Effects of Ginseng Saponins in Energy Metabolism, Memory, and Anti-neurotoxicity

Lawrence C.H. Wang and Tze-fun Lee

Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G2E9, Canada

Abstract

Ginseng has been used as a key constituent in traditional medicine prescriptions for centuries. Other than its well-known anti-stress and adaptogenic properties, ginseng has also been shown to be very effective in treating age-related deterioration in metabolic and memory functions. Although it is generally believed that the saponin (GS) fraction of the ginseng root accounts for the bioactivity of ginseng, a direct demonstration on which ginsenoside does what is still generally lacking. In the past decade, our laboratory has endeavored to identify the active GS components involved in energy metabolism, memory, and anti-neurotoxicity. To examine the ergogenic effects of GS in enhancing aerobic capacity, rats were subjected to either severe cold (-10°C under helium-oxygen, two hours) or exercise workload (70% VO_2max, to exhaustion). Acute systemic injection (i.p.) of ginseng GS (5-20 mg/kg) significantly elevated both the total and maximum heat production in rats and improved their cold tolerance. However, pretreating the animal with the optimal dose (10 mg/kg) of GS devoid of Rg_1 and Rb_1 failed to elicit any beneficial effects in improving cold tolerance. This indicates that either Rb_1 and/or Rg_1 may be essential in exemplifying the thermogenic effect of GS. Further studies showed that only pretreating the animals with Rb_1 (2.5-5 mg/kg), but not Rg_1, resulted in an increase in thermogenesis and cold tolerance. In contrast to the acute effect of GS on cold tolerance, enhancement of exercise performance in rats was only observed after chronic treatment (4 days). Further, we were able to demonstrate that both Rb_1 and Rg_1 are effective in enhancing aerobic endurance by exercise. To illustrate the beneficial effects of GS in learning and memory, a passive avoidance paradigm (shock prod) was used. Our results indicated that the scopolamine-induced amnesia can be significantly reversed by chronically treating (4 days) the rats with either Rb_1 or Rg_1 (1.25 - 2.5 mg/kg). To further examine its underlying mechanisms, the effects of various GS on β-amyloid-modulated acetylcholine (ACh) release from the hippocampal slices

were examined. It was found that inclusion of Rb_1 (0.1 μM), but not Rg_1, can attenuate β-amyloid-suppressed ACh release from the hippocampal slices. Our results demonstrated that Rb_1 and Rg_1 are the key components involved in various beneficial effects of GS but they may elicit their effects through different mechanisms.

Effects of GS on cold tolerance

It has been shown previously that acute treatment with crude extract of ginseng root in rodents can significantly increase their cold resistance (Cheng et al., 1987; Kumar et al., 1996). However, there is no direct evidence that GS is indeed the component in enhancing thermogenesis. To examine this possibility, rats were subjected to severe cold (-10°C under helium-oxygen) for two hours (Wang & Lee, 2000). As shown in Table 1, acute intraperitoneal injection (i.p.) of GS (containing both protopanaxadiol- and protopanaxtriol-type saponins) (5-20 mg/kg) caused a dose-related elevation in the level of thermogenesis. In comparison to saline control, both the total and maximal thermogenesis of rats were significantly enhanced by 11.5 and 10.5% above control values, respectively, after receiving 10 mg/kg GS. Consequently, the decrease in final body temperature (Tb) was also significantly less after this treatment. Increasing the dose of GS, up to 20 mg/kg, did not further enhance the thermogenic effect. Interestingly, pretreating the rats with a special GS preparation, which did not contain Rb_1 and Rg_1, failed to elicit an increase in

Table 1. Effects of intraperitoneal injection of GS, with or without Rb_1 and Rg_1, on maximal heat production (HP), total HP, and change in final body temperature (Tb) on young rats (3-6 months) exposed to cold (Wang & Lee, 2000)

Treatment	Max. HP (kcal/15 min)	Total HP (kcal/105 min)	Tb Change (°C)
Saline 1 ml/kg	1.71±0.03	11.39±0.26	-3.95±0.46
GS 5 mg/kg	1.75±0.07	11.71±0.48	-3.34±0.60
GS 10 mg/g	1.89±0.04*	12.70±0.25*	-2.09±0.54*
GS 20 mg/kg	1.86±0.06*	12.49±0.44*	-2.21±0.61*
GS 10 mg/kg (without Rb_1 and Rg_1)	1.73±0.03	11.54±0.28	-3.91±0.52

Each value represents the mean±s.e.m. from 8 rats.
* Significantly different from corresponding saline control, $p<0.05$

Table 2. Effects of intraperitoneal injection of various doses of Rb_1 and Rg_1, on maximal heat production (HP), total HP, and change in final body temperature (Tb) on young rats (3-6 months) exposed to cold (Wang & Lee, 2000)

Treatment	Max. HP (kcal/15 min)	Total HP (kcal/105 min)	Tb Change (°C)
Saline 1 ml/kg	1.66±0.05	11.24±0.25	-2.95±0.42
Rg_1 2.5 mg/kg	1.62±0.07	10.81±0.41	-3.24±0.67
Rg_1 5.0 mg/kg	1.69±0.03	11.37±0.32	-2.84±0.51
Saline 1 ml/kg	1.61±0.06	10.74±0.32	-3.61±0.39
Rb_1 2.5 mg/kg	1.72±0.04*	12.11±0.47*	-2.33±0.53*
Rb_1 5.0 mg/kg	1.80±0.04*	11.74±0.44*	-2.57±0.47*

Each value represents the mean±s.e.m. from 6 rats.
*Significantly different from corresponding saline control, $p<0.05$

thermogenic response and cold tolerance as compared to those after saline-treatment (Table 1). This indicates that either Rb_1 and/or Rg_1 may be essential in examplifing the thermogenic effect of GS.

Further studies on pretreating the animals with Rb_1 (2.5-5 mg/kg), but not Rg_1, resulted in an increase in thermogenesis and cold tolerance similar to those seen after GS (Table 2). Our results clearly indicate that Rb_1 is the key ingredient in GS-mediated enhancement in thermogenic capacity in young rats. The lack of thermogenic effect of Rg_1 could be due to its opposing metabolic and physiological effects. It has been shown previously that interleukin-1β-induced hyperthermia can be attenuated by Rg_1 in rats (Kang et al., 1995), indicating a central thermolytic role of Rg_1. Thus, it is perhaps not surprising to observe that Rg_1 is ineffective in enhancing thermogenesis.

Effects of GS on exercise performances

Other than enhancing thermogenesis, ginseng has also been reported to increase aerobic performance in rodents. Acute pretreatment with crude extract of ginseng root in mice significantly increased their endurance of swimming (Brekhman & Dradynov, 1969; Grandhi et al., 1994) or running on the rotating rod (Saito et al., 1974). However, none of these studies has verified that GS is the key component involved in enhancing the aerobic performance. To

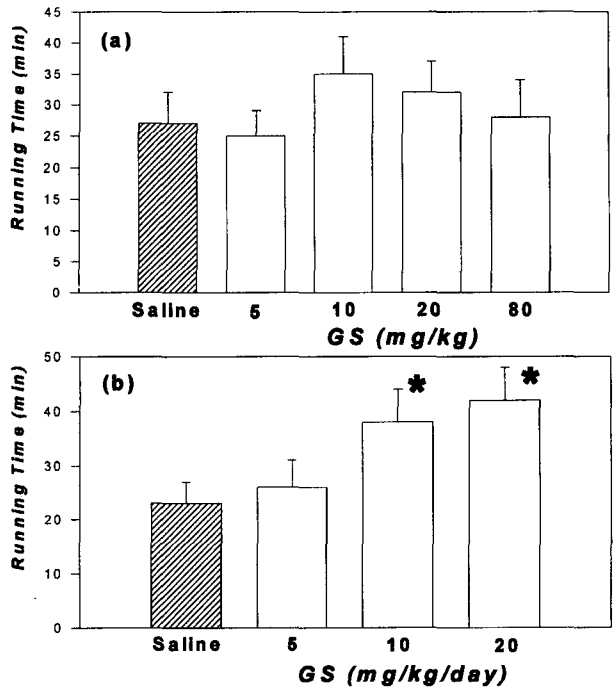

Fig. 1. (a) Effect of single, acute injection of either saline or various doses of ginsenoside (GS) on the exercise endurance (running on the treadmill with 15% incline at 70% of VO_2 max till exhaustion) of rats (n=10); (b) effects of chronic (4 days) daily treatment with either saline or various doses of GS on exercise endurance of rats (n=12). * Significantly different from concurrent control group, $p<0.05$ (Wang & Lee, 1998)

elucidate whether GS is indeed the active ingredient to elicit the ergogenic effect, rats were trained to run on a treadmill (70% VO_2max till exhaustion) with a 15% incline (Wang & Lee, 1998). As shown in Figure 1(a), single, acute injection of GS (5-80 mg/kg) did not cause any significant change in overall exercise performance of the rats. In contrast, a dose-related increase in running time was achieved after a 4-day daily chronic treatment with GS (5-20 mg/kg/day, i.p.)(Fig. 1b). It is unlikely that the failure of acute injection of GS to improve endurance is due to an insufficient dosage as the highest dose of GS (80 mg/kg) used, which was equivalent to the 4-day treatment dose in total, also did not elicit any ergogenic effect. Instead, it seems that GS-elicited ergogenic effects required both time and repeated treatments to develop.

Pretreating the rats for 4 days with the optimal dose (20 mg/kg/day) of special GS preparation which did not contain Rb_1 and Rg_1 failed to improve the endurance of the animal (saline = 29.4±8.3 min; GS without Rb_1 and Rg_1 = 39.2±8.7 min; n=8), indicating that either Rb_1 and/or Rg_1 may be essential for eliciting the beneficial ergogenic effect of GS. To further investigate this

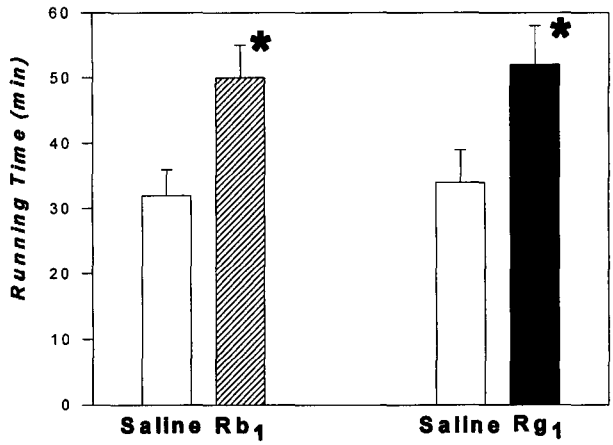

Fig. 2. Effects of chronic (4 days) daily treatment with either saline or 2.5 mg/kg/day Rb_1 (shaded column) or Rg_1 (closed column) on exercise endurance of rats (n=8). *Significantly different from concurrent control group, $p<0.05$ (Wang & Lee, 1998)

possibility, purified Rb_1 and Rg_1 were employed. Similar to those observed with GS, chronic (4 days) treatment with either 2.5 mg/kg/day Rb_1 or Rg_1 significantly increased the running time of the rat by 56.3% and 52.9%, respectively (Fig. 2). Based on this observation, it appears that both Rb_1 and Rg_1 are essential for exerting the beneficial ergogenic effects of GS.

Effects of GS on learning and memory

In addition to increasing physical performance, ginseng has also been shown to improve cognitive function in various animal studies. Treating the animals with ginseng extract significantly reversed the memory deficits either induced by pharmacological manipulations (Hsieh et al., 2000; Lee et al., 2000) or age-related deterioration (Nitta et al., 1995; Zhong et al., 2000). Recently, it has been shown that the effectiveness of ginseng in reversing memory deficits depends on the ratio of protopanaxadiol and protopanaxatriol saponin (Jin et al., 1999). Similarly, Yamaguchi et al. (1995, 1996) demonstrated that not all GS can ameliorate memory deficit induced by scopolamine. Thus, it is of necessarsity to identify which specific ginsenosides are of particular efficacy in enhancing cognitive function.

To illustrate the beneficial effect of various GS in learning and memory, a passive avoidance paradigm (shock prod) was used (Benishin et al., 1991). As shown Figure 3, treating the animals with scopolamine (1 mg/kg, i.p.) markedly impaired their performance in avoiding the shock

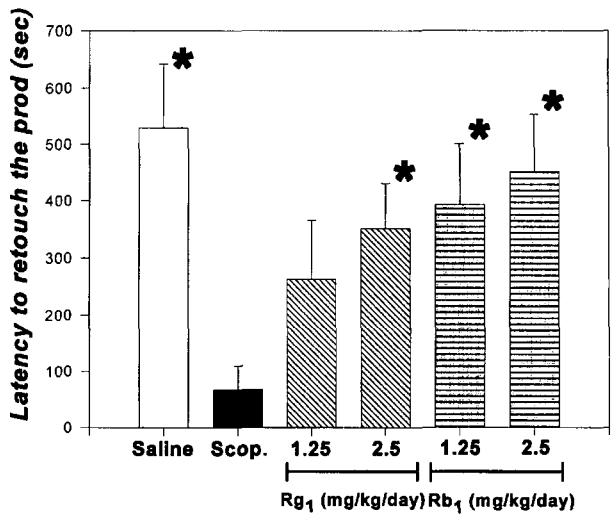

Fig. 3. Effects of chronic treatment (4 days) with either Rg_1 or Rb_1 on scopolamine (scop., 1 mg/kg, i.p.)-induced amnesia. Each bar represents the mean±sem for 12 rats. *Significantly different from scopolamine control, $p<0.05$

prod. The scopolamine-induced amnesia can be significantly reversed by chronically treating (4 days) the rats with either Rb_1 or Rg_1 (Fig. 3). In contrast to previous reports that only Rg_1, but not Rb_1, can improve memory deficit induced by scopolamine (Yamaguchi et al., 1995), our results indicated that both Rb_1 and Rg_1 are effective in improving learning and memory. Other than differences in methodology, the discrepancy may also be due to different treatment regimen. Chronic treatment with various GS was used in our study whereas acute single treatment was employed in previous studies.

Effects of GS on neurotoxicity of β-amyloid protein

Alzheimer's disease (AD) is characterized by the progressive loss of short-term memory and β-amyloidogenesis has been suggested to be critical in the pathogenesis of AD (Clippingdale et al., 2001; Selkoe, 1994; Sisodia & Gallagher, 1998). Recently, it has been shown both *in vitro* (Kar et al., 1996) and *in vivo* (Olariu et al., 2001) that addition of β-amyloid peptides potently suppressed hippocampal and cortical acetylcholine (ACh) release. The suppression of cholinergic transmission by the β-amyloid peptide has been suggested to be part of the mechanisms through which the amyloid peptides elicit their neurotoxic effect. Previously, we have shown that GS,

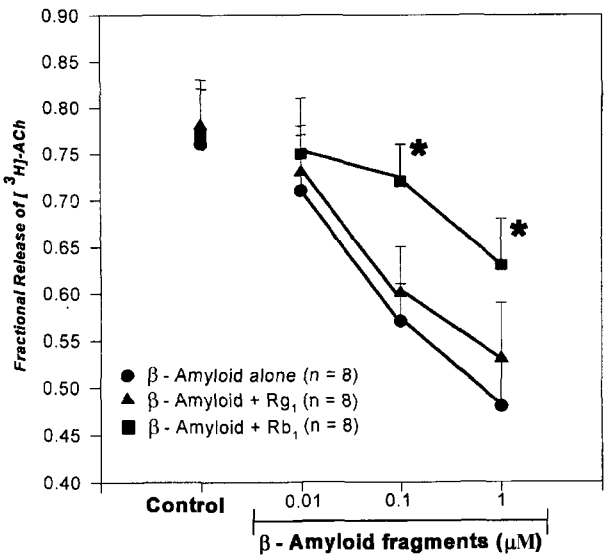

Fig. 4. Effects of ginsenosides (0.1 μM Rb_1 or Rg_1) on β-amyloid$_{25-35}$ inhibited ACh release from hippocampal slices. The results are expressed as mean±s.e.m. from 8 animals. *Significantly different from corresponding control group, $p<0.05$ (Lee at al., 2001)

particularly Rb_1, can facilitate central cholinergic metabolism (Benishin et al., 1991), Thus, it is possible that GS may alleviate some age-related dysfunction by minimizing the neurotoxic effects induced by β-amyloid peptide. To further examine this possibility, the effects of various GS on β-amyloid-modulated ACh release from the hippocampal slices were examined (Lee et al., 2001).

As shown in Fig. 4, addition of β-amyloid fragment$_{25-35}$ (0.01 - 1 μM) in the superfusion medium suppressed the K^+-evoked [^{3}H]-ACh release from the rat hippocampal slices in a concentration-related manner and about 40% reduction in ACh outflow was observed when incubating with the highest concentration of an amyloid fragment (1 μM). To examine the effects of GS on β-amyloid-suppressed [^{3}H]-ACh release, a submaximal concentration of Rb_1 (0.1 μM), which only elicited an insignificant increase in ACh outflow, was used. Inclusion of Rb_1 in the superfusion medium caused a rightward shift of the concentration-response curve of β-amyloid (Fig. 4). The reversal of β-amyloid-inhibited ACh release by Rb_1 was not affected by the voltage-dependent sodium channel blocker tetrodotoxin (TTX)(1 μM)(S_2/S_1 ratio: 1 μM β-amyloid + 0.1 μM Rb_1 = 0.67±0.06; β-amyloid + Rb_1 + TTX = 0.65±0.05, n = 5) indicating that the interaction occurs at the cholinergic synapse rather than via interneurons.

In contrast to that observed with Rb_1, addition of the same concentration of Rg_1 (0.1 μM) in the medium had no effect on β-amyloid-inhibited ACh release (Fig. 4). Since both Rb_1 and Rg_1 have been reported to have similar neuroprotective effects (Liao et al., 2002; Liu et al., 1995), the insensitivity of β-amyloid to Rg_1 in modulating ACh release is unexpected. However, the discrepancy could be due to their various effects on the cholinergic activities. Previously, an increase in acetyltransferase activity was observed in the medial septum after repeated intraperitoneal injections of Rg_1, but not Rb_1, in rats (Yamaguchi et al., 1997). Further, we have observed that inclusion of Rb_1 in the superfusion medium alone induced a concentration-related increase in hippocampal ACh outflow, whereas Rg_1 did not cause any significant change (Lee et al., 2001). Even though GS has been shown to improve learning and memory in aged rats (Nitta et al., 1995; Zhong et al., 2000), it is quite possible that Rb_1 and Rg_1 may alleviate these age-related dysfunctions through different mechanisms. In view of the fact that formation of amyloid plaque is the most frequent cause of neruo-degenerative process in AD, it is possible that one of the mechanisms through which Rb_1 ameliorates the impairment of learning performance observed in AD is to minimize the toxic effect of β-amyloid peptide.

Conclusion

Our cumulative data clearly demonstrated that GS, particularly Rb_1 and Rg_1, are the key components involved in improving physical performance and well being reported with ginseng root extract. However, it is noteworthy that Rb_1 and Rg_1 elicit their physiological effects differently. Rb_1 appears to be effective in all our animal models, whereas Rg_1 can only improve exercise performance and cognitive function. This could partly explain the contradictory reports on the efficacy of ginseng root extract in various clinical trials. As some 30 or more individual ginsenosides have been described, the concentration of individual ginsenosides could vary because of the source (wild or cultivated), the different parts of the plant as well as between species and commercial brands (Ma et al., 1995; Soldati & Tanaka, 1985). In view of the fact that each ginsenoside could behave differently, the actual composition of ginseng preparation presents a persistent problem. To complicate this matter further, our studies also demonstrate that the effectiveness of GS could also depend on the dosage regimen. For instance, the exercise endurance of the rat can only be enhanced after chronic, but not acute, treatment with GS preparation. In order to elucidate the effectiveness of GS extract in various physical performance

and well being, it is imperative that standardization of the materials employed as well as the dosage regimen be considered in future clinical studies.

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