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

Chihiro Tohda, Noriaki Matsumoto, Kun Zou, Meselhy R. Meselhy and Katsuko Komatsu*

Research Center for Ethnomedicines, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan

Received May 8, 2002 Accepted August 5, 2002

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 Rb1 and Rb3, and notoginsenosides R4 and Fa isolated from Ye-Sanchi extract extended neurites, while protopanaxatriol-, ocottillol- 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 Rb1 and Rb3, and notoginsenosides R4 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 Rb1 and Rg1, improved spatial learning in normal mice (7), and ginsenoside Rb1 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.

*Corresponding author. FAX: +81-76-434-5064
E-mail: katsukok@ms.toyama-mpu.ac.jp
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 x 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 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 compounds were isolated. Although notoginsenoside R2, ginsenosides Rg2, and Rg2 and Re, and chikusetsusaponin IV were examined, the other 5 compounds were not sufficient for testing. Chemical structures of isolated compounds were determined by IR, 1H-NMR, 13C-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% CO2.

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 | Jinning county, Yunnan province, China | 19759 |
| Zhezishen       | 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 $8.0 \times 10^3$ 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 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, fluorescein isothiocyanate (FITC)-labeled donkey anti mouse IgG (dilution 1:100, Chemicon), and Cy3-labeled donkey anti rabbit IgG (dilution 1:100, Chemicon). The slides were washed with PBS, mounted with Aqua Poly Mount (Poly-science, 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. stipuleanatus 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. stipuleanatus had no effect.

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
Axon and Dendrite Extension by Diol Saponins

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

| Compound name     | Type       | R₁      | R₂      | R₃      | R₄      |
|-------------------|------------|---------|---------|---------|---------|
| Ginsenoside Rb₁   | Diol       | glc-2-glc |        | glc-6-glc |        |
| Ginsenoside Rb₃   | Diol       | glc-2-glc |        | glc-6-xyI |        |
| Notoginsenoside R₄ | Diol       | glc-2-glc |        | glc-6-glc-6-xyI |        |
| Notoginsenoside Fa | Diol       | glc-2-glc-2-xyI |        | glc-6-glc |        |
| Yesanchinoside J  | Diol       | glc(6-Ac)-2-glc |        | glc-6-glc-6-xyI |        |
| Ginsenoside Rg₁   | Triol      | H       |        | glc     |        |
| Ginsenoside Rg₂₅  | Triol      | H       |        | glc-2-rha | H       |
| Ginsenoside Re    | Triol      | H       |        | glc-2-rha | glc     |
| Notoginsenoside R₁ | Triol      | H       |        | glc-2-xyI | glc     |
| Notoginsenoside R₂₅ | Triol      | H       |        | glc-2-xyI | H       |
| 20-O-Glc-ginsenoside Rf | Triol | H       |        | glc-2-glC | glc     |
| Majonoside R₂     | Ocotillol  | H       |        | glc-2-xyI |        |
| (24S)-Pseudoginsenoside RT₄ | Ocotillol | H       |        | glc     |        |
| (24S)-Pseudoginsenoside F₁₁ | Ocotillol | H       |        | glc-2-rha |        |
| Vina-ginsenoside R₁ | Ocotillol | H       |        | glc(6-Ac)-2-rha |        |
| Vina-ginsenoside R₂ | Ocotillol | H       |        | glc(6-Ac)-2-xyI |        |
| Vina-ginsenoside R₆ | Ocotillol | H       |        | glc(6-Ac-6-xyI)-2-xyI |        |
| Ginsenoside Ro₆   | Oleanane   | gluA-2-glC |        | glc     |        |
| Chikusetusaponin 1Va₆ | 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 Rb₁ and Rb₃, and notoginsenosides R₄ 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 ocatillol-type and 2 oleanane-type saponins showed no effect on neurite outgrowth in SK-N-SH cells (Fig. 2).

Time courses of ginsenosides Rb₁ and Rb₃ and notoginsenosides R₄ 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).
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-
Axon and Dendrite Extension by Diol Saponins

Number 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 $0.10 \pm 0.07$, whereas they were $0.93 \pm 0.21$, $0.80 \pm 0.08$, $0.53 \pm 0.13$ and $0.33 \pm 0.11$ in ginsenoside $R_{b_1}$, ginsenoside $R_{b_3}$, notoginsenoside $R_{a}$ 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 $100 \mu M$ of each of ginsenosides $R_{b_1}$ and $R_{b_3}$, notoginsenoside $R_{a}$ and Fa, and the methanol extract of Ye-Sanchi at $50 \mu g/ml$ (Fig. 6). Immunostaining with phosphorylated NF-H antibody

**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 $R_{b_1}$ and $R_{b_3}$, notoginsenosides $R_{a}$ and Fa and the methanol extract of Ye-Sanchi at a concentration of $100 \mu M$ and $50 \mu g/ml$, respectively, or with vehicle (Control) at the start of culture. Neurite outgrowth activity was measured 5 days later. Scale bar = $50 \mu m$. 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).
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 Rb₁ and Rb₃, and notoginsenosides R₄ 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 Rb₁ and Rb₃, and notoginsenosides R₄ 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 immuno-fluorescent-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.
Axon and Dendrite Extension by Diol Saponins  

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 Rb₁ and Rb₂, and notoginsenosides R₂ and Fa, were found to show neurite outgrowth activity, whereas protopanaxatriol-, ocottillol- 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 Rb₁ 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 Rb₁, 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 trkA, a high affinity receptor for NGF (our unpublished data). We are investigating whether NGF contents and the expression level of trkA 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 meaningful experiment to perform. Our preliminary data showed that treatment by ginsenoside Rb₁ 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.

Acknowledgments

We would like to thank Miss Shu Zhu for the gene analysis of Ginseng drugs. This work was supported by Grant-in-Aid for Scientific Research (B) No. 11695086 in 1999 – 2001 from Japan Society for the Promotion of Science and in part by the Kampo Science Foundation.

REFERENCES

1 Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Van deventer C, Walker S, Wogulis M, Yednock T, Games D and Seubert P: Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 400, 173 – 177 (1999)
2 Potter H and Dressler D: The potential of BACE inhibitors for Alzheimer’s therapy. Nature Biotech 18, 125 – 126 (2000)
3 Li YM, Xu M, Lai MT, Huang Q, Castro JL, DiMuzio-Mower J, Harrison T, Lellis C, Nadin A, Neduvellil JG, Register RB, Sardana MK, Shearman MS, Smith AL, Shi XP, Yin KC, Shafer JA and Gardell SJ: Photoactivated γ-secretase inhibitors directed to the active site covalently label presenilin 1. Nature 405, 689 – 694 (2000)
4 Zhao R and MaDaniel WF: Ginseng improves strategic learning by normal and brain-damaged rats. Neuroreport 9, 1618 – 1624 (1998)
5 Zhong YM, Nishijo H, Uwano T, Tamura R, Kawanishi K and Ono T: Red ginseng ameliorated place navigation deficits in young rats with hippocampal lesions and aged rats. Physiol Behav 69, 511 – 525 (2000)
6 Sugaya A, Yuzurihara M, Tsuda T, Yasuda K, Kajiwara K and Sugaya E: Proliferative effect of ginseng saponin on neurite extension of primary cultured neurons of the rat cerebral cortex. J Ethnopharmacol 22, 173 – 181 (1988)
7 Moook-Jung I, Hong HS, Boo JH, Lee KH, Yun SH, Cheong MY, Joo I, Huh K and Jung MW: Ginsenoside Rb1 and Rg1 improve spatial learning and increase hippocampal synaptophysin level in mice. J Neurosci Res 63, 509 – 515 (2001)
8 Saito H, Suda K, Schwab M and Thoenen H: Potentiation of the NGF-mediated nerve fiber outgrowth by ginsenoside Rb1 in organ cultures of chicken dorsal root ganglia. Jpn J Pharmacol 27, 445 – 451 (1977)
9 Nishiyama N, Cho SL, Kitagawa I and Saito H: Malonylginsenoside Rb1 potentiates nerve growth factor (NGF)-induced neurite outgrowth of cultured chick embryonic dorsal root ganglia. Biol Pharm Bull 17, 509 – 513 (1994)
10 Zou K, Shu Z, Cai S and Komatsu K: Constituents from the underground part of Panax plants: Ye San Qi and Kou Zi Qi. The 121st Annual Meeting of the Pharmaceutical Society of Japan, Abstract, p 110 (2001)
11 Soldati F and Sticher O: HPLC separation and quantitative determination of ginsenosides from Panax ginseng, Panax quinquefolium and from ginseng drug preparations. 2nd communication. Planta Med 39, 348 – 357 (1980)
12 Yoshikawa M, Murakami T, Ueno T, Yashiro K, Hirokawa N, Murakami N, Yamahara J, Matsuda H, Sajioh R and Tanaka O: Bioactive saponins and glycosides. VIII. Notoginseng (1): new dammarane-type triterpene oligoglycosides, notoginsenosides-A, -B, -C, and -D, from the dried root of Panax notoginseng (Burk.) FH CHEN. Chem Pharm Bull (Tokyo) 45, 1039 – 1045 (1997)
13 Takemoto Y, Ueyama T, Saito H, Horio S, Sanada S, Shoji J, Yahara S, Tanaka O and Shibata S: Potentiation of nerve growth factor-mediated nerve fiber production in organ cultures of chicken embryonic ganglia by ginseng saponins: structure-activity relationship. Chem Pharm Bull (Tokyo) 32, 3128 – 3133 (1984)
14 Taylor EM, Yan R, Hauptmann N, Maher TJ, Djahandideh D and Glasky AJ: AIT-082, a cognitive enhancer, is transported into brain by a nonsaturable influx mechanism and out of brain by a saturable efflux mechanism. J Pharmacol Exp Ther 293, 813 – 821 (2000)
15 Di Iorio P, Virgilio A, Giuliani P, Ballerini P, Viamale G, Middlemiss PJ, Rathbone MP and Ciccarelli R: AIT-082 is neuroprotective against kainate-induced neuronal injury in rats. Exp Neurol 169, 392 – 399 (2001)
16 Hurko O and Walsh FS: Novel drug development for amyotrophic lateral sclerosis. J Neurol Sci 180, 21 – 28 (2000)
17 Sidell N, Sarafian T, Kelly M, Tsuchida T and Haussler M: Retinoic acid-induced differentiation of human neuroblastoma: a cell variant system showing two distinct responses. Exp Cell Biol 54, 287 – 300 (1986)
18 Chuang W-C, Wu H-K, Sheu S-J, Chiu S-H, Chang H-C and Chen Y-P: A comparative study on commercial samples of Ginseng Radix. Planta Med 61, 459 – 465 (1995)