqueous extract of roots	41

*To whom correspondence should be addressed. e-mail : chihiro@ms.toyama-mpu.ac.jp

withanoside IV and withanoside VI which are also constituents in Ashwagandha, are shown to have strong activity for regeneration of the neuronal network, and improve memory disorder. This review focuses on effects of Ashwagandha on neurological disorder, especially dementia, and proposes usefulness of several constituents based on scientific researches.

II. Effect of Ashwagandha in normal animals

In Wistar strain albino rats, withaferin A, sitoindoside IX or sitoindosideX was given per os at 50 mg/kg for 3 consecutive days.[3] Learning acquisition and memory retention were assessed by the passive avoidance test. Sitoindoside IX and sitoindosideX facilitated short-term memory acquisition, and long-term memory consolidation in old (14 - 16 months) as well as young (5 - 6 months) rats. Withaferin A showed no effect.

An equimolar mixture of sitoindosides VII, VIII, IX and X, and withaferin A was injected intraperitoneally for 7 days at 40 mg/kg in adult Wistar rats.[4] Acetylcholinesterase staining was significantly increased in with the lateral septum and globus pallidus in rats which were injected the mixture. Binding levels of muscarinic acetylcholine receptors (mAChR) M1 and M2 were also determined in the rats. M1-mAChR binding sites were increased in the frontal cortex, lateral and medial septum following treatment with the mixture. Whereas, M2-mAChR binding sites were increased by treatment the mixture in the cingulated cortex, the frontal cortex, the hindlimb and forelimb areas, the parietal cortex, and the piriform and retrosplenial cortex. $GABA_A$, benzodiazepine, NMDA and AMPA receptors in any of the cortical or subcortical regions were not changed by injection of the mixture.

III. Effect of Ashwagandha in disorder model animals - stress, scopolamine, and iboteinic acid models

Water-soluble fraction of 50% ethanol extract of roots of Ashwagandha was administered in adult Wistar rats per os at 25 or 50 mg/kg for 21 days.[5] This fraction contains sitoindosides VII, VIII, IX and X, and withaferin A. The rats were subjected to once daily 1 h footshock through a grid floor for 21 days. The chronic stress significantly and adversely affected retention of learning in the transfer latency (elevated plus maze test) and passive avoidance test. Treatment with the water-soluble fraction showed memory-enhancing effect in a dose dependent manner.

Scopolamine, anticholinergic agent, produced deficits in learning as well as in memory retention. Authenticated root extract of Ashwagandha was administered per os at 50 - 200 mg/kg in albino mice.[6] In a passive avoidance test, treatment with the extract 30 min after scopolamine (0.3 mg/kg) injection significantly reversed scopolamine-induced delay in the latency to reach the shock-free zone dose-dependently. In an elevated plus maze test, scopolamine-induced increase in transfer latency was reduced by

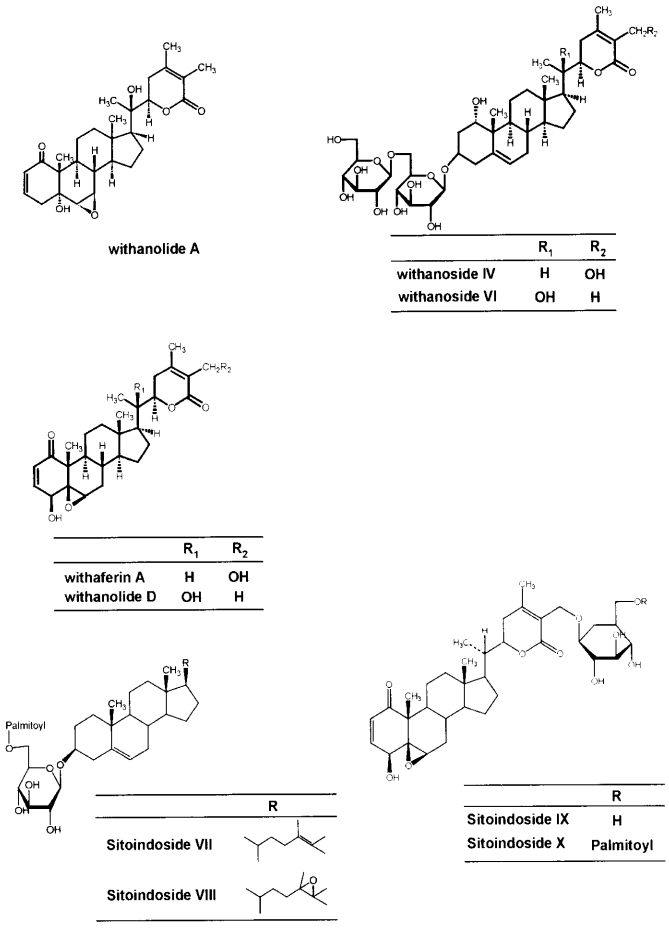

Fig. 1 Structures of withanolide A, withanoside IV, withanoside VI, withaferin A, withanolide D and sitoindosides VII - X.

prior treatment with the extract dose-dependently.

Ibotenic acid (10 μg) was injected into the right side of nucleus basalis magnocellularis (NBM) of adult Wistar rats. This iboteinic acid lesion of NBM caused significantly a deficit in the memory retention in an active avoidance test, a marked reduction of acetylcholine concentrations and choline acetyltransferase (ChAT) activity and number of mAChR in the frontal cortex and the hippocampus. An equimolar mixture of sitoindosides VII, VIII, IX and X, and withaferin A was orally administered at 20 or 50 mg/kg for 7 or 14 days after iboteinic acid lesion.[7] Cognitive deficit, reductions of cholinergic markers induced by iboteinic acid lesion were reversed dose-dependently by treatment with the mixture.

IV. Effect of Ashwagandha in disorder model animals - Alzheimer's disease model

Amyloid β is a major pathological cause of Alzheimer's disease due to the formation of a β-sheet structure[8]; amyloid forms deposits in the brain, and subsequently induces neuronal cell death,[9] neuritic atrophy,[10,11] and synaptic loss.[12] Aβ (25-35) is an active partial fragment of amyloid β. This fragment also forms a β-sheet structure[13] and induces neuronal cell death,[13,14] neuritic atrophy,[15,16] synaptic loss[15-17] and memory impairment.[16-18] Aβ (25-35) (25 nmol) was

injected into the right ventricle of ddY mice. Seven days after an i.c.v. injection of Aβ (25-35), withanolide A (10 μmol/kg),[19] withanoside VI (10 μmol/kg) or the vehicle (0.5% gum arabic solution) was administered orally once daily for 13 days. It was previously confirmed that neuritic and synaptic losses occurred in the hippocampus and the cerebral cortex of mice 7 days after the i.c.v. administration of Aβ (25-35), and these losses continued for at least 30 days after the i.c.v. administration of Aβ (25-35). Furthermore, we also confirmed that spatial memory deficit was maintained even 30 days after the i.c.v. administration of Aβ (25-35). We therefore started p.o. administration of withanolide A from 7 days after the i.c.v. administration of Aβ (25-35), when neuronal and synaptic losses had already occurred. Mice were trained in the water maze for 6 days, starting 7 days after the p.o. administration of drugs, i.e., 14 days after the i.c.v. administration of Aβ (25-35). All the mice reduced the time to reach the platform (escape latency) training-day dependently. At training day 5, Aβ (25-35)-injected mice tended to increase the escape latencies compared with control mice (Fig. 2A), while the administration of withanolide A[19] or withanoside VI decreased the escape latencies compared with administration of the vehicle. From the day after the last training day, serial drug administration was discontinued, and then 7 days after the last training day, the retention test was performed. In the retention test, the number of crossings over the platform position significantly decreased in the Aβ (25-35)-injected mice compared with the control mice (Fig. 2B), while administrations of withanolide A[19] and withanoside VI increased the crossing numbers compared with administration of the vehicle.

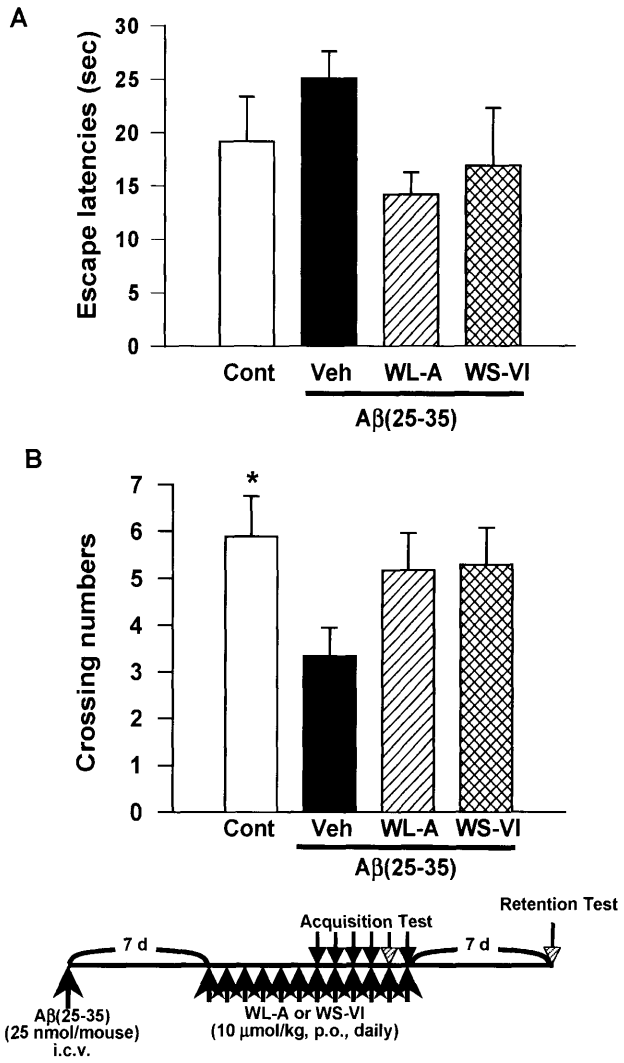

Fig. 2 The effect of withanolide A and withanoside VI on spatial memory deficit induced by Aβ (25-35)

(A)Escape latencies of four trials are shown on training day 5 in a Morris water maze.

(B) Crossing numbers over the position where the platform had been located were measured for 60 s at 7 days after the last training day. This was also 7 days after the discontinuance of drug treatment. The time schedule of the experiment is shown below the Figure. The values represent the means and s.e.m. of 6-9 mice. *$p < 0.05$ when compared with Veh.

After the retention test, the expression levels of phosphorylated NF-H, MAP2, synaptophysin, and PSD-95 were measured in the mouse brain. We observed two cortical regions (parietal cortex and temporal cortex) and three hippocampal regions (CA1, CA3, and dentate gyrus) where neuronal degeneration occurred also in Alzheimer's disease patients[20,21] and Alzheimer's disease model mice.[22] In Aβ (25-35)-injected mice, phosphorylated NF-H- (axon marker), MAP2- (dendrite marker), and synaptophysin-positive areas (presynapse marker) were remarkably decreased in most regions compared with control mice, while the administration of withanolide A[19] and withanoside VI increased NF-H- (Fig. 3A), MAP2- (Fig. 3B), and synaptophysin (Fig. 3C)-positive areas, compared with administration of the vehicle. It can be considered that these increases of neuritic and synaptic marker proteins contribute to the recovery of memory deficits of mice induced by Aβ (25-35).

V. Neuritic and synaptic regeneration by withanolide A, withanoside IV and withanoside VI

Rat cortical neurons were cultured only with Aβ (25-35) for 4 days, after that, withanolide A (1 μM), withanoside VI (1 μM), NGF (100 ng/ml), or the vehicle (0.1% DMSO) was added. After the drug treatment for 4 days, the cells were fixed and immunostained for phosphorylated NF-H or MAP2. Lengths of axons and dendrites in the neurons treated with the vehicle were shorter than the control at 8 days after treatment with Aβ (25-35), whereas treatments with withanolide A[19] and withanoside VI significantly increased the lengths of both axons and dendrites, as compared with treatment with the vehicle (Fig. 4).

It is crucial to determine whether regenerated neurites are also able to reconstruct synapses. Since withanolide A and withanoside VI were shown to regenerate axons and dendrites, we tested the effects of these drugs on presynaptic and post-synaptic maturation. Four days after the addition of Aβ (25-35), the cells were fixed and immunostained with an antibody for synaptophysin or PSD-95. Dendritic shafts were visualized by double-immunostaining with a MAP2 antibody. The number and fluorescence intensity of synaptophysin- and PSD-95-positive puncta on dendrites were obviously decreased by

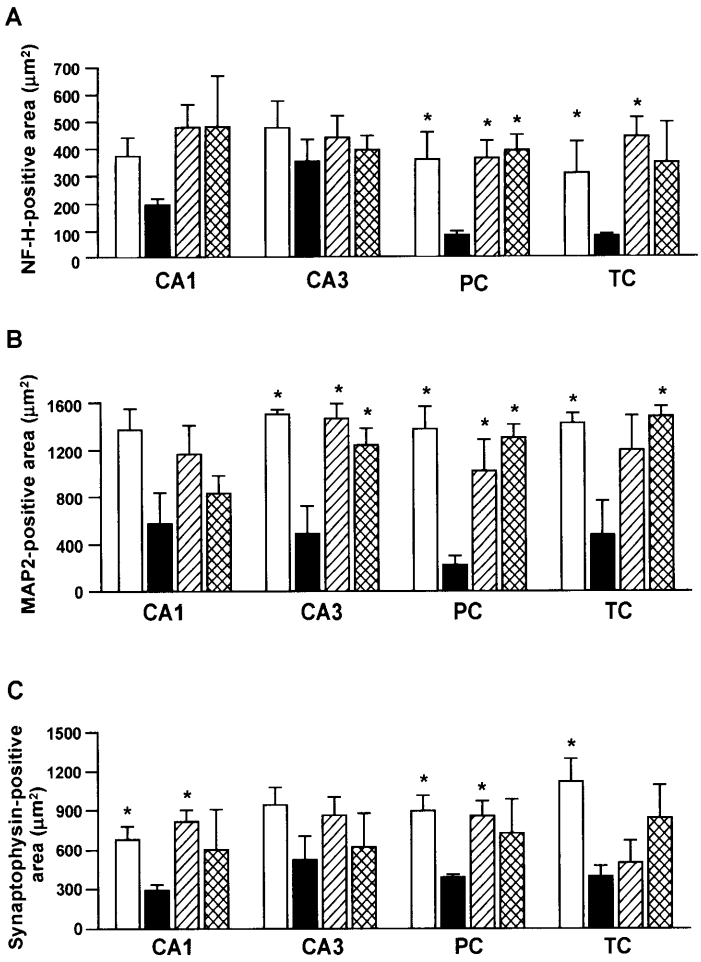

Fig. 3 Quantified effects of withanolide A and withanoside VI on neuritic atrophy and synaptic loss *in vivo* induced by Aβ (25-35)

NF-H- (A), MAP2- (B) and synaptophysin- (C) positive areas were measured in the parietal cortex (PC) and temporal cortex (TC), and the hippocampal CA1, CA3. Control mice (open columns) were treated with an i.c.v. injection of saline and p.o. administration of the vehicle. Aβ (25-35) i.c.v.-injected mice were treated with p.o. administration of the vehicle (closed columns), withanolide A (hatched columns) or withanoside VI (cross hatched columns). The values represent the means and s.e.m. of 3 mice. $*p < 0.05$ when compared with Aβ (25-35) plus the vehicle-treated group.

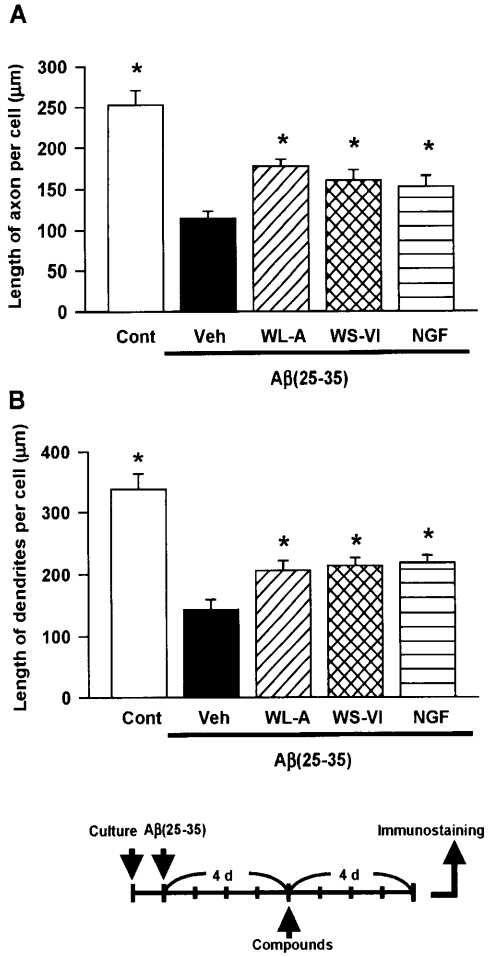

Fig. 4 The effects of withanolide A and withanoside VI on axonal and dendritic regeneration after Aβ (25-35)-induced atrophy

Cortical neurons were cultured for 24 h, and were then treated with or without (Cont) 10 M Aβ (25-35). Four days after the administration of Aβ (25-35), the cells were treated with withanolide A (WL-A) or withanoside VI at a concentration of 1 M; or NGF at a concentration of 100 ng/ml; or the vehicle (Veh). Four days after treatment, the cells were fixed and immunostained for phosphorylated NF-H or MAP2. The lengths of NF-H-positive (A) or MAP2-positive (B) neurites were measured in each treatment. The values represent the means and s.e.m. of 30 cells. $*p < 0.05$ when compared with Veh.

treatment with Aβ (25-35). Quantified areas of synaptophysin- and PSD-95-positive puncta were significantly decreased by treatment with Aβ (25-35) for 4 days (53.4% and 66.9% of the control, respectively). These results indicate that Aβ (25-35) induced the losses of both pre-synaptic and post-synaptic structures in long term-cultured cortical neurons.

Withanolide A and withanoside VI, NGF and the vehicle were added to the culture medium after treatment with A β (25-35) for 4 days when synaptic loss had already occurred. Seven days after addition of the test drug, the cells were fixed and immunostained for synaptophysin or PSD-95. Synaptophysin-positive (Fig. 5A) areas and PSD95-positive (Fig. 5B) areas in the neurons treated with the vehicle continued to decrease 11 days after treatment with A β (25-35) compared with control neurons. On the other hand, treatment with withanolide A and withanoside VI significantly increased in synaptophysin and PSD-95 expressions, as compared with treatment with the vehicle. These results indicate that withanolide A and withanoside VI facilitated the reconstruction of pre-synaptic and post-synaptic regions in neurons in which severe synaptic loss had already occurred. Withanoside IV also showed the same activities of neuritic and synaptic regeneration.

VI. Effect and safety of constituents of Ashwagandha

Neurite extension activity, a basis of synaptic formation, was assessed using 18 compounds isolated from the methanol extract of Ashwagandha. Besides withanolide A, withanoside IV and withanoside VI, 3 other compounds ((20*S*, 22*R*)-4β, 5β, 27-trihydroxy-3α, 6α-epoxy-l-oxowitha-24-enolide, (20*S*, 22*R*)-4β 5β, 6α, 27-tetrahydroxy-l-oxowitha-2, 24-dienolide, coagulin Q) had also neurite

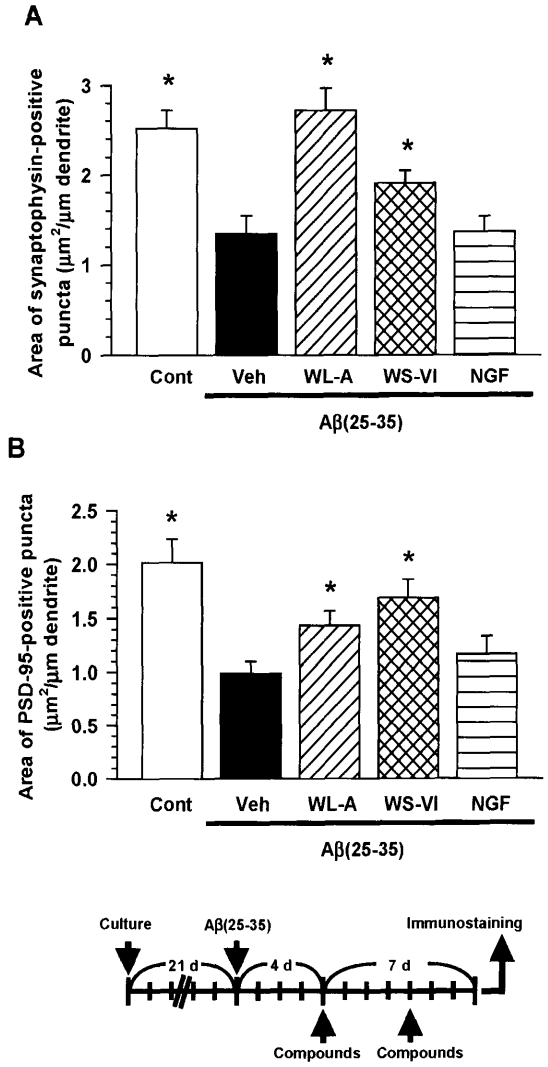

Fig. 5 The effects of withanolide A and withanoside VI on pre-synaptic and post-synaptic reconstruction after Aβ (25-35)-induced synaptic loss

After culture for 21 days, the cortical neurons were treated with or without (Cont) Aβ (25-35). The cells were then treated with withanolide A (WL-A) or withanoside VI at a concentration of 1 M; or NGF at a concentration of 100 ng/ml; or with the vehicle (Veh). Seven days after treatment, the cells were double-immunostained for synaptophysin or PSD-95, plus MAP2. Areas of synaptophysin- (A) PSD-95- (B) positive puncta per μm of dendrites were measured. The values represent the means and s.e.m. of 20-35 dendrites. $*p < 0.05$ when compared with Veh.

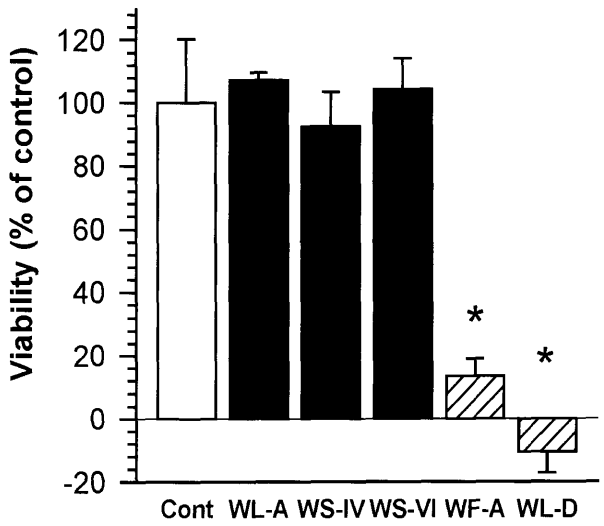

Fig. 6 Effects of withanolide A, withanosides IV and VI, withaferin A and withanolide D on neuronal viability

The cortical neurons were treated with withanolide A (WL-A), withanoside IV (WS-IV), withanoside VI (WS-VI), withaferin A (WF-A), or withanolide D (WL-D) at a concentration of 1 μM. Four days after, cell viability was quantified by a WST-8 assay, and the survived cell rate was shown compared with vehicle treatment (Cont). The values represent the means and s.e.m. of 3 wells. $*p < 0.05$ when compared with Cont.

extension activity at 1 μM.[23] The methanol extract of Ashwagandha roots also induced neurite extension at 5 μg/ml, but cell death at 50 μg/ml.[24] In our investigation, contents of withanolide A, withanoside IV and withanoside VI in the methanol extract was estimated approximately 0.14 - 0.18%, 0.84 - 3.5% and 0.50 - 0.97%, respectively. Among isolated compounds, withaferin A and withanolide D which are famous as major constituents of Ashwagandha, and are contained in the methanol extract at the same level as withanoside IV or more.[25] Although withaferin A and withanolide D were reported to have antitumor activity,[26,27] these showed strong cytotoxicity at 1 μM in primary cultured normal cortical neurons (Fig. 6) and as well as neuroblastoma SH-SY5Y.[23] Traditionally, Ashwagandha has been considered to have no side effects. However, systematic study was hardly seen, which include acute, subacute, or chronic toxicity of powder or extracts of Ashwagandha. Aqueous-methanol extracts of Ashwagandha roots, and equimolar mixture of sitoindosides VII and VIII and withaferin A were studied for acute toxicity in mice.[28] The acute LD_{50} of the extract and the mixture was 1076 ± 78 mg/kg and 1564 ± 92 mg/kg, respectively. In our animal study, 10 μmol/kg of withanolide A, withanoside IV or withanoside VI was effective for memory disorder. If the methanol extract of Ashwagandha were to be taken, a quite high dose, approximately 350 - 1080 mg/kg, of extract would be needed to reach effective doses of the active 3 compounds. Although the extract has a merit, which contains multiple active constituents, it may be accompanied with risk of acute toxicity and neuronal death at high doses, or no effect at low doses at least for anti-dementia purpose.

VII. Conclusion

Among constituents of Ashwagandha, withanolide A, withanoside IV and withanoside VI could facilitate the regeneration of axons and dendrites, and this compound led to the dramatic reconstruction of synapses, when neuron damage had already progressed. Moreover, these compounds could ameliorate the memory deficit in mice, and could generate neurites and synapses in the cerebral cortex and the hippocampus, suggesting that withanolide A, withanoside IV and withanoside VI have potential as an essentially useful drug to treat neurodegenerative diseases when used together with treatments preventing pathogenesis and neuronal death. Other constituents, sitoindoside VII - X

have also activating effects against cholinergic neurons, and cause cognitive improvement. Therefore, Ashwagandha may be useful drug for dementia. However, contents of cytotoxic constituents should be considered carefully.

Acknowledgments

We thank Dr. Jing Zhao, Dr. Meselhy Ragab Meselhy, Dr. Kun Zou, Dr. Norio Nakamura, Prof. Masao Hattori and Mr. Hong Yu Liu. This work was supported by the Uehara Memorial Foundation and Shorai Foundation.

References

1) Chatterjee, A. and Pakrashi, S.C.: *The Treatise on Indian Medical Plants,* **4**, 208-212, 1995.
2) Bone, K.: Clinical Applications of Ayurvedic and Chinese Herbs. *Monographs for the Western Herbal Practitioner.* Phytotherapy Press, Australia, pp. 137-141, 1996.
3) Ghosal, S., Lal, J. and Srivastava, R.: Immunomodulatory and CNS effects of sitoindosides IX and X, two new glycowithanolides from *Withania somnifera. Phytother. Res.,* **3**, 201-206, 1989.
4) Schliebs, R., Liebmann, A., Bhattacharya, S.K., Kumar, A., Ghosal, S. and Bigl, V.: Systemic administration of defined extracts from *Withania somnifera* (Indian Ginseng) and Shilajit differentially affects cholinergic but not glutamatergic and GABAergic markers in rat brain. *Neurochem. Int.,* **30**, 181-190, 1997.
5) Bhattacharya, S.K. and Muruganandam, A.V.: Adaptogenic activity of *Withania somnifera*: an experimental study using a rat model of chronic stress. *Pharmacol. Biochem. Behav.,* **75**, 547-555, 2003.
6) Dhuley, J.N.: Nootropic-like effect of Ashwagandha *(Withania somnifera* L.) in mice. *Phytother. Res.,* **15**, 524-528. 2001.
7) Bhattacharya, S.K. and Kumar, A.: Effects of glycowithanolides from *Withania somnifera* on an animal model of Alzheimer's disease and perturbed central cholinergic markers of cognition in rats. *Phytother. Res.,* **9**, 110-113, 1995.
8) Simmons, L.K., May, P.C., Tomaselli, K.J., Rydel, R.E., Fuson, K.S., Brigham, E.F., Wright, S., Liberburg, I., Becker, G.W., Brems, D.N. and Li, W.Y.: Secondary structure of amyloid β peptide correlates with neurotoxic activity *in vitro. Mol. Pharmacol.,* **45**, 373-379, 1994.
9) Bobinski, M., Wegiel, J., Tarnawski, M., Bobinski, M., Reisberg, B., De Leon, M.J., Miller, D.C. and Wisniewski, H.M.: Relationships between regional neuronal loss and neurofibrillary changes in the hippocampal formation and duration and severity of Alzheimer disease. *J. Neuropath. Exp. Neurol.,* **56**, 414-420, 1997.
10) Canning, D.R., Mc Keon, R.J., De Witt, D.A., Perry, G., Wujek, J.R., Frederickson, R.C. and Silver, J.: β-Amyloid of Alzheimer's disease induces reactive gliosis that inhibits axonal outgrowth. *Exp. Neurol.,* **124**, 289-298, 1993.
11) Knowles, R.B., Wyart, C., Buldyrey, S.V., Cruz, L., Urbanc, B., Hasselmo, M.E., Stanley, H.E. and Hyman, B.T.: Plaque-induced neurite abnormalities: implications for disruption of neural networks in Alzheimer's disease. *J. Neuropathol. Exp. Neurol.,* **96**, 5274-5279, 1999.
12) Terry, R.D., Masliah, E., Salmon, D.P., Butters, N., De Teresa, R., Hill, R., Hansen, L.A. and Katzman, R.: Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann. Neurol.,* **30**, 572-580, 1991.
13) Pike C.J., Walencewicz-Wasserman, A.J., Kosmoski, J., Cribbs, D.H., Glabe, C.G. and Cotman, C.W.: Structure-activity analyses of β-amyloid peptides: contribution of the 25-35 region to aggregation and neurotoxicity. *J. Neurochem.,* **64**, 253-265, 1995.
14) Yankner, B.A., Duffy, L.K. and Kirschner, D.A.: Neurotrophic and neurotoxic effects of amyloid β protein: reversal by tachykinin neuropeptides. *Science,* **250**, 279-282, 1990.
15) Grace, E.A., Rabiner, C.A. & Busciglio, J.: Characterization of neuronal dystrophy induced by fibrillar amyloid β: implications for Alzheimer' disease. *Neuroscience,* **114**, 265-273, 2002.
16) Tohda, C., Matsumoto, N., Zou, K., Meselhy, M.R. and Komatsu, K.: Aβ (25-35)-induced memory impairment, axonal atrophy and synaptic loss are ameliorated by M1, a metabolite of protopanaxadiol-type saponins. *Neuropsycopharmacology,* **29**, 860-868, 2004.
17) Tohda, C., Tamura, T. and Komatsu, K.: Repair of amyloid β(25-35)-induced memory impairment and synaptic loss by a Kampo formula, Zokumei-to. *Brain Res.,* **990**, 141-147, 2003.
18) Maurice, T., Lockhart, B.P. and Privat, A.: Amnesia induced in mice by centrally administered β-amiloid peptides involves cholinergic dysfunction. *Brain Res.,* **706**, 181-193, 1996.
19) Kuboyama, T., Tohda, C. and Komatsu, K.: Neuritic regeneration and synaptic reconstruction induced by withanolide A. *British J. Pharmacol.,* **144**, 961-971, 2005.
20) De Kosky, S. and Scheff, S.W.: Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann. Neurol.,* **27**, 457-464, 1990.
21) Heinonen, O., Lehtovirta, M., Soininen, H., Helisalmi, S., Mannermaa, A., Sorvari, H., Kosunen, O., Paljarvi, L., Ryynanen, M. and Riekkinen, P.J. Sr.: Alzheimer pathology of patients carrying apolipoprotein E epsilon 4 allele. *Neurobiol. Aging,* **16**, 505-513, 1995.
22) Games, D., Adams, D, Alessandrini, R., Barbour, R., Borthelette, P., Blackwell, C., Carr, T., Clemens, J., Donaldson, T., Gillespie, F., Guido, T., Hagopian, S., Johnson-Wood, K., Khan, K., Lee, M., Leibowitz, P., Lieberburg, I., Little, S., Masliah, E., McConlogue, L., Montoya-Zavala, M., Mucke, L., Paganini, L., Pennima, E., Power, M., Schenk, D., Seubert, P., Snyder, B., Soriano, F., Tan, H., Vitale, J., Wadsworth, S., Wolozin, B. and Zhao, J.: Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. *Nature,* **373**, 523-527, 1995.
23) Kuboyama, T., Tohda, C., Zhao, J., Nakamura, N., Hattori, M. and Komatsu, K.: Axon- or dendrite-predominant outgrowth induced by constituents from Ashwagandha. *NeuroReport,* **13**, 1715-1720, 2002.
24) Tohda, C., Kuboyama, T. and Komatsu, K.: Dendrite extension by methanol extract of Ashwagandha (roots of *Withania somnifera*) in SK-N-SH cells. *NeuroReport,* **11**, 1981-1985, 2000.
25) Ganzera, M., Choudhary, M.I. and Khan, I.A.: Quantitative HPLC analysis of withanolides in *Withania somnifera. Fitoterapia,* **74**, 68-76, 2003.
26) Chowdhury, K. and Neogy, R.K.: Mode of action of Withaferin A and Withanolide D. *Biochem. Pharmacol.,* **24**, 919-920, 1975.
27) Jayaprakasam, B., Zhang, Y., Seeram, N.P. and Nair, M.G.: Growth inhibition of human tumor cell lines by withanolides from *Withania somnifera* leaves. *Life Sci.,* **74**, 125-132, 2003.
28) Bhattacharya, S.K., Goel, R.K., Kaur, R. and Ghosal, S.: Anti-stress activity of sitoindosides VII and VIII, new acylsterylglucosides from *Withania somnifera. Phytother. Res.,* **1**, 32-37, 1987.
29) Anbalagan, K. and Sadique, J.: Influence of an Indian medicine (Ashwagandha) on acute-phase reactants in inflammation. *Indian J. Exp. Biol.,* **19**, 245-249, 1981.
30) Anbalagan, K. and Sadique, J.: Role of prostaglandins in acute phase proteins in inflammation. *Biochem. Med.,* **31**, 236-245, 1984.
31) Begum, V.H. and Sadique, J.: Effect of *Withania somnifera* on glycosaminoglycan synthesis in carrageenin-induced air pouch granuloma. *Biochem. Med. Metab. Biol.,* **38**, 272-277, 1987.
32) Begum, V.H. and Sadique, J.: Long term effect of herbal drug *Withania somnifera* on adjuvant induced arthritis in rats. *Indian J. Exp. Biol.,* **26**, 877-882, 1988.
33) Al Hindawi, M.K., al Khafaji, S.H. and Abdul-Nabi, M.H.: Anti-granuloma activity of Iraqi *Withania somnifera. J. Ethnopharmacol.,* **37**, 113-116, 1992.

34) Kaur, K., Rani, G., Widodo, N., Nagpal, A., Taira, K., Kaul, S.C., Wadhwa, R.: Evaluation of the anti-proliferative and anti-oxidative activities of leaf extract from in vivo and in vitro raised Ashwagandha. *Food Chem Toxicol.* **42**, 2015-2020, 2004.
35) Devi, P.U., Sharada, A.C., Solomon, F.E., Kamath, M.S.: In vivo growth inhibitory effect of *Withania somnifera* (Ashwagandha) on a transplantable mouse tumor, Sarcoma 180. *Indian J. Exp. Biol.,* **30**, 169-172, 1992.
36) Devi, P.U.: Withania somnifera Dunal (Ashwagandha): potential plant source of a promising drug for cancer chemotherapy and radiosensitization. *Indian J. Exp. Biol.,* **34**, 927-932, 1996.
37) Grandhi, A., Mujumdar, A.M., Patwardhan, B.: A comparative pharmacological investigation of Ashwagandha and Ginseng. *J. Ethnopharmacol.,* **44**, 131-135, 1994.
38) Panda, S. and Kar, A.: Evidence for free radical scavenging activity of Ashwagandha root powder in mice. *Indian J. Physiol. Pharmacol.,* **41**, 424-426, 1997.
39) Dhuley, J.N.: Effect of Ashwagandha on lipid peroxidation in stress-induced animals. *J. Ethnopharmacol.,* **60**, 173-178, 1998.
40) Dhuley, J.N.: Adaptogenic and cardioprotective action of Ashwagandha in rats and frogs. *J. Ethnopharmacol.,* **70**, 57-63, 2000.
41) Panda, S. and Kar, A.: Changes in thyroid hormone concentrations after administration of Ashwagandha root extract to adult male mice. *J. Pharm.Pharmacol.,* **50**, 1065-1068, 1998.