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Axonal and Dendritic Extension by Protopanaxadiol-Type Saponins From Ginseng Drugs in SK-N-SH Cells

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ABSTRACT—Extension of axons and dendrites in neurons may compensate for and repair damaged neuronal networks in the dementia brain. To find out drugs capable of regenerating the neuronal network, we focused on several herbal drugs belonging to the genus *Panax*, kinds of Ginseng, and investigated neurite outgrowth activity of their extracts and compounds. We found that the methanol extracts of Ginseng (root of *P. ginseng*), Notoginseng (root of *P. notoginseng*) and *Ye-Sanchi* in Chinese (rhizome of a relative to *P. vietnamensis*) increased neurite outgrowth in SK-N-SH cells. The protopanaxadiol-type saponins, ginsenosides Rb_1 and Rb_3, and notoginsenosides R_4 and Fa isolated from *Ye-Sanchi* extract extended neurites, while protopanaxatriol-, ocotillol- and oleanane-type saponins had no effect on the neurite outgrowth. The percentage of cells with multipolar neurites and number of varicosities were intensely high in cells treated with the methanol extract of *Ye-Sanchi* as well as ginsenosides Rb_1 and Rb_3, and notoginsenosides R_4 and Fa. Both phosphorylated NF-H-expressing neurites and MAP2-expressing ones were extended by treatment with those saponins and the extract. Especially, longer neurites were mainly positive for phosphorylated NF-H. These results suggest that protopanaxadiol-type saponins enhance axonal and dendritic formation activity.

Keywords: Ginseng, Protopanaxadiol saponin, Axon, Dendrite, SK-N-SH cell

Despite the growing social problem of dementia, there is not yet any drug available for reliable treatment against dementia. Cholinomimetic agents in the form of acetylcholine esterase inhibitors are primarily used in the treatment of dementia patients. These drugs, however, just slow down the progression of dementia rather than actually restoring brain function. Regardless of the type (Alzheimer's or cerebrovascular), dementia is induced by neuronal degeneration and atrophy. Inhibiting the cause of the disease, an accumulation of amyloid β in Alzheimer's brain (1 – 3), and attempts for neuroprotection have lately been considered attractive. Such protective measures may reduce progression of dementia, but can not recover severe disfunction of the brain. Therefore, one strategy for achieving an irreversible amelioration of dementia may be reconstruction of synaptic formation in the brain. Although it is difficult to repair neurons or to increase cell number after neurodegeneration in the central nervous system, new synapses could possibly be formed through the activation of remaining immature and mature neurons. Since synaptic formation is based on neurite outgrowth and dendrite and axon maturation, drugs activating these steps could possibly initiate a recovery of brain function.

Ginseng, root of *Panax ginseng*, is the most famous drug in traditional medicine as a tonic and an anti-amnesic agent. It has been reported that significant improvement in learning and memory was observed in brain-damaged rats (4, 5) and aged rats (5) after oral administration of ginseng powder. Furthermore, neurites of rat cultured cerebral cortex were extended by ginseng saponins (6). The major ginseng saponins, ginsenosides Rb_1 and Rg_1, improved spatial learning in normal mice (7), and ginsenoside Rb_1 potentiated the nerve growth factor (NGF)-mediated neurite outgrowth of chick dorsal root ganglia (8, 9). These findings encouraged us to investigate several Ginseng drugs to search for candidates that can ameliorate dementia. The present study deals with the neurite outgrowth effects of various Ginseng drugs in human neuroblastoma SK-N-SH cells as well as of constituents isolated from their extracts.

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MATERIALS AND METHODS

Materials

Ginseng drugs and plant used in this study are listed in Table 1. Two drugs contained in these preparations, *Ye-Sanchi* and *Kouzichi* in Chinese reading, were identified as a relative to *Panax vietnamensis* and *P. japonicus* var. *major*, respectively, by morphological study and gene analysis in our laboratory (10). Voucher specimens of each sample were deposited in the Museum of Materia Medica, Research Center for Ethnomedicines of our University.

Preparation of herbal drug extracts

Fifty grams of each were extracted three times with 300 ml methanol (each for 2 h, under reflux) and filtered. The filtrate was concentrated under reduced pressure and freeze-dried. These extracts were dissolved in vehicle solution, dimethyl sulfoxide and used in the present assay.

Isolation of constituents

Dried rhizomes (500 g) of *Ye-Sanchi* were pulverized and extracted three times with methanol (2 L × 3) under reflux for 3 h for the first time and then for 2 h each. The combined extracts were evaporated on a rotatory evaporator to give a viscous residue. This residue was suspended in water and lyophilized to give 150.2 g of dry extract. The extract was suspended in 1500 mL water and partitioned with ethyl acetate and *n*-butanol saturated with water to give a butanol-soluble fraction (72.3 g) after evaporation. This fraction was subjected to column chromatography on silica gel (SiO_2 60, 0.040 – 0.063 mm; Merck, Darmstadt, Germany). Elution with chloroform-methanol-water gradient gave 27 fractions. After repeated column chromatography over RP-18 and Sephadex LH-20 and final purification by HPLC, 30 compounds were isolated from *Ye-Sanchi*. Fifteen compounds (ginsenosides Rb_1, Rb_3, Rg_1 and Re, notoginsenosides R_4, Fa and R_1, Yesanchinoside J, 20-*O*-glc-ginsenoside R_f, majonoside R_2, (*24S*)-pseudoginsenoside RT_4 and F_{11}, vina-ginsenosides R_1, R_2 and R_6) were used in the present assays. Other compounds were isolated in small amounts, which were not sufficient for testing.

Dried rhizomes (1000 g) of *Kouzichi* were pulverized and extracted three times with 4 L methanol under reflux for 3 h each. The combined extracts were concentrated under reduced pressure and freeze-dried to give 264 g of dry extract. One hundred grams of this extract was suspended in 800 mL water and partitioned with ethyl acetate and *n*-butanol to give a butanol-soluble fraction (32 g). This fraction was subjected to column chromatography on silica gel, eluting with chloroform-methanol-water gradient, to obtain 56 fractions. After repeated column chromatography over silica gel and Sephadex LH-20, and purification by HPLC if necessary, 9 compouds were isolated. Although notoginsenoside R_2, ginsenosides Rg_2 and Ro, and chikusetsusaponin IVa were examined, the other 5 compounds were not sufficient for testing. Chemical structures of isolated compounds were determined by IR, ^{1}H-NMR, ^{13}C-NMR, APIMS, APIMS/MS analyses, melting point and specific rotation.

Cell culture

A human neuroblastoma cell line, SK-N-SH (Riken, Tsukuba), was maintained as a monolayer culture in minimum essential medium (Gibco BRL, Rockville, MD, USA) supplemented with 5% fetal bovine serum at 37°C in a humidified atmosphere of 95% air / 5% CO_2.

Table 1. Ginseng drugs and plant used in this experiment

Crude drug name	Scientific name	Used part	Place of collection (Market)	TMPW No.*
Ginseng	*Panax ginseng* C.A.MEYER	Root	Nagano prefecture, Japan (Tochimoto Tenkaido Co., Ltd.)	19899
Red Ginseng	*P. ginseng* C.A.MEYER	Root (steamed)	Nagano prefecture, Japan (Tochimoto Tenkaido Co., Ltd.)	19898
Notoginseng	*P. notoginseng* (Burk.) F.H.CHEN	Root	Wenshan county, Yunnan province, China	18214
Ye-Sanchi	Relative to *P. vietnamensis* HA et GRUSHV	Rhizome & root	Jingping county, Yunnan province, China	19759
Zhuzishen	*P. japonicus* C.A.MEYER var. *major* C.Y.WU et FENG	Rhizome	Lijiang county, Yunnan province, China	19626
Kouzichi	*P. japonicus* C.A.MEYER var. *major* C.Y.WU et FENG	Rhizome	Hubei province, China	19896
(Plant)	*P. stipuleanatus* H.T.TSAI et K.M.FENG	Rhizome & root	Pingbian county, Yunnan province, China	K. Komatsu et al., Y283#

*The registration number of the Museum of Materia Medica, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University.
#Collector and voucher number.

Quantification of neurite outgrowth

Cells were plated with a plating density of 8.0×10^3 cells/cm^2 in 60-mm diameter culture dishes with 2-mm grids (Corning, Acton, MA, USA). Extracts or compounds were applied to the culture medium once at the start of culture. The vehicle solution was 0.1% dimethyl sulfoxide (DMSO). Cells (100 – 300 cells) were counted in four or eight areas of 650×430 μm per one dish, and the percentage of cells with neurites was calculated. All neurites exceeding 50 μm in length were counted.

Immunocytochemistry

Cells were cultured in 8-chamber slides (Becton Dickinson Labware, Franklin Lakes, NJ, USA) for 6 days in the presence of the methanol extract of *Ye-Sanchi*, compounds or vehicle. The slides were rinsed in phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde for 30 min at room temperature, and rinsed again with PBS containing 0.2% TritonX-100. Antiserum to phosphorylated neurofilament-H (dilution 1:1000, anti-NF-H rabbit antiserum; Affiniti, Exeter, UK) and monoclonal antibody to MAP2 (MAP2a and 2b) (dilution 1:200; Chemicon, Temecula, CA, USA) were used. PBS containing 0.3% TritonX-100, antibodies to phosphorylated NF-H and MAP2, and 1% normal donkey serum were added to the fixed slides. After incubation for 1 day at 4°C, the slides were rinsed with PBS containing 0.2% TritonX-100 and incubated for 30 min with PBS containing 0.3% TritonX-100, fluorescein isothiocyanate (FITC)-labeled donkey anti mouse IgG (dilution 1:100, Chemicom), and Cy3-labeled donkey anti rabbit IgG (dilution 1:100, Chemicon). The slides were washed with PBS, mounted with Aqua Poly Mount (Polysciences, Warrington, PA, USA), and viewed with a confocal laser scanning microscope (LSM-GB200-IMT-2; Olympus, Tokyo).

Data processing

Statistical comparisons were made by the Student's *t*-test or repeated measured two-way analysis of variance (ANOVA) and post hoc Student-Newman-Keuls multiple comparisons. $P<0.05$ was considered as significant. The means of data are presented together with S.E.M.

RESULTS

Effects of methanol extracts of Ginseng drugs on neurite extension

Methanol extracts of 6 kinds of Ginseng drugs and the plant material of *Panax stipuleanatus* were added to the culture medium of SK-N-SH cells at a concentration of 50 μg/ml, and the neurite outgrowth activity was observed 6 days later (Fig. 1). Values represent the means and S.E.M. of three experiments. Since extracts of *Zhuzishen* (dried rhizome of *P. japonicus* var. *major*) and dried rhizome of *P. stipleanatus* were toxic to cell viability, they were tested at lower doses, 5 μg/ml. The methanol extracts of Ginseng (dried root of *P. ginseng*), Red Ginseng (steamed and then dried root of *P. ginseng*), Notoginseng (dried root of *P. notoginseng*) and *Ye-Sanchi* (dried rhizome of a relative to *P. vietnamensis*) increased neurite outgrowth, and Red Ginseng and *Ye-Sanchi* showed especially significant effects. Neurite outgrowth activities of these 4 extracts were increased dose-dependently, and effects of extracts of Red Ginseng and *Ye-Sanchi* were significant even at 5 μg/ml (data not shown). Water extracts of these 4 kinds of Ginseng drugs also showed neurite outgrowth effects, but they were weaker than the methanol extracts (data not shown). The methanol extracts of *Zhuzishen*, *Kouzichi* and rhizome of *P. stipleanatus* had no effect.

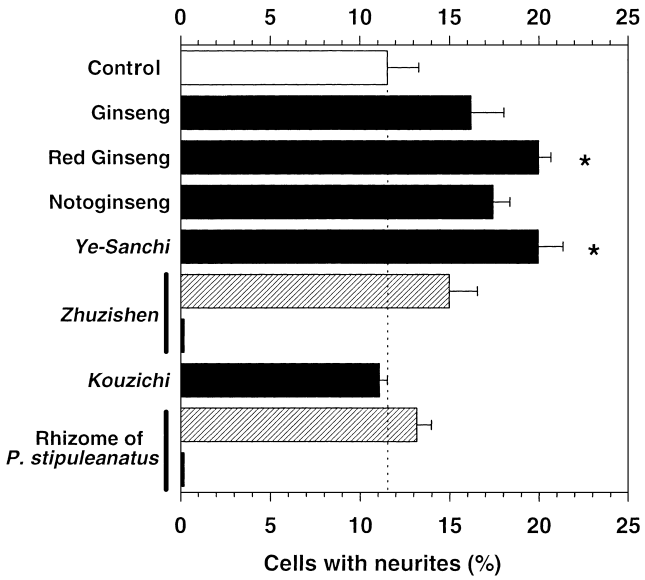
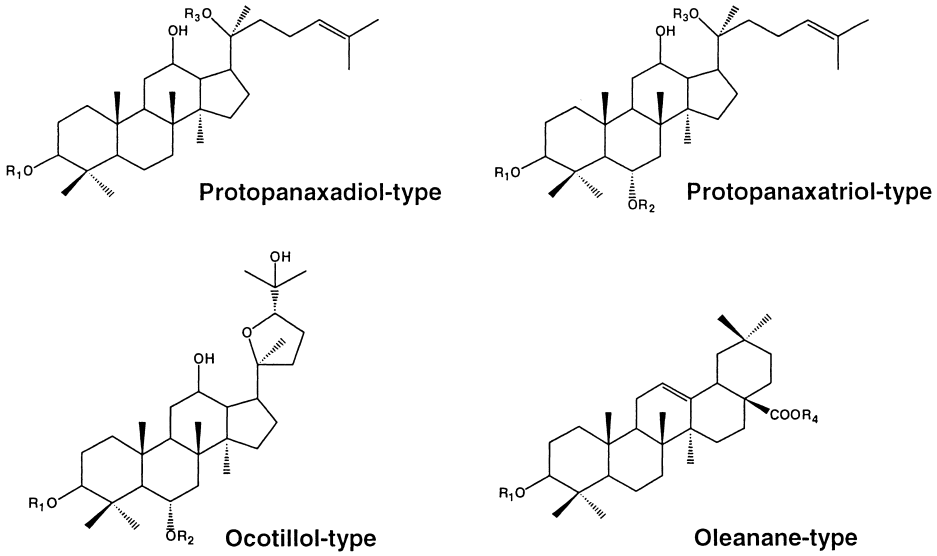


Fig. 1. Effects of extracts from Ginseng drugs on neurite outgrowth in SK-N-SH cells. Cells were treated with the methanol extract of Ginseng, Red Ginseng, Notoginseng, *Ye-Sanchi*, *Zhuzishen*, *Kouzichi* and rhizome of *P. stipuleanatus* at a final concentration of 50 μg/ml (closed columns, n = 3) or vehicle (0.1% DMSO, n = 6) at the start of culture. The extracts of *Zhuzishen* and rhizome of *P. stipuleanatus* were also used at 5 μg/ml (hatched columns), since they were toxic at a dose of 50 μg/ml. Neurite outgrowth activity was measured 6 days later. Values represent the means and S.E.M. of 3 experiments. *$P<0.05$, when compared with the vehicle (Student's *t*-test).

Effects of isolated saponins on neurite extension

Of the methanol extracts of Red Ginseng and *Ye-Sanchi* which were significantly effective to promote neurite outgrowth, many more kinds of constituents were detected in *Ye-Sanchi* by a preliminaly TLC. With the aim of comparing activities of many constituents, we isolated saponins from *Ye-Sanchi* (Table 2). We also isolated oleanane-type

Table 2. Structures of compounds isolated from *Ye-Sanchi* and *Kouzichi* extracts



Compound name	Type	R_1	R_2	R_3	R_4
Ginsenoside Rb_1	Diol	glc-2-glc		glc-6-glc	
Ginsenoside Rb_3	Diol	glc-2-glc		glc-6-xyl	
Notoginsenoside R_4	Diol	glc-2-glc		glc-6-glc-6-xyl	
Notoginsenoside Fa	Diol	glc-2-glc-2-xyl		glc-6-glc	
Yesanchinoside J	Diol	glc(6-Ac)-2-glc		glc-6-glc-6-xyl	
Ginsenoside Rg_1	Triol	H	glc	glc	
Ginsenoside Rg_2[#]	Triol	H	glc-2-rha	H	
Ginsenoside Re	Triol	H	glc-2-rha	glc	
Notoginsenoside R_1	Triol	H	glc-2-xyl	glc	
Notoginsenoside R_2[#]	Triol	H	glc-2-xyl	H	
20-*O*-Glc-ginsenoside Rf	Triol	H	glc-2-glc	glc	
Majonoside R_2	Ocotillol	H	glc-2-xyl		
(24*S*)-Pseudoginsenoside RT_4	Ocotillol	H	glc		
(24*S*)-Pseudoginsenoside F_{11}	Ocotillol	H	glc-2-rha		
Vina-ginsenoside R_1	Ocotillol	H	glc(6-Ac)-2-rha		
Vina-ginsenoside R_2	Ocotillol	H	glc(6-Ac)-2-xyl		
Vina-ginsenoside R_6	Ocotillol	H	glc(6-α-glc)-2-xyl		
Ginsenoside Ro[#]	Oleanane	gluA-2-glc			glc
Chikusetsusaponin lVa[#]	Oleanane	gluA			glc

[#]: Compounds isolated from *Kouzichi* extract.

saponins from *Kouzichi*, because they were only present in small amounts in Ginseng, Red Ginseng, Notoginseng and *Ye-Sanchi*. Values represent the means and S.E.M. of three experiments.

Out of 5 protopanaxadiol-type saponins, ginsenosides Rb_1 and Rb_3, and notoginsenosides R_4 and Fa extended significantly the neurites in SK-N-SH cells at a concentration of 100 μM (Fig. 2). Neurite outgrowth activities of these 4 compounds were increased dose-dependently (data not shown). The novel compound Yesanchinoside J was found to be toxic at 100 μM, and no effect was observed at the lower concentration of 10 μM. Six protopanaxatriol-type, 6 ocotillol-type and 2 oleanane-type saponins showed no effect on neurite outgrowth in SK-N-SH cells (Fig. 2).

Time courses of ginsenosides Rb_1 and Rb_3 and notoginsenosides R_4 and Fa showed that neurite extension by these compounds increased time-dependently and reached a maximum at 5 days after the start of treatment (Fig. 3).

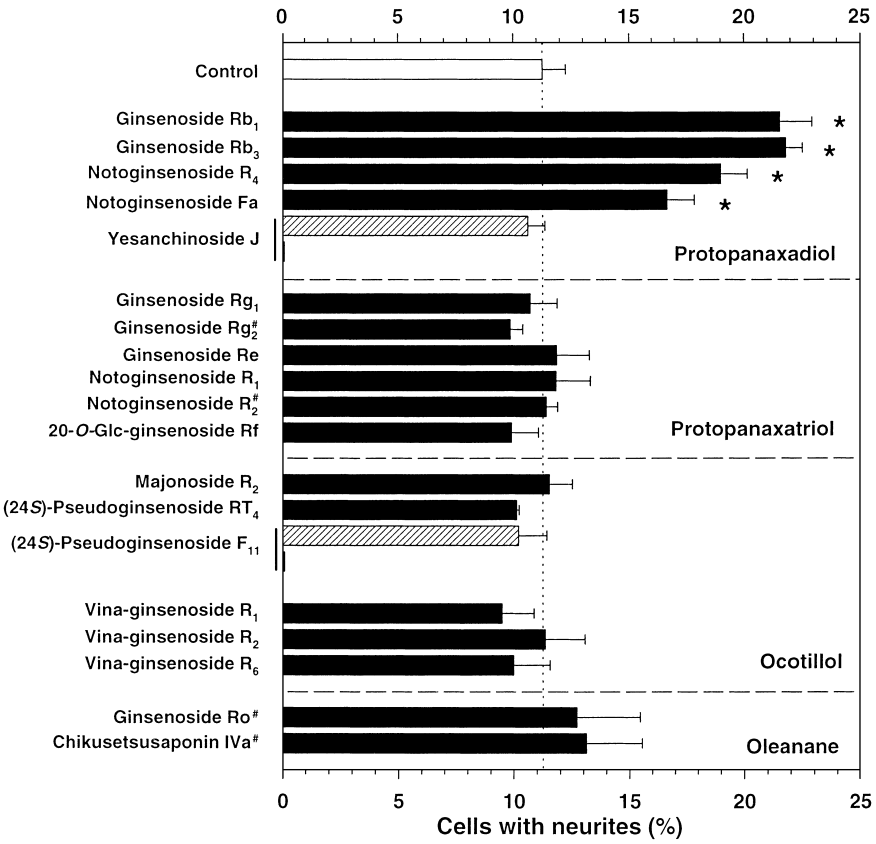


Fig. 2. Effects of compounds isolated from the methanol extracts of *Ye-Sanchi* and *Kouzichi* on neurite outgrowth in SK-N-SH cells. Cells were treated with 5 compounds of protopanaxadiol-, 4 compounds of protopanaxatriol- and 6 compounds of ocotillol-type saponins isolated from *Ye-Sanchi*, 2 compounds of protopanaxatriol- and 2 compounds of oleanane-type saponins isolated from *Kouzichi* (marked #) at a concentration of 100 μM (closed columns) or vehicle (0.1% DMSO, open column) at the start of culture. Yesanchinoside J and (*24S*)-pseudoginsenoside F_{11} were also used at 10 μM (hatched columns), since they were toxic at a dose of 100 μM. Neurite outgrowth activity was measured 5 days later. Values represent the means and S.E.M. of 3 experiments. Three to ten dishes were observed. *P<0.05 when compared with vehicle (Student's t-test).

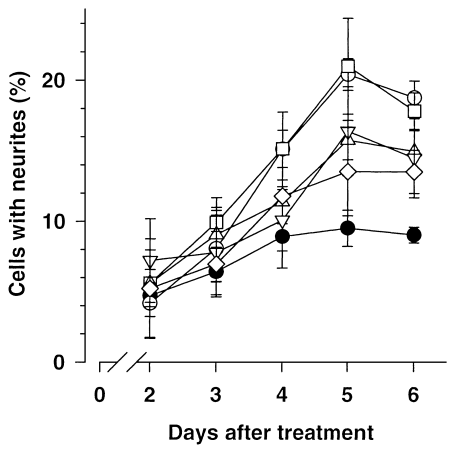


Fig. 3. Time-course of the active protopanaxadiol saponins and the methanol extract of *Ye-Sanchi* on neurite outgrowth in SK-N-SH cells. Cells were treated with ginsenoside Rb_1 (open circles), ginsenoside Rb_3 (open squares), notoginsenoside R_4 (open reverse triangles) and notoginsenoside Fa (open triangles) at a concentration of 100 μM, the methanol extract of *Ye-Sanchi* at a concentration of 50 μg/ml (open diamonds) or with vehicle (closed circles), and the time course was measured up to 6 days. Repeated measured by two-way ANOVA and post hoc Student-Newman-Keuls multiple comparisons were done.

Repeated measured two-way ANOVA revealed significant effects of ginsenosides Rb_1 ($F(1,6) = 16.01$, $P = 0.0071$) and Rb_3 ($F(1,6) = 34.39$, $P = 0.0011$) and notoginsenosides R_4 ($F(1,6) = 13.37$, $P = 0.0106$) and Fa ($F(1,6) = 21.55$, $P = 0.0035$). Significant interactions of treatment x time were shown for ginsenosides Rb_1 ($F(4,24) = 5.04$, $P = 0.0043$) and Rb_3 ($F(4,24) = 2.68$, $P = 0.0500$). At a concentration of 100 μM, these 4 compounds did not affect cell growth and cell survival (data not shown).

Four active compounds belong to the protopanaxadiol-type saponins with a common aglycone structure (see Table 2). Among them, ginsenosides Rb_1 and Rb_3 had comparatively strong effects for neurite extension. The chain length of the sugar moiety at C-20 or C-3 in ginsenosides Rb_1 and Rb_3 is shorter than that in notoginsenosides R_4 and Fa.

Morphology of cells treated with 4 protopanaxadiol-type saponins

SK-N-SH cells treated with ginsenosides Rb_1 and Rb_3 and notoginsenosides R_4 and Fa at 100 μM as well as the methanol extract of *Ye-Sanchi* markedly extended the neurites multipolarly (Fig. 4A). The percentages of cells with multipolar neurites were intensely high in cells treated with these 4 compounds and the extract, whereas the num-

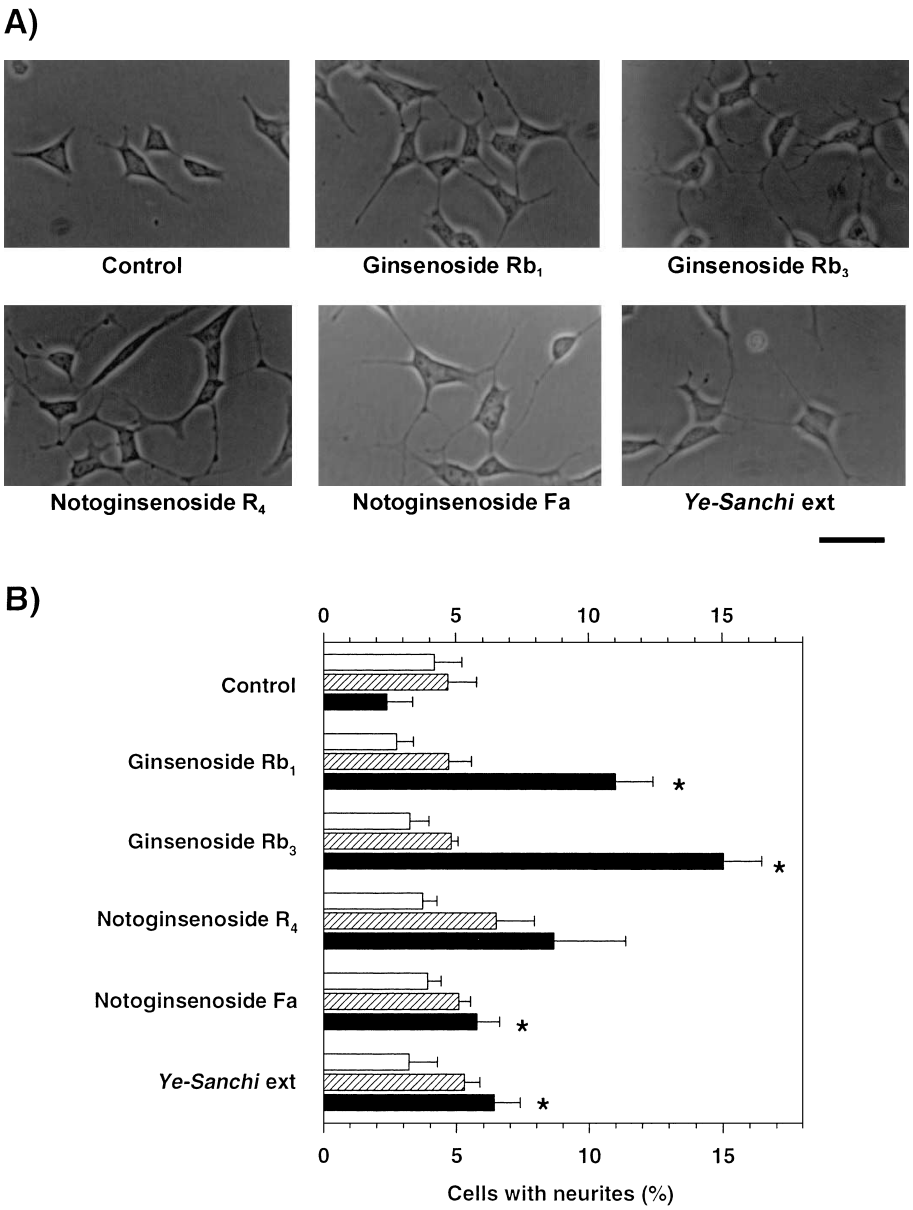


Fig. 4. Extension of multipolar neurites by the active protopanaxadiol-type saponins and the methanol extract of *Ye-Sanchi* in SK-N-SH cells. A) Cells were treated with ginsenosides Rb_1 and Rb_3, notoginsenosides R_4 and Fa and the methanol extract of *Ye-Sanchi* at a concentration of 100 μM and 50 μg/ml, respectively, or with vehicle (Control) at the start of culture. Neurite outgrowth activity was measured 5 days later. Scale bar = 50 μm. B) Quantitative analysis of neurites extending from cells treated with the active protopanaxadiol-type saponins and the methanol extract of Ye-Sanchi 5 days after the administration. The percentage of cells with uni- (open columns), bi- (hatched columns) or multipolar (closed columns) neurites are shown. Values represent the means and S.E.M. of four sites. *P<0.05, when compared with vehicle (Student's t-test).

ber of cells with uni- and bi-polar neurites was not increased (Fig. 4B).

Number of varicosities, which would be the site of synaptic connection, was counted to assess neurite maturity. In vehicle-treated cells, the number of varicosities per cell was 0.10 ± 0.07, whereas they were 0.93 ± 0.21, 0.80 ± 0.08, 0.53 ± 0.13 and 0.33 ± 0.11 in ginsenoside Rb_1-, ginsenoside Rb_3-, notoginsenoside R_4- and notoginsenoside Fa-treated cells, respectively (Fig. 5).

To investigate the expression of axonal and dendritic markers in extended neurites, double-staining for phosphorylated neurofilament-H (NF-H) and MAP2 was performed 6 days after treatment with 100 μM of each of ginsenosides Rb_1 and Rb_3, notoginsenoside R_4 and Fa, and the methanol extract of *Ye-Sanchi* at 50 μg/ml (Fig. 6). Immunostaining with phosphorylated NF-H antibody

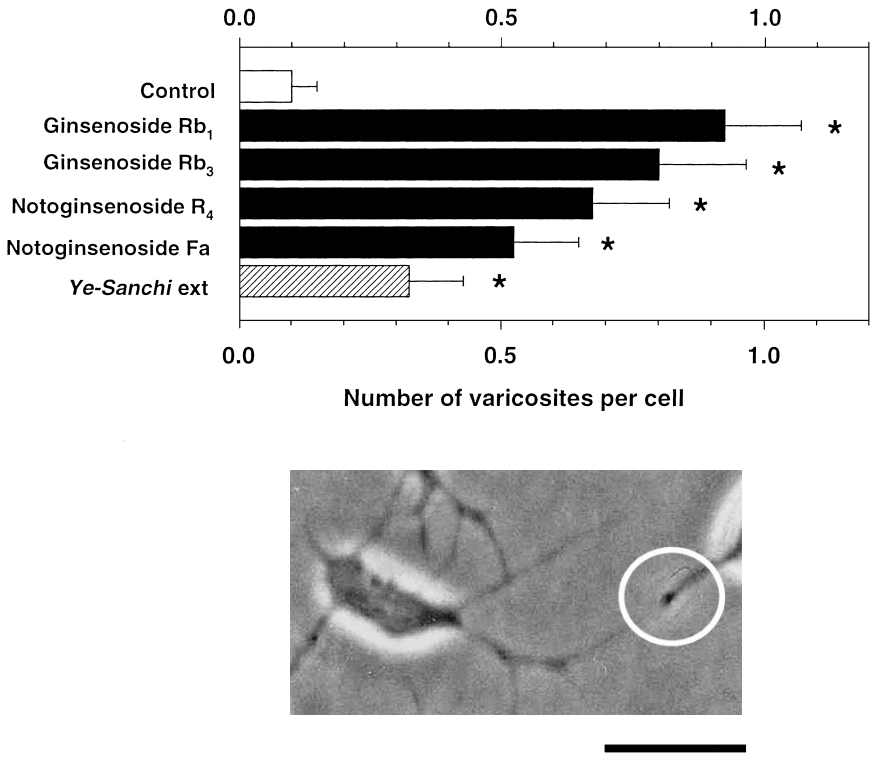


Fig. 5. Formation of varicosities by the active protopanaxadiol-type saponins and the methanol extract of *Ye-Sanchi* in SK-N-SH cells. Cells were treated with ginsenosides Rb_1 and Rb_3, and notoginsenosides R_4 and Fa (closed columns) at a concentration of 100 μM, the methanol extract of *Ye-Sanchi* at a concentration of 50 μg/ml (hatched column) or with vehicle (open column) at the start of culture. Neurite outgrowth activity was observed 5 days later. Numbers of varicosities on neurite processes were counted and represented as values per cell. Values represent the means and S.E.M. of 40 cells. *P<0.05, when compared with vehicle (Student's t-test). Scale bar = 50 μm. A structure in a circle of a photograph is regarded as a varicosity.

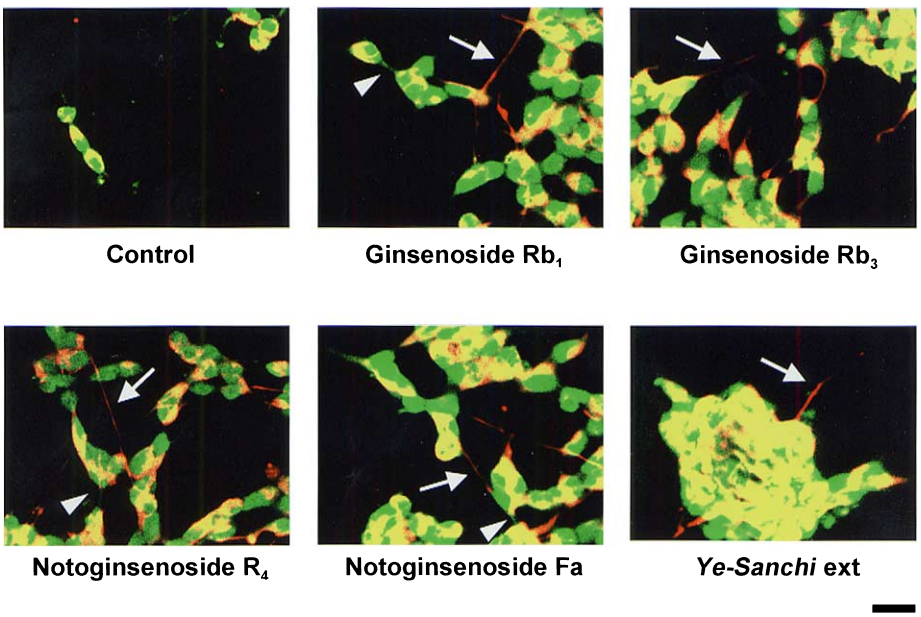


Fig. 6. Effect of the active protopanaxadiol-type saponins and the methanol extract of *Ye-Sanchi* on the expression of axonal and dendritic marker proteins in SK-N-SH cells. Cells were treated with ginsenosides Rb_1 and Rb_3 and notoginsenosides R_4 and Fa at a concentration of 100 μM, the methanol extract of *Ye-Sanchi* at a concentration of 50 μg/ml or with vehicle (Control) at the start of culture. Neurite outgrowth activity was measured 6 days later. Then, fixed cells were double-stained with immunofluorescent-labeled antibodies for phosphorylated neurofilament-H (red color) and MAP2 (green color). Arrows and arrowheads indicate typical neurites stained in red and green, respectively. Scale bar = 20 μm.

(axonal marker) is shown by the red color, whereas the green stain indicates MAP2 (dendritic marker) expression. A merged yellow color indicates coexpression of both. Both phosphorylated NF-H-expressing neurites and MAP2-expressing neurites were extended by treatment with these compounds and the extract. In particular, the long neurites were phosphorylated NF-H-positive.

DISCUSSION

The present study demonstrated neurite outgrowth activity of the methanol extracts of Ginseng, Red Ginseng, Notoginseng and *Ye-Sanchi*, a relative to *P. vietnamensis*. Among several saponins in *Ye-Sanchi*, 4 of the protopanaxadiol-type compounds, ginsenosides Rb_1 and Rb_3, and notoginsenosides R_4 and Fa, were found to show neurite outgrowth activity, whereas protopanaxatriol-, ocotillol- and oleanane-type saponins had no effect. The cells treated with the methanol extract of *Ye-Sanchi* and 4 of the protopanaxadiol-type saponins formed a lot of varicosities and extended both axons and dendrites, suggesting that this extract and saponins may enhance synaptic formation. Since Ginseng, Red ginseng (11) and Notoginseng (12) as well as *Ye-Sanchi* also contained protopanaxadiol-type saponins at comparatively rich levels, they might show neurite outgrowth activities. In *Zhuzishen* and a rhizome of *P. stipuleanatus* that inhibited cell viability, some cytotoxic compounds may be present.

It was reported that ginsenoside Rb_1 increased neurite outgrowth in chick dorsal root ganglia neurons and potentiated NGF-induced neurite outgrowth (8, 9). We here demonstrated for the first time that protopanaxadiol-type saponins, not only ginsenoside Rb_1, stimulated the development of axons and dendrites. Takemoto et al. (13) reported that several protopanaxadiol-type saponins potentiated NGF-induced neurite outgrowth, but not protopanaxatriol- and oleanane-type saponins. In the present experiment, however, Yesanchinoside J, one of the protopanaxadiol-type saponins was found to be toxic at 100 μM, and no effect was observed at 10 μM. In the case of Yesanchinoside J, a toxic pathway may be stimulated by the acetyl group in its side chain at C-3. The intracellular mechanism of the 4 active compounds is yet unknown. It should be investigated whether an aglycone structure of these compounds is a common active core structure or not, and differences of sugar moiety in side chains are involved in the efficacy and toxicity.

NGF-independent neurite extension by protopanaxadiol-type saponins in the present results is also a new finding concerning ginsenosides. Although several NGF agonists, such as neotrofin (14, 15) and xaliproden (16) are studied as anti-Alzheimer's disease drugs, other drugs that make neurites extend NGF-independently are also useful for restoring a variety of aspects of neurons in the damaged brain. We can not deny completely that protopanaxadiol-type saponins facilitated NGF signal transduction, and then neurites extended, because SK-N-SH cells express NGF and *trk*A, a high affinity receptor for NGF (our unpublished data). We are investigating whether NGF contents and the expression level of *trk*A are changed by treatment with protopanaxadiol-type saponins in SK-N-SH cells or not. Although SK-N-SH cells are differentiated by retinoic acid-treatment (17), we used undifferentiated cells for a neurite outgrowth assay. Since we think that immature neurons as well as mature neurons need to participate in regeneration of the neuronal network in the dementia brain, an assay using undifferential cells is a meanful experiment to perform. Our preliminary data showed that treatment by ginsenoside Rb_1 extended axons also in cultured cortical neurons of rat (data not shown).

Except for protopanaxadiol-type saponins, other compounds tested had no effect on neurite outgrowth. The content of each saponin depends on differences of species (18), the growing area and the season of collection. Therefore, the quality evaluation of total content of protopanaxadiol-type saponins will be necessary to choose a more effective Ginseng drug for dementia. On the other hand, to develop novel anti-dementia drugs from Ginseng drugs, the individual active compounds must be investigated with regards to the mechanism of the effect, drug metabolism, the possibility for it to pass through the blood brain barrier, and so on.

In the present study, it was clarified that protopanaxadiol-type saponins have axonal and dendritic formation activity. Identifying binding proteins to these saponins in neurons and the mechanism of neurite formation of active compounds are now underway in our laboratory. We need to perform further analyses at both the tissue and animal levels to elucidate whether extended neurites by these compounds have significant contribution to synaptic formation and restoration of the neuronal network or not.

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