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Search for Natural Products Related to Regeneration of the Neuronal Network

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Key Words
Neuritic atrophy · Synaptic loss · Dendrite · Axon · Alzheimer's disease · Amyloid-beta · Ginseng · *Withania somnifera* · Ashwagandha · Coffee bean

Abstract
The reconstruction of neuronal networks in the damaged brain is necessary for the therapeutic treatment of neurodegenerative diseases. We have screened the neurite outgrowth activity of herbal drugs, and identified several active constituents. In each compound, neurite outgrowth activity was investigated under amyloid-β-induced neuritic atrophy. Most of the compounds with neurite regenerative activity also demonstrated memory improvement activity in Alzheimer's disease-model mice. Protopanaxadiol-type saponins in Ginseng drugs and their metabolite, M1 (20-*O*-β-*D*-glucopyranosyl-(20*S*)-protopanaxadiol), showed potent regeneration activity for axons and synapses, and amelioration of memory impairment. Withanolide derivatives (withanolide A, withanoside IV, and withanoside VI) isolated from the Indian herbal drug Ashwagandha, also showed neurite extension in normal and damaged cortical neurons. Trigonelline, a constituent of coffee beans, demonstrated the regeneration of dendrites and axons, in addition to memory improvement.

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Introduction

Despite the great number of ongoing investigations, neurodegenerative diseases remain incurable. The drugs currently available for dementia, such as donepezil, an acetylcholinesterase inhibitor, are efficacious in the temporary treatment of memory dysfunction, but do not prevent or reverse the underlying neurodegeneration [1]. In patients with Alzheimer's disease, neuritic atrophy and synaptic loss are considered the major causes of cognitive impairment, based on the results of neuropathological postmortem studies of the brain [2–4]. In the brains of patients suffering from other neurodegenerative diseases, such as Parkinson's disease, Huntington's disease, and Creutzfeldt-Jakob disease, neurite atrophy has also been observed [5–7]. Such atrophy leads to the destruction of neuronal networks, and subsequently to the fatal dysfunction of brain systems in these patients. The exclusion of, or at least a decrease in the magnitude of, the causes of each disease may prevent the progression of symptoms, but such inhibition is not associated with the repair of already severely damaged brain function. We hypothesized that the reconstruction of neuronal networks in the injured brain would be the most necessary step in the fundamental recovery of brain function, requiring neuritic regeneration and synaptic reconstruction.

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Table 1. Natural medicine-oriented compounds which enhance neurite outgrowth

Compound	Main botanical source	Cell used	Effective dose	Function	Reference
Ginsenoside Rb1	*Panax ginseng* *Panax notoginseng*	rat cortical neuron	0.1–100 μ*M*	axon extension synaptogenesis memory improvement	14, 21
Metabolite 1*	(protopanaxadiol-type saponins)	rat cortical neuron	0.01–1 μ*M*	axon extension synaptogenesis memory improvement	21
Withanolide A	*Withania somnifera*	rat cortical neuron	1 μ*M*	axon extension dendrite extension synaptogenesis memory improvement	36 37
Withanoside IV	*Withania somnifera*	rat cortical neuron	1 μ*M*	axon extension dendrite extension synaptogenesis memory improvement	36
Withanoside VI	*Withania somnifera*	rat cortical neuron	1 μ*M*	axon extension dendrite extension synaptogenesis memory improvement	36
Trigonelline	coffee bean	rat cortical neuron	30–100 μ*M*	axon extension dendrite extension memory improvement	41
Honokiol	*Magnolia obovata* *Magnolia officinalis*	rat cortical neuron	0.1–10 μ*M*	neurite outgrowth	43
(–)-3,5-Dicaffeoyl-*muco*-quinic acid	*Aster scaber*	PC12	1–10 μ*M*	neurite outgrowth	44
Catalpol	*Rehmannia glutinosa*	PC12h	0.1–1 μg/ml	neurite outgrowth	45
Geniposide	*Gardenia jasminoides*	PC12h	0.1–10 μg/ml	neurite outgrowth	45
Gardenoside	*Gardenia jasminoides*	PC12h	0.1–10 μg/ml	neurite outgrowth	45
Picroside I	*Picrorhiza scrophulariiflora*	PC12D	10–100 μ*M*	potentiating NGF-induced neurite outgrowth	46
Picroside II	*Picrorhiza scrophulariiflora*	PC12D	0.1–100 μ*M*	potentiating NGF-induced neurite outgrowth	46
Nardosinone	*Nardostachys chinensis*	PC12D	0.1–100 μ*M*	potentiating NGF-induced neurite outgrowth	47

* 20-*O*-β-*D*-Glucopyranosyl-(20*S*)-protopanaxadiol.

Natural Products Enhancing Neurite Outgrowth

Neurite outgrowth is the first step in the construction of the neuronal network, and neurite outgrowth activity has been investigated in many crude drugs. Of these extracts, several constituents have been identified as active compounds (table 1). It is critical that extended neurites have specific functions, such as axons and dendrites, and can make circuits by synaptic connections. However, the identification of axons and dendrites and the mea-

surement of synaptogenesis have not been undertaken in studies of natural products, apart from in our research. Ginseng drugs, Ashwagandha and coffee beans contain interesting compounds with potent neurite regeneration, synaptic reconstruction and memory improvement activities.

Ginseng Drugs

Neurite Outgrowth Using Methanol Extracts and Isolated Saponins in SK-N-SH Cells

Ginseng, the root of *Panax ginseng*, is widely used as a tonic throughout the world, and is efficacious in the treatment of amnesia. In addition, significant improvement in learning and memory has been observed in brain-damaged [8, 9] and aged rats [9] after the oral administration of Ginseng powder, and the major Ginseng saponins, ginsenoside Rb_1 and Rg_1, are known to improve spatial learning in normal mice [10]. Regarding the effects on neuronal cells, it has been shown that neurite outgrowth of cultured rat cerebral cortical neurons is enhanced by crude Ginseng saponins [11], and that ginsenoside Rb_1 potentiates the nerve growth factor (NGF)-mediated neurite outgrowth of chick dorsal root ganglia [12, 13].

We tested the neurite outgrowth activity of methanol extracts of 6 types of Ginseng drugs and *P. stipuleanatus* plant material in SK-N-SH cells [14]. The methanol extracts of Ginseng (dried root of *P. ginseng*), Red Ginseng (steamed and dried root of *P. ginseng*), Notoginseng (dried root of *P. notoginseng*) and Ye-Sanchi (dried rhizome and root of *P. vietnamensis* var. *fuscidiscus*) increased neurite outgrowth, with the effects of Red Ginseng and Ye-Sanchi being particularly significant.

Thirty saponins were isolated from Ye-Sanchi and structurally elucidated [15, 16]. Oleanolic acid-type saponins were also isolated from Kouzichi (dried rhizome of *P. japonicus* var. *major* from Hubei province) [17], and 19 saponins (ginsenosides Rb_1, Rb_3, Rg_1 and Re, notoginsenosides R_4, Fa and R_1, Yesanchinoside J, 20-*O*-glc-ginsenoside Rf, majonoside R_2, (24*S*)-pseudoginsenoside RT_4 and F_{11}, vina-ginsenoside R_1, R_2 and R_6 from Ye-Sanchi, notoginsenoside R_2, ginsenoside Rg_2 and Ro, chikusetsusaponin IVa from Kouzichi) were tested. Protopanaxadiol (ppd)-type saponins, ginsenosides Rb_1 and Rb_3, and notoginsenosides R_4 and Fa significantly extended the neurites in SK-N-SH cells at a concentration of 100 μM, and their activity increased dose-dependently. On the other hand, protopanaxatriol, ocotillol and oleanolic acid-type saponins showed no effect [14]. This suggests that ppd-type saponins are active compounds. Ginseng, Red Ginseng, Notoginseng and Ye-Sanchi, which showed neurite outgrowth activity, have been demonstrated to contain comparatively rich ppd-type saponins in our quantitative study [18]. This suggested that the effects of these drugs could be mainly attributed to ppd-type saponins. However, Zhuzishen (dried rhizome of *P. japonicus* var. *major* from Yunnan province) and a rhizome of *P. stipuleanatus*, which inhibited cell viability, may contain some cytotoxic compounds.

Effect of M1, a Metabolite of Protopanaxadiol-Type Saponins, on Aβ(25–35)-Induced Memory Impairment, Axonal Atrophy and Synaptic Loss in Mice

When taken orally, ppd-type saponins are mostly metabolized by intestinal bacteria to ppd monoglucoside, 20-*O*-β-*D*-glucopyranosyl-(20*S*)-protopanaxadiol (M1) [19, 20] (fig. 1). As Ginseng is generally taken orally, a metabolite of ppd-type saponins, M1, should be investigated to determine the active constituent of Ginseng responsible for its major effects. We therefore conducted experiments to determine whether treatment with ginsenoside Rb_1, as a representative of ppd-type saponins, and its metabolite, M1, can induce recovery from memory disorder, axonal atrophy, and synaptic loss induced by the active fragment of the amyloid-β peptide (Aβ(25–35)) [21].

Male ddY mice (6 weeks old) were prepared to create a mouse model of Alzheimer's disease (AD). Seven days after an i.c.v. injection of Aβ(25–35), ginsenoside Rb_1 (10 μmol/kg), M1 (10 μmol/kg), donepezil hydrochloride (DNP, 0.5 mg/kg), or the vehicle (tap water) was administered orally once daily for 14 days. Mice were trained in the water maze for 7 days starting 14 days after the i.c.v. administration of Aβ(25–35) (fig. 2a). The escape latency to find the platform in the Aβ(25–35)-injected group significantly increased compared with the saline-injected group, whereas the escape latencies of the groups administered ginsenoside Rb_1 and M1 p.o. significantly decreased as compared with the vehicle-administered group. The donepezil-administered group showed no significant shortening of the escape latency.

In the retention test (fig. 2b), the number of crossings over a previous platform position was significantly decreased in the Aβ(25–35)-injected group compared with the saline-injected group. The number of crossings recovered after treatment with ginsenoside Rb_1 and M1. Treatment with donepezil showed the smallest effect in the retention test. All mice showed normal swimming performance and a constant increase in body weight. Locomotor activity did not differ among groups.

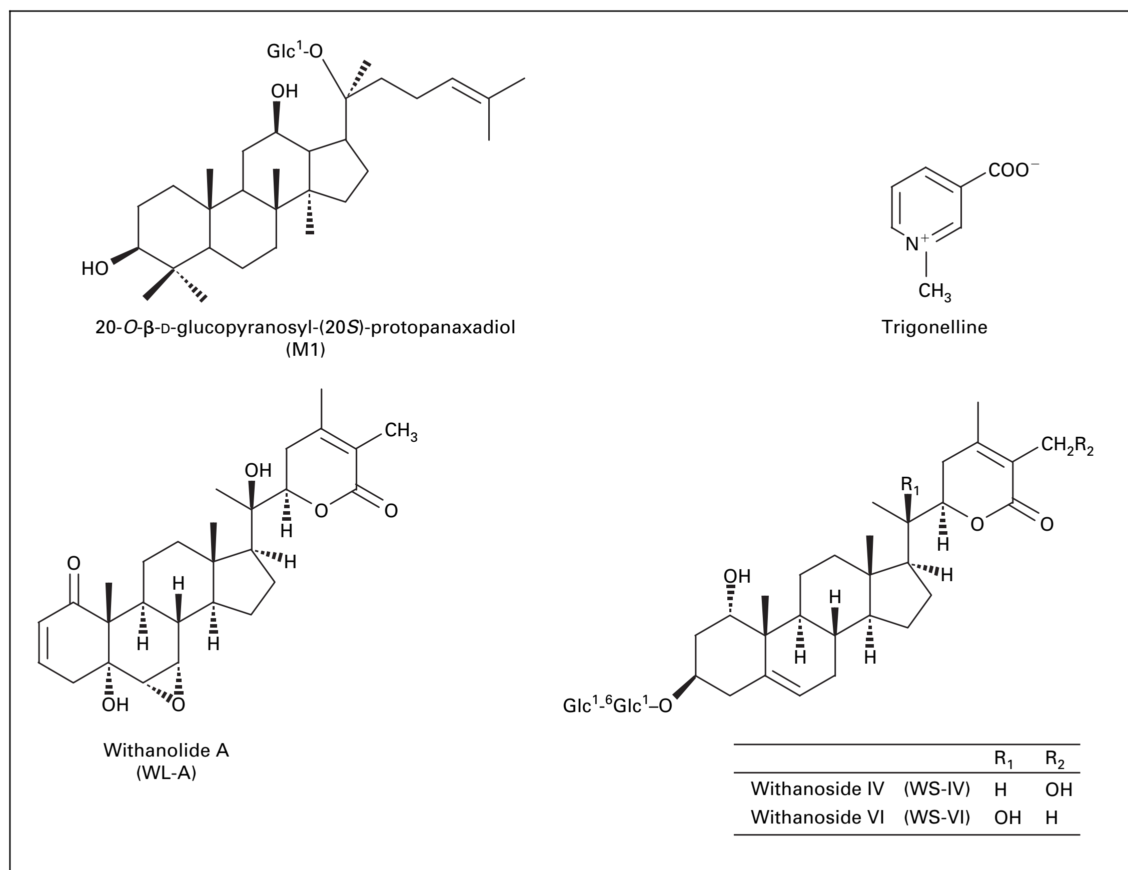

Fig. 1. Chemical structures of compounds that regenerate the neuronal network.

After the retention test, the expression levels of phosphorylated NF-H (axonal marker), synaptophysin (synaptic marker) and MAP2 (dendritic marker) were measured in mouse brains. We observed two cortical areas (parietal cortex and temporal cortex) and three hippocampal areas (CA1, CA3, and the dentate gyrus), as it is known that synaptic loss occurs primarily in the cerebral cortex and hippocampus in AD patients [22, 23] and in AD model mice [24]. The phosphorylated NF-H levels were remarkably reduced in these five areas of the brain in Aβ(25–35)-injected compared with saline-injected mice (fig. 3a). Significant decreases were seen in the parietal cortex, CA1 and CA3; however, the expression levels of phosphorylated NF-H were nearly equal to those of the control in ginsenoside Rb_1- and M1-treated mice. Donepezil treatment had no effect on the levels of phosphorylated NF-H. The synaptophysin levels were also reduced in these five areas of the brain in Aβ(25–35)-injected compared with saline-injected mice (fig. 3b). Significant decreases were seen in the temporal cortex and CA1. In all areas, the synaptophysin levels were almost equal to or higher than control levels in ginsenoside Rb_1- and M1-treated mice. Donepezil treatment had no effect on the synaptophysin levels. The MAP2 levels were also reduced in the cerebral cortex and CA1 of the brain in Aβ(25–35)-injected compared with saline-injected mice (fig. 3c). Significant decreases were seen in the temporal cortex; however, these decreases in the expression levels of MAP2 were not clearly recovered by ginsenoside Rb_1, M1 or donepezil. Although treatment with M1 tended to increase the MAP2 level in the temporal cortex, the effect was weak. No differences in neuronal density were observed among the groups in any brain areas. Treatment with M1, a metabolite of ginsenoside Rb_1, results in the recovery of impaired learning and memory in Aβ(25–35)-injected mice with degenerated axons and synapses. The maintained retention of spatial memory was also seen after the discontinuation of ginsenoside Rb_1 and M1 administration. These results

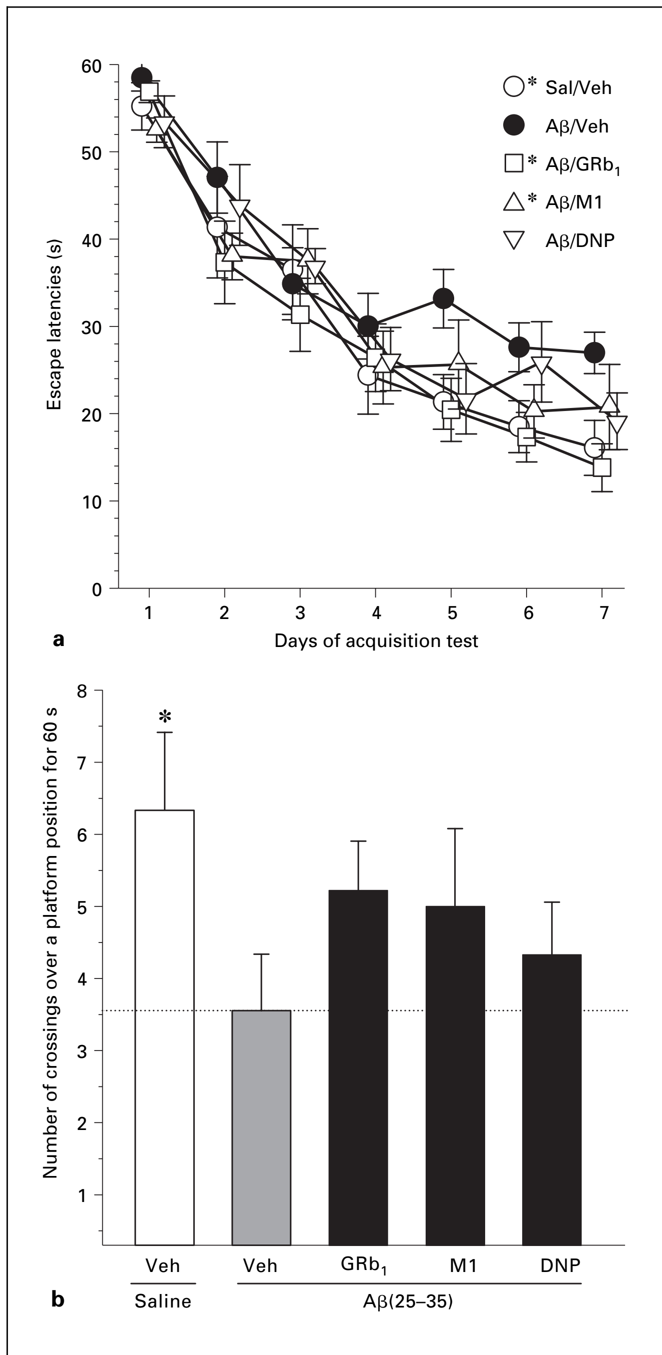

Fig. 2. Effects of ginsenoside Rb_1 and M1 on the impairment of spatial memory induced by Aβ(25–35) injection. **a** Escape latencies per group in four trials were tested in a Morris water maze over 7 days. Vehicle was administered p.o. to saline-i.c.v.-injected mice. To Aβ(25–35)-i.c.v.-injected mice (5 nmol), the vehicle, ginsenoside Rb_1 (10 μmol/kg), M1 (10 μmol/kg), or donepezil (0.5 mg/kg) was administered p.o. for 14 days. Values represent the means and SEM of 9 mice. * $p < 0.05$ when compared with the Aβ(25–35) plus vehicle-treated group. Two-way repeated measure analysis of variance was carried out, followed by Dunnett's post hoc test. **b** The number of crossings over the previous position of a platform had previously been measured over 60 s, 6 days after the last acquisition test. This was also 6 days after the discontinuance of drug treatment. Vehicle was administered p.o. to saline-i.c.v.-injected mice. To Aβ(25–35)-i.c.v.-injected mice, vehicle (Veh), ginsenoside Rb_1 (GRb_1), M1, or donepezil (DNP) was administered p.o. Values represent the means and SEM of 9 mice. * $p < 0.05$ when compared with the Aβ(25–35) plus vehicle-treated group. One-way analysis of variance was carried out, followed by Dunnett's post hoc test.

suggest that ginsenoside Rb_1 and M1 may induce the structural repair of neuronal connections.

In the rat large intestine, ginsenoside Rb_1 is completely metabolized to M1 3 h after administration [25]. In mice, only M1 is continuously detected in the blood from 30 min to 16 h after oral administration of ginsenoside Rb_1 [26]. In humans, M1 is detected in plasma from 7 h after the ingestion of Ginseng, and in urine from 12 h after intake, and aglycone is not detected in either plasma or urine [20]. These results suggest that M1 is the final metabolite of ppd-type saponins. The recovery potency in Aβ(25–35)-injected mice by p.o.-administered ginsenoside Rb_1 and M1 was almost identical, indicating that the majority of orally administered ginsenoside Rb_1 was metabolized into M1. Considering that most ppd-type saponins are metabolized to M1, which is the active principal, the total content of ppd-type saponins is possibly an important index of the anti-AD activity of Ginseng.

Effect of M1 on Aβ(25–35)-Induced Axonal Atrophy in Rat Cortical Neurons

In in vitro experiments, M1 demonstrated an axonal regeneration effect. To investigate the Aβ(25–35)-induced damage to the neuronal network and the reconstructive activity of drugs, 10 μ*M* Aβ(25–35) was added to the cortical neurons on day 7, and after 3 days the medium was replaced by fresh medium, including drugs. Although the cortical neurons connected with each other during the 7-day culture, some of the connections were lost 3 days after Aβ(25–35) treatment. At 4 days, both phosphorylated NF-H-positive (fig. 4a) and MAP2-positive (fig. 4b) neurites were significantly shortened by Aβ(25–35) treatment. Treatment with 0.01 μ*M* M1 (to 78.5% of the control) significantly increased the recovery of the length of phosphorylated NF-H-positive neurites (fig. 4a), while MAP2-positive neurites were not extended (fig. 4b). NGF significantly enhanced the lengths of phosphorylated NF-

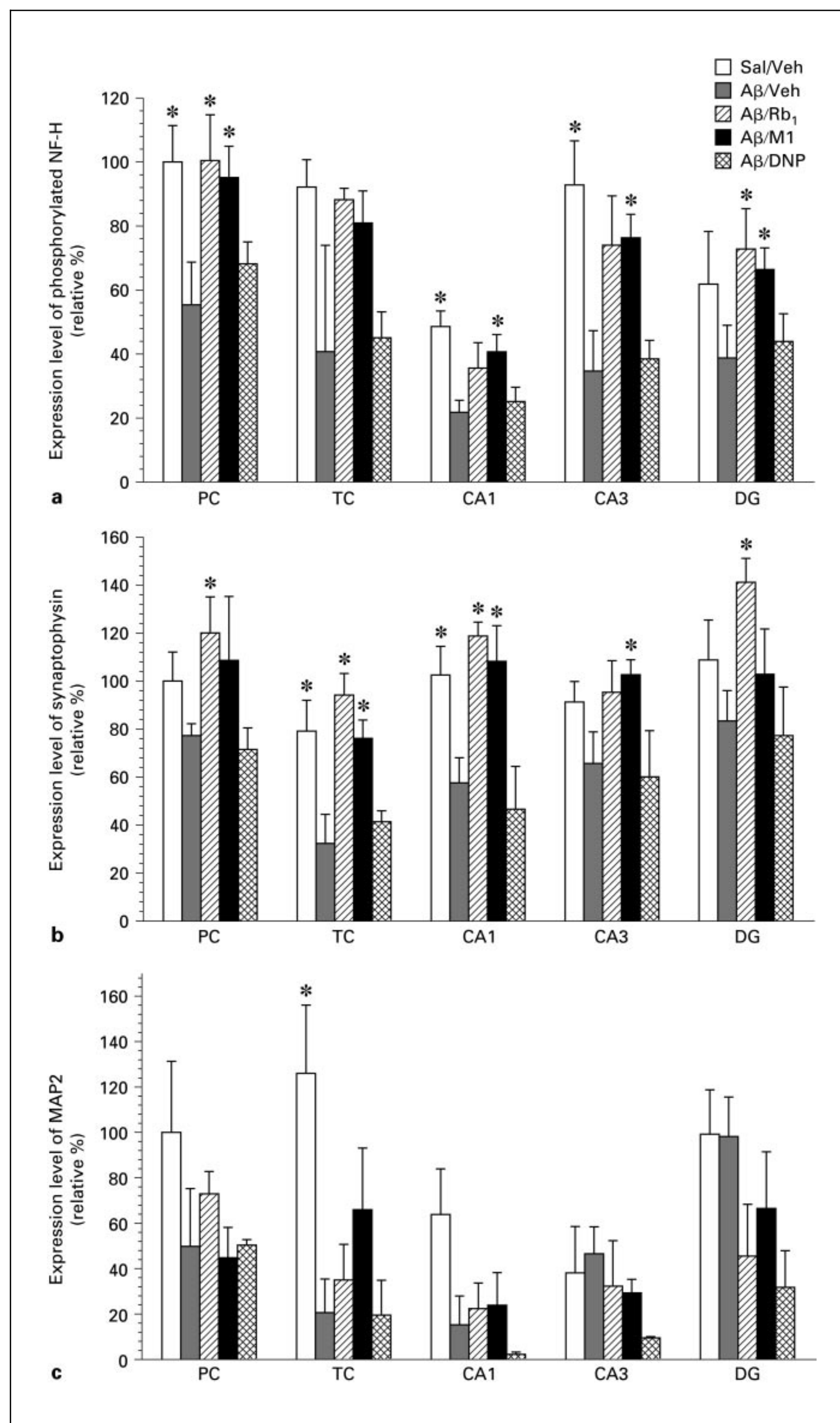

Fig. 3. Effects of ginsenoside Rb_1 and M1 on axonal atrophy and synaptic loss induced by Aβ(25–35) injection. Expression levels of phosphorylated NF-H (**a**), synaptophysin (**b**) and MAP2 (**c**) in brain slices were quantified. Vehicle was administered p.o. to saline-i.c.v.-injected mice. To Aβ(25–35)-i.c.v.-injected mice, vehicle, ginsenoside Rb_1 (10 μmol/kg), M1 (10 μmol/kg), or donepezil (0.5 mg/kg) was administered p.o. for 14 days. The parietal cortex (PC), temporal cortex (TC), hippocampal CA1 and CA3, and dentate gyrus (DG) were observed. The fluorescence intensities of six areas in each slice were measured. Values represent the means and SEM of three mice. * $p < 0.05$ when compared with the Aβ(25–35) plus vehicle-treated group. One-way analysis of variance was carried out, followed by Dunnett's post hoc test.

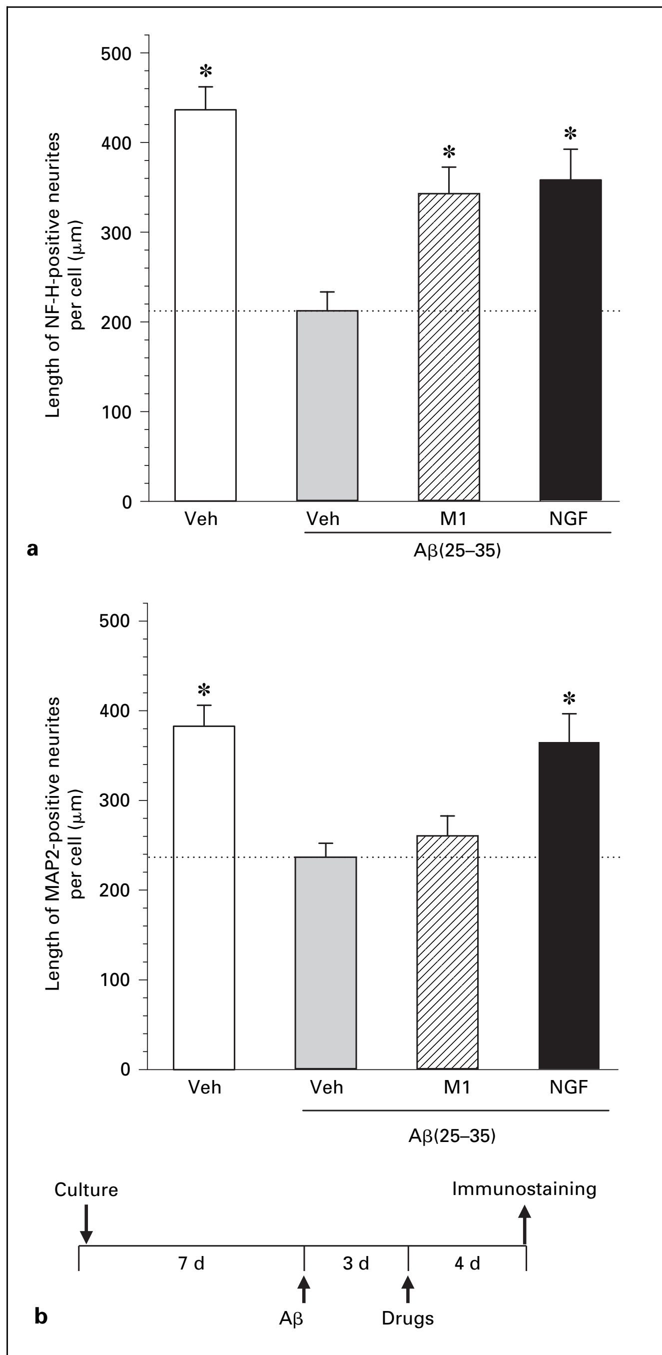

Fig. 4. Effect of post-treatment with M1 on Aβ(25–35)-induced axonal and dendritic atrophy. Aβ(25–35) (10 µ*M*) was added to rat cortical neurons at 7 days in vitro. Three days later, the medium was replaced by new medium containing M1 (0.01 µ*M*), NGF (100 ng/ml) or the vehicle (Veh, DMSO). Four days later, the cells were fixed and immunostained for phosphorylated NF-H (**a**) and MAP2 (**b**). The lengths of neurites positive for phosphorylated NF-H or MAP2 per cell were measured. Values represent the means and SEM of 30 cells. * $p < 0.05$ when compared with the Aβ(25–35) plus vehicle-treated group. One-way analysis of variance was carried out, followed by Dunnett's post hoc test.

H-positive (to 82.0% of the control) and MAP2-positive (to 95.3% of the control) neurites. In addition, M1 increased in pre-synaptic density to the control level after Aβ(25–35)-induced synaptic loss occurred [our unpublished data].

Neuritic atrophy by Aβ(1–40) and Aβ(25–35) has been reported in chick sympathetic neurons [27] and rat cortical neurons [28]. As neurite atrophy is thought to be due to unusual cell adhesion [27, 29], M1 may be capable of normalizing the adhesive mechanism. Although Aβ is known to cause neuronal death through increased [Ca^{2+}] neurons [30], increased peroxynitrites in microglias [31], and mitochondrial dysfunction in neurons [32], the death pathway has been shown to be mediated by separate molecular mechanisms of a neuritic dystrophy event [27–29]. Since ginsenoside Rb_1 did not inhibit neuronal death induced by Aβ(25–35), the mechanism of rescuing axonal atrophy may not be identical to that for recovery from Aβ-induced neuronal death.

Ashwagandha

Neurite Outgrowth with Methanol Extract and Isolated Withanolides

Ashwagandha (root of *Withania somnifera* Dunal) is the most popular herbal drug in Ayurvedic medicine, and has been used traditionally and commonly as a tonic and nootropic agent. It has also been reported as associated with improvements in scopolamine-induced memory deficits in mice [33]. Treatment with a methanol extract of Ashwagandha induced neurite extension [34]. We further identified 6 withanolide derivatives from methanol extract (withanolide A, withanoside IV, withanoside VI, etc.; fig. 1), which induced neurite outgrowth in human neuroblastoma SH-SY5Y cells [35]. In normal cortical neurons, the predominant dendritic outgrowth was induced by treatment with withanoside IV or withanoside VI, whereas predominant axonal outgrowth was observed in treatment with withanolide A in normal cortical neurons [36].

Effect of Withanolides on Aβ(25–35)-Induced Neuritic Atrophy and Synaptic Loss

In Aβ(25–35)-induced damaged cortical neurons, withanolide A, withanoside IV, and withanoside VI showed neuritic regeneration and synaptic reconstruction. 24 h after culture initiation, 10 µ*M* Aβ(25–35) was added to the culture medium simultaneously with the drugs. Four days later, Aβ(25–35) treatment significantly

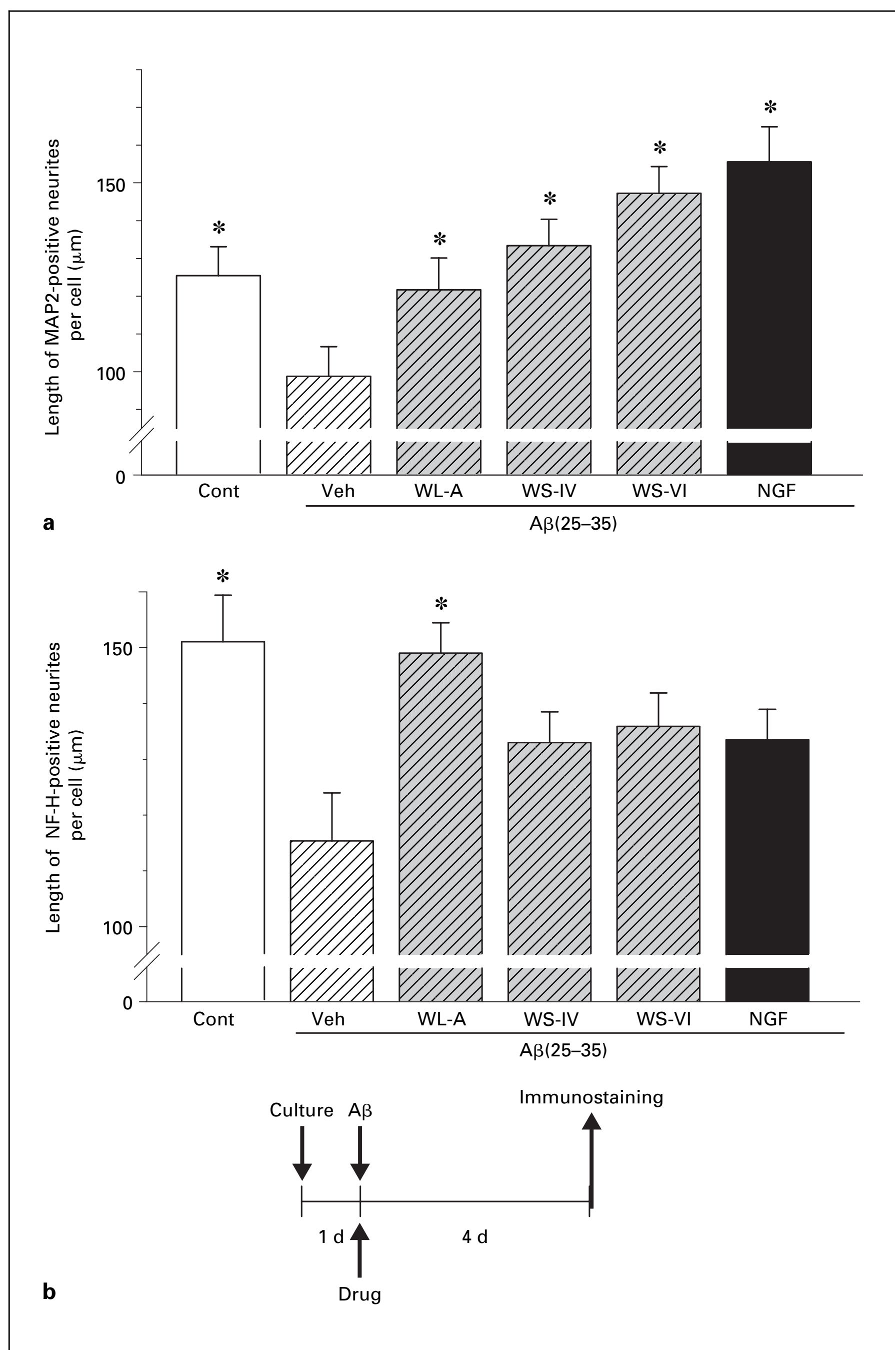

Fig. 5. Effects of withanolide A, withanoside IV, and withanoside VI on the prevention of Aβ(25–35)-induced dendritic and axonal atrophy. Cortical neurons were cultured for 24 h, and then the cells were treated simultaneously with 10 μ*M* Aβ(25–35), and withanolide A (WL-A), withanoside IV (WS-IV), or withanoside VI (WS-VI) at a concentration of 1 μ*M*; or NGF or BDNF at a concentration of 100 ng/ml; or vehicle (Veh); or with vehicle alone (Cont). Four days after treatment, the cells were fixed and immunostained for MAP2 or phosphorylated NF-H. Lengths of MAP2-positive neurites (**a**) and phosphorylated NF-H-positive neurites (**b**) were measured in each treatment. The values represent the means and SEM of 30 cells. * $p < 0.05$ when compared with the Aβ(25–35) plus vehicle-treated group. One-way analysis of variance was carried out, followed by Dunnett's post hoc test.

inhibited the outgrowth of both MAP2-positive neurites and phosphorylated NF-H-positive neurites, showing that Aβ(25–35) induced both dendritic and axonal atrophy in rat cortical neurons. Simultaneous treatment with Aβ(25–35) and withanolide A, withanoside IV, or withanoside VI at a concentration of 1 μ*M* prevented both dendritic and axonal atrophy induced by Aβ(25–35). Dendritic atrophy was completely prevented by treatment with withanolide A (97.0% of the control), withanoside IV (106.3% of the control), or withanoside VI (117.4% of the control) (fig. 5a). In particular, treatment with withanosides IV and VI tended to induce the growth of longer dendrites than treatment with withanolide A.

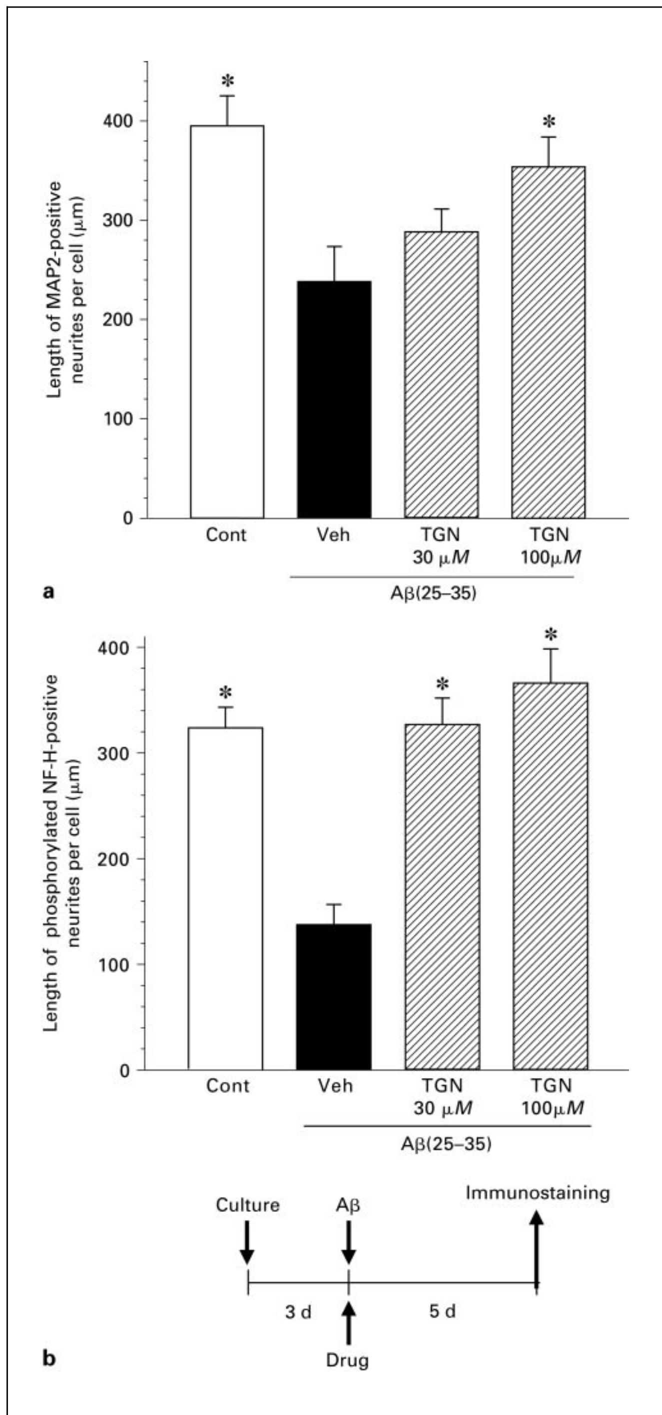

Fig. 6. The effect of trigonelline on the prevention of Aβ(25–35)-induced dendritic and axonal atrophy. Cortical neurons were cultured for 3 days, and then the cells were treated simultaneously with 10 μ*M* Aβ(25–35), and trigonelline at a concentration of 30 or 100 μ*M*, or vehicle (Veh), or with the vehicle alone (Cont). Five days after treatment, the cells were fixed and immunostained for MAP2 or phosphorylated NF-H. Lengths of MAP2-positive neurites (**a**) and phosphorylated NF-H-positive neurites (**b**) were measured in each treatment. The values represent the means and SEM of 12–20 cells (**a**) or 14–22 cells (**b**). * $p < 0.05$ when compared with the Aβ(25–35) plus vehicle-treated group. One-way analysis of variance was carried out, followed by Dunnett's post hoc test.

Axonal atrophy was partially prevented by treatment with withanoside IV (88.0% of the control) and withanoside VI (90.0% of the control), whereas treatment with withanolide A (98.6% of the control) completely prevented axonal atrophy (fig. 5b).

To determine whether regenerated neurites are able to reconstruct synapses, the expressions of synaptic markers were investigated. Rat cortical neurons were cultured for 21 days to construct mature synapses in vitro, and after the culture period, Aβ(25–35) was added to the samples. Four days later, the cells were immunostained with an antibody for post-synaptic density, (PSD)-95 (post-synaptic marker), or with synaptophysin (pre-synaptic marker). PSD-95- and synaptophysin-positive puncta were significantly decreased by treatment with Aβ(25–35) [37]. Withanolide A, withanoside IV, withanoside VI, or NGF was added to the culture medium after 4 days of treatment with Aβ(25–35) after synaptic loss had occurred. Seven days after the addition of the drug, the cells were fixed and immunostained for PSD-95 or synaptophysin. Treatment with withanolide A, withanoside IV, or withanoside VI significantly induced both PSD-95 and synaptophysin expression, as compared with treatment with the vehicle. These results indicate that withanolide A, withanoside IV, and withanoside VI facilitated the reconstruction of both post-synaptic and pre-synaptic regions in neurons in which severe synaptic loss had already occurred. This increase in post-synaptic structures tended to be significant following treatment with withanoside IV (86.0% of the control) and withanoside VI (83.6% of the control), as compared with withanolide A treatment (68.0% of the control). However, reconstruction of the pre-synaptic region was induced significantly and markedly by treatment with withanolide A (108.1% of the control), as compared with withanoside IV (81.3% of the control) and withanoside VI (75.8% of the control) treatments. Treatment with NGF did not lead to an increase in the development of either the post-synapses (57.7% of the control) or the pre-synapses (54.4% of the control).

Although NGF extended both axons and dendrites (fig. 4, 5), it has no effect on synaptogenesis. Since NGF itself is not able to pass through the blood-brain barrier, low-molecular-weight substances that mimic NGF action have been developed as anti-dementia drugs. However, such NGF-like drugs are not expected to cure dementia because of a lack of synaptogenesis activity.

Coffee Beans

Neurite Outgrowth with Trigonelline

Coffee is consumed as a drink, and is known to stimulate the central nervous system as well as the heart and circulation [38]. It is thought that these effects are mainly caused by caffeine [39] but the effects of other coffee constituents on the central nervous system have hardly been reported. Coffee beans are crude drugs, used in the traditional system of Unani medicine [40].

Among the extracts of raw and roasted coffee beans, a methanol-soluble fraction of the ethanol extract (1 μg/ml) of raw beans significantly increased the percentage of cells with neurites in human neuroblastoma SK-N-SH cells [41]. It was demonstrated that the neurite outgrowth activity of the methanol fraction decreased depending on the extent of roasting. Among subfractions of this methanol fraction, the basic fraction had significant neurite outgrowth activity. In this basic fraction, trigonelline was identified as an active constituent (fig. 3). It is known that a decrease in trigonelline is related to the degree of roasting [42].

In rat cortical neurons, trigonelline showed dendritic and axonal regeneration. Three days after initiation of the culture, Aβ(25–35) was added to the culture medium with trigonelline. Trigonelline (30 and 100 μ*M*) treatment dose-dependently prevented both dendritic (fig. 6a) and axonal (fig. 6b) atrophy induced by Aβ(25–35).

Effect of Trigonelline on Aβ(25–35)-Induced Memory Impairment

Fourteen days after the i.c.v. injection of Aβ(25–35) in male ddY mice (6 weeks old), trigonelline (500 mg/kg), donepezil hydrochloride (0.5 mg/kg), or the vehicle (tap water) was administered orally once daily for 15 days. Mice were trained in the water maze for 5 days, starting 21 days after the i.c.v. administration of Aβ(25–35). Six days after the last acquisition test, the retention test was performed (fig. 7). The number of crossings over a previous platform position was significantly decreased in the Aβ(25–35)-injected group compared with the saline-injected group. The number of crossings was recovered by treatment with trigonelline, suggesting that memory retention is improved by trigonelline.

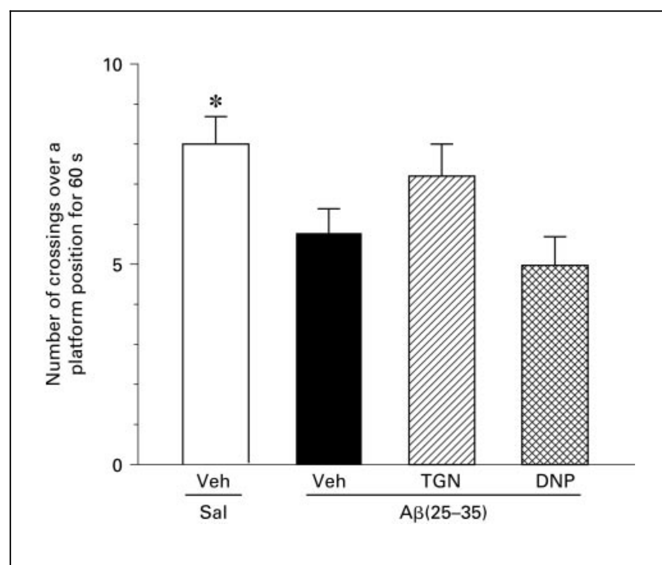

Fig. 7. Effect of trigonelline on the impairment of spatial memory induced by Aβ(25–35) injection. The number of crossings over the previous position of a platform was measured over 60 s, 6 days after the last acquisition test in a Morris water maze. This was also 6 days after the discontinuance of drug treatment. Vehicle was administered p.o. to saline-i.c.v.-injected mice. To Aβ(25–35)-i.c.v.-injected mice (4.7 nmol), the vehicle (Veh), 500 mg/kg trigonelline (TGN), or 0.5 mg/kg donepezil (DNP) was administered p.o. Values represent the means and SEM of 9 mice. * $p < 0.05$ when compared with the Aβ(25–35) plus vehicle-treated group. One-way analysis of variance was carried out, followed by Dunnett's post hoc test.

Conclusions

The ppd-type saponins of Ginseng drugs and M1 (a metabolite of ppd-type saponins by intestinal bacteria) induced significant recovery from memory impairment, axonal atrophy and synaptic loss in mice. The effect of M1 on axonal reconstruction was further confirmed in cultured cortical neurons. These results suggest that orally administered ppd-type saponins potentially ameliorate dementia by reconstructing the neuronal network. Withanolide A, withanoside IV, and withanoside VI, which were isolated from Ashwagandha, facilitated the regeneration of dendrites and axons, and led to the dramatic construction of synapses, although the neuron damage was

profound and severe. Trigonelline also had dendritic and axonal regeneration activity, and improved memory retention. These compounds, sourced from natural products, and used with treatments preventing pathogenesis and neuronal death, are expected to play an important role as new categorized drugs in curing neurodegenerative diseases in the near future. Although we have shown the high potential of neuronal regeneration from compounds isolated from Ginseng drugs, Ashwagandha and coffee beans, it is dangerous to simply imply that these herbal drugs are expected to be excellent anti-dementia drugs. When taking herbal drugs, the risk of side effects brought by other constituents, and sufficient efficacy compared with isolated compounds should be investigated and carefully considered. However, drugs used in traditional medicine may offer a treasury of new medicines to treat intractable diseases with the use of novel study concepts and the application of objective scientific analyses.

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