Full Paper

Improvement of Memory in Mice and Increase of Hippocampal Excitability in Rats by Ginsenoside Rg1's Metabolites Ginsenoside Rh1 and Protopanaxatriol

Yu-Zhu Wang^{1,†}, Ji Chen^{2,†}, Shi-Feng Chu^{2,†}, Yong-Sheng Wang², Xiao-Ying Wang³, Nai-Hong Chen², and Jun-Tian Zhang^{2,*}

¹Center for Drug Evaluation, State Food and Drug Administration, Jia 1 Fuxing Road, Haidian District, Beijing 100038, China

²Institute of Materia Medica, ³Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, 1 Xiannongtan Street, Xuanwu District, Beijing 100050, China

Received March 5, 2008; Accepted December 26, 2008

Abstract. Ginsenoside Rg1 has been reported to improve cognitive function in many memory-impaired animal models. However, little is known about the bioactivity of its metabolites in the central nervous system in vivo. In the present study, we employed the step through test and electrophysiological approach to investigate the effects of ginsenoside Rg1's primary metabolite ginsenoside Rh1 and end metabolite protopanaxatriol (Ppt) on learning and memory as well as hippocampal excitability. The behavioral study showed that both ginsenoside Rh1 and Ppt significantly ameliorated memory-impaired models induced by scopolamine in mice. Consistently, the electrophysiological work revealed that ginsenoside Rh1 and Ppt as well as their precursor ginsenoside Rg1 all increased hippocampal excitability in the dentate gyrus of anesthetized rats. These results demonstrated that both ginsenoside Rh1 and Ppt had similar but more potent actions than ginsenoside Rg1 in improving memory and hippocampal excitability, suggesting the role of ginsenoside's sugar moieties in biological activities is not as necessary as traditionally considered.

Keywords: ginsenoside Rg1, ginsenoside Rh1, protopanaxatriol, cognition, hippocampal excitability

Introduction

Ginsenosides (ginseng saponins) have been considered as the primary active ingredients in ginseng. More than forty ginsenoside analogues have been identified so far. All ginsenosides share a four-ring, steroid-like structure but with a different number of sugar moieties (glycones). Based on the number and position of their sugar moieties, ginsenosides are classified into two major groups: the 20(S)-protopanaxadiol and 20(S)-protopanaxatriol saponins. Therefore, the wide range of pharmacological actions of ginseng may be due to the variety of its active ingredients – ginsenosides. Because ginseng

products are orally administered in most situations, ginsenosides are metabolized into analogues with fewer sugar moieties by gastrointestinal bacteria. For example, the 20(S)-protopanaxatriol ginsenoside Rg1 (Rg1) with two sugar moieties is normally metabolized into ginsenoside Rh1 (Rh1), the primary metabolite with one sugar moiety, and 20(S)-protopanaxatriol (Ppt), the end metabolite with no sugar moiety (1) (Fig. 1), whereas the 20(S)-protopanaxadiol ginsenoside Rg3 (with three sugar moieties) is hydrolyzed into ginsenoside Rh2 (with two sugar moieties) and 20(S)-protopanaxadiol (Ppd, with no sugar moiety). Thus the diverse actions of ginsenosides perhaps also result from their metabolites in vivo, if the metabolites have any bioactivity.

Although it has been proposed that only ginsenosides with sugar moieties were the active components in vivo, a growing body of information from experimental studies starts to challenge this classical view. The

[†]These authors equally contributed to the work.

*Corresponding author. zhangjt@imm.ac.cn

Published online in J-STAGE

doi: 10.1254/jphs.08060FP

Fig. 1. Structures of ginsenoside Rg1, Rh1, and Ppt.

pharmacokinetic studies revealed that the orally administered ginsenosides including ginsenoside Rb1 and Rg1 were poorly absorbed (2–4), but the deglycosylated metabolic products of these ginsenosides reached relatively high concentration in the systemic circulation (5). In pharmacological studies, the anti-tumor activities of 20(S)-protopanaxadiol ginsenoside Rb2, Rg3, and Rh2 (6–9) had been proposed to be mediated by their aglycone metabolite Ppd (10, 11). These experimental data lead to a hypothesis that the aglycone metabolite Ppd is the actual molecule exerting the anti-tumor actions of the ginsenosides.

In contrast to the anti-tumor action of other ginsenosides, Rg1 also exhibited significant effects on the brain function both in vivo and in vitro. It improved learning and memory in ten animal models including aged (12) and brain ischemia-reperfusion rats (13). Rg1 was further proved to facilitate the acquisition, consolidation, and retrieval of learning and memory evaluated by passive avoidance test and Morris water maze test (14). At the cellular level, Rg1 increased hippocampal excitability (15). However, the low bioavailability of Rg1 and detectable level of Rh1 and Ppt in the systemic circulation or even in the brain tissue after orally administrated Rg1 raise the following two open questions: (1) Which component, Rg1, Rh1, or Ppt, contributes to Rg1's effect on the central nervous system (CNS) function? (2) Does the bioactivity of Rg1 and its metabolites in the CNS correlate to their number of sugar moieties?

To address the above questions, the present study assessed the effects of Rh1 and Ppt on learning and

memory as well as hippocampal excitability with behavioral and electrophysiological approaches.

Materials and Methods

Animals

Male Kunming (KM) mice (18–22 g) were obtained from the Animal Center of the Chinese Academy of Medical Sciences (Beijing, China). Adult male Sprague-Dawley rats (250–280 g) were provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). After arrival, the animals were individually caged with food and water ad libitum and maintained at controlled environment (temperature: 22°C; moisture: 40%–50%) with a 12-h light-dark cycle. After 3 days of habituation to the step-through apparatus, animals then started to perform the passive avoidance experimental procedures. All experiments were carried out with the approval of the local laboratory animal care and use committee. Efforts were made to minimize animal suffering.

Drugs and agent preparation

Rg1, Rh1, and Ppt, all with purity of 95%, were purchased from Jilin University (Changchun, Jilin Province, China). For step-through and tail-flick tests, Rh1 and Ppt were individually triturated into fine powder before they were dissolved in double-distilled water (DDW) at the concentration of 1 mg/ml. For electrophysiological experiments, Rg1, Rh1, and Ppt powder were individually dissolved in physiological saline (0.9% NaCl, N.S.) to make stock solution at 4×10^{-2} M and stored at -20°C . Stock solutions were then diluted to 4×10^{-5} M or 4×10^{-6} M with N.S. before application. HPLC-grade acetonitrile and ammonium formate were from Fisher Scientific (Fair Lawn, NJ, USA). Scopolamine was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Donepezil hydrochloride was obtained from Xincat Pharmaceutical Co., Ltd., Zibo City, Shandong Province in China.

Behavioral test

Step-through passive avoidance test: The learning ability of each mouse was evaluated by the single trial passive avoidance test according to the literature (16). Briefly, in the experimental session, each mouse was trained to adapt to the step-through apparatus (Taisho Pharmaceutical, Tokyo). The apparatus for the step-through passive avoidance test was an automated shuttle-box divided into an illuminated safe compartment and a dark shock compartment of the same size, separated by a wall with a guillotine door. An animal was put into the illuminated compartment, facing away

from the dark compartment. After 10 s, the door between these two boxes was opened and the mouse was allowed to move into the dark compartment freely. When the mouse stepped into the dark compartment, an inescapable foot-shock (36 V) was delivered through the grid floor. The experiment was divided into learning and memory trials. During the learning trial, each mouse was permitted to enter the dark compartment and accept the electric shock twice. The retention of passive avoidance response, named the memory trial, was measured at 24 h after the learning trial. During the memory trial, each animal was put into the illuminated compartment and the latency of the first time to enter the dark compartment and the error number within 5 min were recorded. The maximum cut-off time for the latency was 300 s. The latency and the error number were used to assess the animal's memory. All training and testing was performed between 8 AM and 2 PM.

Drug treatment: One hundred and twelve mice were randomly divided into seven groups (16/each group). Scopolamine (1 mg/kg, i.p.) was administrated at 20 min prior to the learning trial. The positive drug control group or four test groups were orally administered donepezil hydrochloride (5 mg/kg), Rh1 (5 or 10 mg/kg), or Ppt (5 or 10 mg/kg) at 1 h before the learning trial with the same volume of DDW as the vehicle control. No animal died during drug administration.

Tail-flick test

The tail-flick test was used to observe the influence of Rg1, Rh1, and Ppt on pain threshold. KM mice were gently restrained on the tail-flick apparatus with tails free to test the tail-flick latency. Radiant heat from a 90-W bulb focused on the point of 2 cm from the tail end of the mouse. The basic latencies of all mice from the onset of the heat stimulus to tail-flick were recorded. Twenty-four hours later, each group of twelve mice was orally administrated Rg1, Rh1, or Ppt at 5 or 10 mg/kg, respectively, and the Control mice were orally administrated DDW. The latencies of tail-flick were recorded again one hour later to compare them with their respective basic latencies.

Electrophysiological approach

Surgical preparation: The animals were prepared as previously described (17, 18). Briefly, rats were anesthetized with urethane carbamate (1.5 g/kg, i.p.) before being placed on an SR-6N stereotaxic apparatus (Narishige Science Instrument, Tokyo). Three holes were sequentially drilled at 0.8-, 3.8-, and 7.5-mm posterior to the bregma and 1.8-, 2.5-, and 4.2-mm lateral to the mid-line for an outer guide cannula, a monopolar recording electrode, and a bipolar stimulat-

ing electrode, respectively. The outer guide cannula, recording electrode, and stimulating electrode were lowered into the lateral cerebral ventricle, the granular cell layer of the dentate gyrus, and the perforant path (PP), respectively. The synaptic responses were monitored by a VC-11 memory oscilloscope (Nihon Kohden, Tokyo). Once the locations of the cannula and electrodes were verified, they were kept in place for the whole experimental duration.

Measurement of evoked potentials: The population spike (PS) amplitude was employed to assess the excitation level of the granular cell population in the dentate gyrus. An evoked response was generated in the dentate gyrus granular cell layer by stimulating the PP at low frequency (0.033 Hz) with single constant current pulses (150 μ s in duration) triggered by an SEN-7203 electrical stimulator (Nihon Kohden) through a SS-202J isolator (Nihon Kohden). After input/output curve determination, the baseline responses were evoked by a stimulus with an intensity to produce 20% of the maximal PS amplitude.

Intracerebroventricular (i.c.v.) drug delivery: Drugs or vehicle injections were delivered via a cannula in the lateral cerebral ventricle after 30 min of electrophysiological baseline measurement from the dentate gyrus of the contralateral hemisphere. The cannula was left in place to the end of the experiment after injection. The drugs or vehicle were injected at a 10- μ l volume over a 5-min period via a Hamilton syringe. Drug doses were calculated on the basis that these drugs would theoretically achieve the brain concentrations required, assuming the brain volume to be approximately 2 ml (19). Thus for an estimated brain concentration of Rg1, Rh1, and Ppt at 2×10^{-7} and 2×10^{-8} M, 10 μ l of each drug of 4×10^{-5} or 4×10^{-6} M was injected with the same volume of vehicle as the control.

Data collection and analysis: For each time point, every five records of evoked responses were averaged. The mean baseline was obtained by averaging the PS amplitudes of 6 time points within 30 min before i.c.v. injection of drugs or vehicle. The data at each time point were normalized and expressed as the percentage of mean baseline.

Cerebrospinal fluid (CSF) collection and pretreatment

Fourteen rats were anesthetized with urethane carbamate (1.5 g/kg, i.p.) and fixed on a stereotaxic apparatus followed by unhairing, sterilizing, and cutting a 2-cm aperture at dorsi-mid of the cervical part. Referring to the literature (20), muscles were slowly and softly separated to avoid hemorrhage and the occipital foramen was exposed. CSF was drawn out from the cisterna magna by a 1-ml injector. All the collected

CSF was centrifuged at 3,000 rpm for 10 min and the supernatant was immediately mixed before being distributed into twenty tubes with 100 μ l in each. All of these CSF samples were then divided into four groups (5 tubes/each group) and labeled as N.S., Rg1/15 min, Rg1/30 min, and Rg1/60 min.

HPLC-MS detection

Waters Acquity UPLC™ BEHC18 was used for high performance liquid chromatography (HPLC) detection. The tandem mass spectrometry (ZQ4000) apparatus was from Waters. The temperature for the UPLC column (2.1 mm $\times$ 50 mm, 1.7 μ m) and sampling was 40°C and 10°C, respectively. The step mobile phase consisted of acetonitrile (A, 10 mM) and ammonium formate (B, pH 3.0). The flow rate was 0.3 ml·min⁻¹ and the injection volume was 10 μ l. Ion sources were ESI⁺ and ESI⁻, Corona: 3 KV. Desolvation and source block temperature was 350°C and 110°C, respectively.

Statistical analyses

All data were shown as the mean $\pm$ S.E.M. Statistical analysis of the data for multiple comparisons was performed by one-way ANOVA or by the *t*-test for two-group comparison. *P*<0.05 was considered statistically significant.

Results

Effects of Rh1 and Ppt on memory-impairment

As shown in Table 1, mice treated with scopolamine alone (animal model group) showed significantly shorter latency and significantly higher error number in the retention test compared to the vehicle control animals, indicating that the animals' memory was successfully impaired by scopolamine. In contrast, mice administered Rh1 (5 or 10 mg/kg), Ppt (5 or 10 mg/kg), or donepezil hydrochloride (5 mg/kg) before scopolamine exhibited

significantly longer latency and significantly lower error number in the retention test compared to the animal model group, suggesting that these three compounds ameliorated the memory-impairment induced by scopolamine. More interestingly, the improving effect of Ppt at 5 mg/kg on both latency and error number were stronger than that of Rh1 at the same concentration. Another interesting aspect was that Ppt at either dose (5 or 10 mg/kg) seemed to produce greater improving effect on both latency and error number than donepezil hydrochloride does even though the comparison was not statistically significant.

Effects of Rg1, Rh1, and Ppt on pain threshold

Figure 2 shows that the latencies of tail-flick in the Control mice were not changed. There were no signifi-

Fig. 2. The effects of ginsenoside Rg1, Rh1, and Ppt (5 and 10 mg/kg) on the latency of pain sense. All values are presented as the mean $\pm$ S.E.M. of 12 rats.

Table 1. Improving effects of ginsenoside Rh1 and Ppt on mouse memory-impairment induced by scopolamine in the step-through test

Groups	Treatment	Latency (s)	Error number
Control	DDW	229 $\pm$ 119**	0.6 $\pm$ 0.2**
Scopolamine	1 mg/kg	68 $\pm$ 23	2.1 $\pm$ 0.9
Donepezil hydrochloride	5 mg/kg	129 $\pm$ 99*	1.3 $\pm$ 1.0*
Ginsenoside Rh1	5 mg/kg	115 $\pm$ 71*	1.1 $\pm$ 0.5*
	10 mg/kg	147 $\pm$ 101**	1.0 $\pm$ 0.6**
Ppt	5 mg/kg	177 $\pm$ 121** #	0.8 $\pm$ 0.8** #
	10 mg/kg	178 $\pm$ 117**	0.7 $\pm$ 0.8**

P*<0.05, *P*<0.01 vs. scopolamine group and #*P*<0.05 vs. Rh1/5 mg/kg group by one-way ANOVA. All values are expressed as the mean $\pm$ S.E.M. of 16 mice.

Fig. 3. Enhancing effects of ginsenoside Rg1, Rh1, and Ppt (2×10^{-7} M, i.c.v.) on hippocampal excitability in the dentate gyrus of anesthetized rats. The vertical distance from the minimal point (a) to the tangent line (bc) is employed as the population spike (PS) amplitude. Time course of PS amplitude is shown. All values are presented as the mean $\pm$ S.E.M. of 6 rats, $**P<0.01$ vs. N.S. control by one-way ANOVA.

cant differences in the latencies of tail-flick between pre- and post-treatment of the mice that were orally administered Rg1, Rh1, or Ppt (5 or 10 mg/kg), which indicated that Rg1, Rh1, and Ppt influenced the cognitive function in the step-through test without effects on pain sense.

Effects of Rg1, Rh1, and Ppt on hippocampal excitability of anesthetized rats

After baseline responses were collected, Rg1, Rh1, Ppt, or N.S. was injected into the lateral cerebral ventricle. As shown in Figs. 3 and 4, the PS amplitude did not change after N.S. administration over a 60-min recording period, indicating the hippocampal excitability was not affected. However, the PS amplitude increased after 5-min injection of Rg1, Rh1, or Ppt at dose of 2×10^{-7} M and the increase was maintained for at least 60 min in each case. The enhancement of PS amplitude at 5 min after the injection of Rh1 or Ppt at dose of 2×10^{-8} M is shown in Fig. 4. Specifically, for 2×10^{-7} M, the PS amplitude at 10, 30, and 60 min was $257 \pm 45\%$, $298 \pm 45\%$, and $321 \pm 56\%$, respectively, in the Rg1 group ($n=6$, $P<0.01$ vs. N.S.); $365 \pm 60\%$, $435 \pm 68\%$, and $462 \pm 80\%$, respectively, in the Rh1 group ($n=6$, $P<0.01$ vs. N.S.); and $400 \pm 66\%$, $450 \pm 68\%$, and $488 \pm 72\%$, respectively, in the Ppt group ($n=6$, $P<0.01$ vs. N.S.). The increase of PS

Fig. 4. Enhancing effects of ginsenoside Rh1 and Ppt (2×10^{-8} M, i.c.v.) on hippocampal excitability in the dentate gyrus of anesthetized rats. All values are presented as the mean $\pm$ S.E.M. of 6 rats. $*P<0.05$, $**P<0.01$ vs. N.S. control by one-way ANOVA.

amplitude after Rh1 or Ppt treatment was even larger than that in the Rg1-treatment group, which was also seen in Fig. 4. There were no significant influence in PS amplitude for Rg1 at dose of 2×10^{-8} M; and the PS amplitude at 10, 30, and 60 min was $114 \pm 9\%$, $128 \pm 18\%$, and $138 \pm 17\%$, respectively. The more than 200% increase (compared to baseline) in PS amplitude for longer than 30 min indicated the formation of Rg1, Rh1, or Ppt-induced LTP in the hippocampal dentate gyrus.

Metabolism of Rg1 in CSF in vitro

Rg1 was added to 15 individual 100 μ l CSF tubes to a final concentration of 2×10^{-7} M, the same concentration as used in the hippocampal excitability experiments. The same volume of N.S. was added to 5 individual 100- μ l CSF tubes for the control. The three groups (5 tubes/each) of Rg1 samples were each incubated in a water bath at 37°C for 15, 30, or 60 min before they were stored at -20°C for later assay. After thawing at room temperature, Rg1, Rh1, or Ppt in 10 μ l CSF was detected by HPLC-MS. As shown in Table 2, no matter how long (15, 30, or 60 min) Rg1 was incubated in CSF, Rh1 was detected at extremely low levels and Ppt was not detectable at all in contrast to Rg1 that still remained at high concentration, indicating that Rg1 was not significantly metabolized into Rh1 and Ppt in CSF in vitro.

Table 2. Metabolites of ginsenoside Rg1 after incubation in cerebrospinal fluid for different times in vitro

Time (min)	Rg1 (M)	Rh1 (M)	Ppt (M)
15	$1.99 \times 10^{-7} \pm 0.49 \times 10^{-7}$	$2.12 \times 10^{-16} \pm 0.16 \times 10^{-16}$	ND
30	$2.00 \times 10^{-7} \pm 0.01 \times 10^{-7}$	$2.00 \times 10^{-16} \pm 0.25 \times 10^{-16}$	ND
60	$2.00 \times 10^{-7} \pm 0.79 \times 10^{-7}$	$1.72 \times 10^{-16} \pm 0.12 \times 10^{-16}$	ND

ND: not detected. All values are expressed as mean $\pm$ S.E.M. of 5 rats.

Furthermore, at a concentration as low as that in the incubated CSF, Rh1 did not affect the hippocampal excitability at all (data not shown). These results suggested that the effects of Rg1, Rh1, and Ppt on hippocampal excitability in the present study were exerted by themselves but not their downstream metabolites. In other words, Rg1, Rh1, and Ppt could improve hippocampal excitability as well as learning and memory by themselves.

Discussion

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by impairment of cognitive function that has increasingly produced heavy economic and social burdens throughout the world. Unfortunately, there are currently only a few drugs such as donepezil and piracetam, with different shortcomings, available for clinic application. The discovery of one drug with both an effect to specifically improve memory and also low toxicity has been anticipated for years. Ginsenoside Rg1 was reported to have versatile effects on AD and could be a potential candidate for such a drug (21).

The present study proved that Rg1, Rh1, and Ppt did not influence the animals' pain threshold, confirming the nootropic effects of Rg1, Rh1, and Ppt in the step-through test. In our study, the animals had been orally administered Rh1 or Ppt of 2.5 mg/kg in the step-through test, which showed no effects on the cognitive function. The recently finished toxicological studies confirmed that no significant toxicity was observed after dogs were orally administered Rg1 at 500 mg/kg for two months. Mice were orally administered Rg1 at 20 g/kg for seven days and none of them died. From all the above-described observations showing the very low toxicity of Rg1, Rh1 and Ppt were deduced to be highly safe like their precursor Rg1.

The present study demonstrated that Ppt had similar effects on CNS function as its precursor Rg1, which resembled the anti-tumor action of Ppd mentioned above (10, 11). Rg1, Rh1, and Ppt exhibited improving effects on both memory-impairment and hippocampal excitability in behavioral tests and electrophysiological study, respectively. The result of Rg1's metabolism in CSF *in vitro* indicated that prototype (Rg1) and metabolites (Rh1 and Ppt) exerted similar bioactivities by themselves. This is of particular interest since the potency and duration of the pharmacological effect of Rg1 on CNS functions can be further enhanced by its metabolites *in vivo*. These results also provide an explanation for why a low dose (5–10 mg/kg) (14) of Rg1 with poor absorption (3, 4) produced so many pharmacological actions. Because Rg1, Rh1, and Ppt have progressively

decreased number of sugar moieties, these results further suggest that there is no correlation between sugar moieties and the bioactivities of ginsenosides. Moreover, fewer sugar moieties provide Rh1 and Ppt with higher liposolubility and stronger capability of passing through the blood brain barrier, and thus increase the concentration and enhance the pharmacological action of these ginsenoside metabolites in the CNS. In other words, metabolites Rh1 and Ppt, especially Ppt, could be more potential candidates as drugs with activity in the CNS.

LTP is one form of neural plasticity and provides a good model for studying learning and memory at the cellular and synaptic levels (22). LTP has also been used as a tool for screening nootropic drug candidates. Exercise-induced improvements in behaviors of learning and memory have been directly associated with an increase in synaptic plasticity (23, 24). Conceivably, a drug showing activity to induce LTP should also improve learning and memory *in vivo*. Consistently, the present study demonstrated that Rg1, Rh1, and Ppt improved both memory and hippocampal excitability. This might represent one more example to support the correlation between LTP and memory.

Extraction from ginseng is the only way to obtain ginsenosides at present. No ginsenoside has yet been successfully synthesized, although many attempts have been made. One of the difficulties is the synthesis of the sugar moieties attached to the steroid-like structure. The present results showing that ginsenoside analogues with fewer or even no sugar moieties produced a similar or even more potent effect on CNS functions may provide an experimental basis for synthesizing ginsenoside alternatives without facing the difficulty of sugar synthesis.

Investigations showed that Rg1 increased the level of acetylcholine, M-receptor density, cAMP, PKC, p-CREB, BDNF, and Fos from the first to the fourth messengers (25–27), which were considered to contribute to cellular mechanism of LTP and cognitive function (22). The mechanisms for Rh1 and Ppt have been studied by many researchers. Because the effect of scopolamine was due to its anti-cholinergic effect, we deduced that Rh1 and Ppt could influence the cholinergic systems.

In conclusion, both ginsenoside Rh1 and Ppt have similar memory-improving effects to their prototype ginsenoside Rg1 in the behavioral test and electrophysiological study. Rg1, Rh1, and Ppt exhibited their CNS actions by themselves. Moreover Ppt and Rh1 seem to be more potent candidates than Rg1.

Acknowledgment

The work was supported by the National Natural Science Foundation of China, NO. 30672501.

References

- 1 Tawab MA, Bahr U, Karas M, Wurglics M, Schubert-Zsilavecz M. Degradation of ginsenosides in humans after oral administration. *Drug Metab Dispos.* 2003;31:1065–1071.
- 2 Odani T, Tanizawa H, Takino Y. Studies on the absorption, distribution, excretion and metabolism of ginseng saponins. III. The absorption, distribution and excretion of ginsenoside Rb1 in the rat. *Chem Pharm Bull.* 1983;31:1059–1066.
- 3 Odani T, Tanizawa H, Takino Y. Studies on the absorption, distribution, excretion and metabolism of ginseng saponins. II. The absorption, distribution and excretion of ginsenoside Rg1 in the rat. *Chem Pharm Bull.* 1983;31:292–298.
- 4 Xu QF, Fang XL, Chen DF. Pharmacokinetics and bioavailability of ginsenoside Rb1 and Rg1 from *Panax notoginseng* in rats. *J Ethnopharmacol.* 2003;84:187–192.
- 5 Hasegawa H. Proof of the mysterious efficacy of ginseng: basic and clinical trials: metabolic activation of ginsenoside: deglycosylation by intestinal bacteria and esterification with fatty acid. *J Pharmacol Sci.* 2004;95:153–157.
- 6 Wu CF, Bi XL, Yang JY, Zhan JY, Dong YX, Wang JH, et al. Differential effects of ginsenosides on NO and TNF- α production by LPS-activated N9 microglia. *Int Immunopharmacol.* 2007;7:313–320.
- 7 Keum YS, Han SS, Chun KS, Park KK, Park JH, Lee SK, et al. Inhibitory effects of the ginsenoside Rg3 on phorbol ester-induced cyclooxygenase-2 expression, NF- κ B activation and tumor promotion. *Mutat Res.* 2003;523–524:75–85.
- 8 Kim HS, Lee EH, Ko SR, Choi KJ, Park JH, Im DS. Effects of ginsenosides Rg3 and Rh2 on the proliferation of prostate cancer cells. *Arch Pharm Res.* 2004;27:429–435.
- 9 Jia WW, Bu X, Philips D, Yan H, Liu G, Chen X, et al. Rh2, a compound extracted from ginseng, hypersensitizes multidrug-resistant tumor cells to chemotherapy. *Can J Physiol Pharmacol.* 2004;82:431–437.
- 10 Liu GY, Bu X, Yan H, Jia WW. 20S-protopanaxadiol-induced programmed cell death in glioma cells through caspase-dependent and -independent pathways. *J Nat Prod.* 2007;70:259–264.
- 11 Yu Y, Zhou Q, Hang Y, Bu X, Jia W. Antiestrogenic effect of 20S-protopanaxadiol and its synergy with tamoxifen on breast cancer cells. *Cancer.* 2007;109:2374–2382.
- 12 Liu M. [Studies on the anti-aging and nootropic effects of ginsenoside Rg1 and its mechanisms of actions.] Sheng Li Ke Xue Jin Zhan. 1996;27:139–142. (text in Chinese with English abstract)
- 13 Zhang YG, Liu TP. Influences of ginsenosides Rb1 and Rg1 on reversible focal brain ischemia in rats. *Acta Pharmacol Sin.* 1996;17:44–48.
- 14 Mook-Jung I, Hong HS, Boo JH, Lee KH, Yun SH, Cheong MY, et al. Ginsenoside Rb1 and Rg1 improve spatial learning and increase hippocampal synaptophysin level in mice. *J Neurosci Res.* 2001;63:509–515.
- 15 Wang XY, Zhang JT. NO mediates ginsenoside Rg1-induced long-term potentiation in anesthetized rats. *Acta Pharmacol Sin.* 2001;22:1099–1102.
- 16 Zarrindast MR, Hadi M, Homayoun H, Postami P, Shafaghi B, Khavandgar S. The role of α -adrenoceptors in the amnesic effect of intracerebroventricular dexamethasone. *Pharmacol Res.* 2002;46:339–344.
- 17 Liu SL, Zhang JT. Effects of naloxone on l-clausenamide-induced long-term potentiation in dentate gyrus of anesthetized rats. *Acta Pharmacol Sin.* 1999;20:112–116.
- 18 Zhao MR, Zhang JT. Effects of 7-nitroindazole on long-term potentiation induced by l-clausenamide and high-frequency stimulation in rat hippocampus in vivo. *Acta Pharmacol Sin.* 1999;20:319–323.
- 19 Manahan-Vaughan D, Reymann KG. 1S, 3R-ACPD dose dependently induces a slow onset potentiation in the dentate gyrus in vivo. *Eur J Pharmacol.* 1995;294:497–503.
- 20 Cao YD, Zhang LZ, Wang MS, Tang TY, Su WH. [Two methods to collect cerebrospinal fluid in SD rats.] *Acta Academiae Medicinae Xuzhou.* 2005;25:317–319. (text in Chinese with English abstract)
- 21 Radadl K, Gille G, Liu LL, Rausch WD. Use of ginseng in medicine with emphasis on neurodegenerative disorders. *J Pharmacol Sci.* 2006;100:175–186.
- 22 Miyamoto E. Molecular mechanism of neuronal plasticity: induction and maintenance of long-term potentiation in the hippocampus. *J Pharmacol Sci.* 2006;100:433–442.
- 23 Cotman CW, Berchtold NC. Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends Neurosci.* 2002;25:295–301.
- 24 Farmer J, Zhao X, van Praag H, Wodtke K, Gage FH, Christie BR. Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague-Dawley rats in vivo. *Neuroscience.* 2004;124:71–79.
- 25 Wang XY, Chen J, Zhang JT. Effect of ginsenoside Rg1 on learning and memory impairment induced by β -amyloid peptide (25–35) and its mechanism of action. *Acta Pharmaceut Sin.* 2001;36:1–4.
- 26 Zhang JT, Liu Y, Qu ZW, Zhang XL, Xiao HL. Effects of ginsenoside Rg1 and Rb1 on central neurotransmitter receptors and protein. *Acta Pharmaceut Sin.* 1988;2:12–16.
- 27 Wang YZ, Wang YS, Chu SF, Chen J, Chen NH, Zhang JT. Nootropic signal transduction of ginsenoside Rg1. *Chin Pharmacol Bull.* 2008;24:740–743.