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Ginseng total saponin potentiates acute U-50,488H-induced analgesia and inhibits tolerance to U-50,488H-induced analgesia in mice

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Abstract

In the present study, an attempt has been made to investigate whether the potentiating effect of U-50,488H (U50)-induced analgesia by ginseng total saponin (GTS) is playing a role in inhibiting the tolerance to U50-induced analgesia as measured using the tail-flick test in mice. GTS (100 and 200 mg/kg ip), on acute administration, potentiated the U50 (40 mg/kg ip)-induced analgesia in U50-naive mice. Twice daily administration of U50 (40 mg/kg ip) for 6 days resulted in tolerance to U50-induced analgesia in mice. Chronic administration (Days 4–6) of GTS (50, 100, and 200 mg/kg ip) to U50-tolerant mice dose-dependently inhibited the tolerance to U50-induced analgesia. On the other hand, chronic administration of GTS (50, 100, and 200 mg/kg ip) dose-dependently potentiated the U50-induced analgesia in U50-naive mice. The dose–response curve to U50-induced analgesia in U50-tolerant mice was shifted rightward (2.6-fold) as compared to U50-naive mice, indicating the development of tolerance to U50-induced analgesia. GTS (100 mg/kg ip od), on chronic administration, prevented the rightward shift of dose–response curve to U50-induced analgesia in U50-tolerant mice, whereas in U50-naive mice it resulted in leftward shift (0.6-fold). It can be concluded that acute and chronic administration of GTS potentiates the U50-induced analgesia in U50-naive mice. The potentiating effect of GTS on U50-induced analgesia may be partially responsible in the inhibition of tolerance to U50-induced analgesia in mice.

Keywords: Ginseng total saponin; U-50,488H; Analgesia; Tolerance; Potentiation

1. Introduction

Chronic administration of morphine and related opioid drugs produces a state of cellular tolerance, indicated by loss of drug potency, physical dependence, and appearance of withdrawal syndrome. In clinical situations, opioid tolerance and dependence can limit the usefulness of opioids in the management of severe pain syndrome (Foley, 1991). Selective kappa opioids such as U-50,488H (U50), unlike morphine, do not produce physical dependence or produce very mild degree of dependence (Szmuszkovicz, 1999). However, chronic administration of U50 leads to development of tolerance to analgesic effect (Bhargava et al., 1989; Von Voigtlander et al., 1984). Biochemical and molecular mechanisms in opioid tolerance and dependence are not yet precisely known. Several agents have been reported to inhibit the tolerance to opioid mediated responses (Bhargava, 1994). However, none of these agents was found to be ideal in the inhibition of opioid tolerance and dependence.

Many agents of natural origin have been reported to inhibit the tolerance to morphine-induced analgesia in rodents (Bhargava and Ramarao, 1991; Cao and Bhargava, 1997; Kim et al., 1987; Ramarao et al., 1995; Viswanthan et al., 1990). *Panax ginseng* C.A. Meyer (Araliaceae), a well-known traditional oriental medicine, has been demonstrated to inhibit tolerance to morphine (a mu-opioid)-induced analgesia in rodents (Bhargava and Ramarao, 1991; Kim et al., 1987). Further, standardized ginseng extract G115 has been reported to inhibit tolerance to morphine-induced analgesia (Kim et al., 1989). Ginseng saponins, the main active constituents of *P. ginseng* (Attele et al., 1999; Kaku et al., 1975a,b), were classified in to three groups namely panaxadiol (PD), panaxatriol (PT), and oleonolic acid groups based on their chemical structures. Recently, we reported that ginseng total saponin (GTS) (a mixture of

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all saponins), PD, and PT potentiates U50-induced analgesia and inhibits tolerance to U50-induced analgesia in mice (Nemmani and Ramarao, 2000). Chronic administration of COX inhibitors and the NMDA receptor antagonist, MK-801, was reported to potentiate the morphine-induced analgesia (Dunbar and Yaksh, 1996; Powell et al., 1999). The sensitization effect of morphine-induced analgesia upon chronic administration by these agents may possibly be the underlying mechanism in inhibition of the tolerance to morphine-induced analgesia (Dunbar and Yaksh, 1996; Powell et al., 1999). Recently, it has been proposed that potentiation of morphine-induced analgesia by fenfluramine plays a role in the inhibition of tolerance to morphine-induced analgesia (Arends et al., 1998). In the present study, effect of chronic administration of GTS on U50-induced analgesia was studied in U50-naive and -tolerant mice. To determine the influence of potentiation effect of U50-induced analgesia by GTS, dose–response curves to U50-induced analgesia were constructed in U50-naive and -tolerant mice treated with vehicle or GTS.

2. Method

2.1. Animals

Swiss male mice (Central Animal Facility, NIPER, India), weighing 20–26 g, were housed six per cage in a room with controlled temperature (22 ± 1 °C), humidity (50 ± 10%), and light (0600–1800 h). Food and water were made available ad libitum. All experiments were performed between 1000 and 1800 h to minimize diurnal variations.

2.2. Drugs

U50 (*trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzene acetamide methane sulfonate) was a gift sample from Pharmacia and Upjohn (Kalamazoo, MI) and GTS is mixture of ginsenosides and the ratios of various ginsenosides were: Rb_1, 18.26%; Rb_2, 9.07%; Rc, 9.65%; Rd, 8.24%; Re, 9.28%; Rf, 3.48%; Rg_1, 6.42%; Rg_2, 3.63%; Rg_1, 4.70%; R_0, 3.82%; Ra, 2.91%; and other minor ginsenosides was gift from Korea Ginseng and Tobacco Research Institute (Taejon, South Korea). Both substances were dissolved in distilled water and administered intraperitoneally in a volume of 10 ml/kg in mice.

2.3. Analgesia testing

Analgesic response was assessed by a radiant heat tail-flick method (D'Amour and Smith, 1941). The tail-flick latencies to thermal stimulation were determined at 0, 30, 60, 90, 120, and 180 min after the drug administration of U50 as described previously (Nemmani and Ramarao, 2000). To use each animal's basal tail-flick latency as its own control, percentage of maximum possible effect (% MPE) was determined according to the following formula:

$$\%\ \mathrm{MPE} = \{(\text{postdrug latency} - \text{predrug latency}) / (\text{cut off} - \text{predrug latency})\}.$$

The % MPE vs. time was plotted and the area under curve (AUC) was calculated by trapezoidal rule. The data were expressed as mean % MPE ± S.E.M or mean $AUC_{0-180\ min}$ ± S.E.M. To construct the dose–response curve of the U50-induced analgesia, analgesic response was measured at 30 min after the administration of U50 and the data were fitted to sigmoidal E_{max} model.

2.4. Effect of acute administration of GTS on U50-induced analgesia

To determine the effect of acute treatment of GTS on U50-induced analgesia, GTS (50, 100, and 200 mg/kg ip) was treated simultaneously with U50 (40 mg/kg ip) in U50-naive mice. The analgesic response was measured as described above. To prevent chemical interaction, if any, GTS and U50 were administered on the left and right sides of abdomen to mice, respectively.

2.5. Effect of GTS on tolerance to U50-induced analgesia

Mice were rendered tolerant to U50 by twice daily administration of U50 (40 mg/kg ip) for 6 days. Daily U50 was administered in the morning and evening. To study the effect of GTS on U50-induced analgesia, GTS (50, 100, and 200 mg/kg ip od) was administered chronically (Days 4–6) in the evening to U50-naive and -tolerant mice. Analgesic response was determined on Day 7.

2.6. Effect of GTS on dose–response curve to U50-induced analgesia in U50-naive and -tolerant mice

To determine the effect of GTS (100 mg/kg ip) on dose–response curve to U50-induced analgesia, mice were administered either vehicle or GTS and the analgesic response of U50 (5–120 mg/kg ip) was determined at 30 min after the administration.

To investigate whether the potentiation effect of U50-induced analgesia by GTS on chronic administration contributed to the inhibition of tolerance to U50-induced analgesia, dose–response curves were constructed in U50-naive and -tolerant mice administered chronically with vehicle or GTS (100 mg/kg ip od). In this study, mice were divided into four groups and administered with: Group 1, vehicle + vehicle; Group 2, U50 + vehicle; Group 3, vehicle + GTS; and Group 4, U50 + GTS. Groups 2 and 4 received U50 (40 mg/kg ip bid) for 6 days whereas Groups 3 and 4 administered GTS (100 mg/kg ip od) on Days 4, 5, and 6. All the groups were administered the appropriate

Table 1
Effect of acute and chronic administration of GTS on U50-induced analgesia

	Analgesic response to U50 $AUC_{0-180\ min}$	
Treatment[a]	Acute administration[a]	Chronic administration[b]
Vehicle	2186.8 ± 433.3	2039.7 ± 220.9
GTS 50	1773.5 ± 304.5	3421.4 ± 245.9*
GTS 100	9503.9 ± 508.1*	7456.4 ± 227.7*
GTS 200	10218.2 ± 363.2*	7706.7 ± 274.5*

All the values are expressed as mean ± S.E.M., $n = 5$.

[a] Mice were administered GTS (50, 100, and 200 mg/kg ip) along with U50 (40 mg/kg ip) and the analgesic response was measured.

[b] GTS (50, 100, and 200 mg/kg ip) was administered once daily on Days 4–6 and the analgesic response was measured on Day 7.

* $P < .05$ vs. respective vehicle.

vehicle. On Day 7, i.e., 18 h after the last dose, each group of mice was further subdivided in to subgroups of four to six mice each. Each subgroup of mice received appropriate dose of U50 and the analgesic response was measured at 30 min after the administration.

2.7. Effect of acute treatment of GTS on U50-induced analgesia in U50-naive and -tolerant mice

Mice were rendered tolerant U50 as described above. The effect of acute administration of GTS on U50-induced analgesia was determined in U50-naive and -tolerant mice by treating GTS along with U50 on Day 7 in U50-tolerant mice and the analgesic response was determined as mentioned above.

2.8. Statistics

Data describing analgesia are expressed either as % MPE ± S.E.M or as $AUC_{0-180\ min}$ ± S.E.M. Statistical significance ($P < .05$) was determined using one-way ANOVA followed by Scheffe's S test for multiple comparison between groups. U50 dose–response curve is fitted to the sigmoidal E_{max} model using nonlinear regression program Sigma Plot (Sigma Plot Scientific Graphic System, Ver. 4.01).

$$E = [E_{max}D^N]/[ED_{50}^N + D^N]$$

where E is the % MPE, D is the U50 dose, ED_{50} is the dose at half-maximal effect, E_{max} is the maximal effect and was set to 100%. N is a power constant expressing the steepness of the dose–effect relationship. Statistical comparisons of dose–response curves are based on the confidence limits of the ED_{50} values. A dose–response shift is considered significant when the confidence limits of calculated ED_{50} values of one curve falls outside the confidence limits of the ED_{50} value of the curve to which it is being compared. Groups of four to six animals were assigned for each dose. The ratio of mean ED_{50} of one curve to that of vehicle group was calculated to determine the shift (number of folds) in the dose–response curve.

3. Results

3.1. Effect of GTS on U50-induced analgesia

U50 (40 mg/kg ip) produced analgesia in mice and the peak maximal effect was observed at 30 min after the administration of U50. Simultaneous administration of GTS (50 mg/kg ip) with U50 had no effect on U50-induced analgesia. However, higher doses of GTS (100 and 200 mg/kg ip) potentiated the U50-induced analgesia (expressed as $AUC_{0-180\ min}$) in U50-naive mice (Table 1).

3.2. Effect of GTS on tolerance to U50-induced analgesia

Daily administration of U50 (40 mg/kg ip bid) for 6 days resulted in the development of tolerance to U50-induced analgesia in mice. Chronic administration of U50 or its vehicle did not alter the basal tail-flick response. As shown in Table 2, U50 (40 mg/kg ip) produced analgesia in U50-naive mice. However, U50-induced analgesia (expressed as $AUC_{0-180\ min}$) was significantly less in U50-tolerant mice as compared to U50-naive mice. Chronic administration of GTS (50, 100, and 200 mg/kg ip od) dose-dependently inhibited the tolerance to U50-induced analgesia (Table 2). On the other hand, chronic administration of GTS dose-dependently potentiated the U50-induced analgesia in U50-naive mice.

3.3. Effect of GTS on tolerance to dose–response curve of U50-induced analgesia

The extent of tolerance to U50-induced analgesia with vehicle or GTS administration was assessed by U50 dose–response curves at 18 h after the last dose of U50 + vehicle, with vehicle + GTS, with U50 + GTS, and with vehicle + vehicle (Fig. 1 and Table 3). Chronic administration of GTS significantly lowered the ED_{50} of U50-induced analgesia in

Table 2
Effect of chronic administration of GTS on tolerance to U50-induced analgesia

Treatment[a]	Analgesic response to U50 $AUC_{0-180\ min}$
Vehicle + vehicle	1886.3 ± 175.8
U50 + vehicle	338.2 ± 46.9*
U50 + GTS 50	1089.9 ± 127.9#
U50 + GTS 100	1834.9 ± 269.4#
U50 + GTS 200	1989.2 ± 217.0#

All the values are expressed as mean ± S.E.M., $n = 5$.

[a] Mice were rendered tolerant to U50 by twice daily administration of U50 (40 mg/kg ip) for 6 days. Similar treatment was done in another group of animals with vehicle (10 ml/kg ip). GTS (50, 100, and 200 mg/kg ip) was administered daily in the evening to U50-tolerant mice on Days 4–6. The analgesic response to U50 (40 mg/kg ip) was determined on Day 7.

* $P < .05$ vs. vehicle + vehicle.

# $P < .05$ vs. U50 + vehicle.

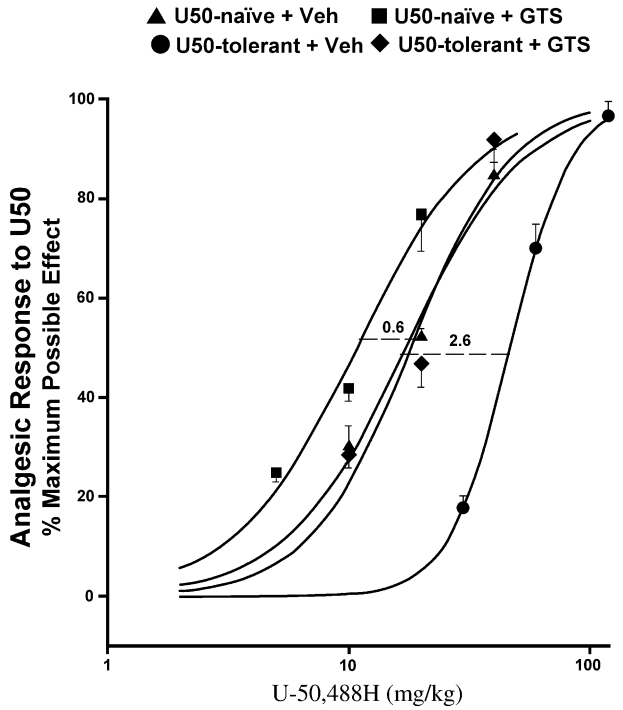

Fig. 1. Effect of chronic administration of GTS (100 mg/kg ip od) on dose–response curve to U50-induced analgesia (expressed as % MPE) in U50-naive and -tolerant mice. All values are expressed as mean ± S.E.M ($n = 4–6$).

U50-naive mice when compared to vehicle administered U50-naive group: ED_{50} of 17.90 ± 0.97 (95% CI 15.20–19.60) vs. 11.01 ± 1.06 (95% CI 7.51–14.51). Daily administration of U50 (40 mg/kg ip bid) for 6 days resulted in rightward shift of U50 dose–response curve indicating the development of tolerance to analgesia in mice. ED_{50} increased to 2.6-fold from 17.90 to 47.34. In comparison, chronic administration of GTS (100 mg/kg ip od) to U50-tolerant mice, inhibited this rightward shift of dose–response curve to U50-induced analgesia indicating its inhibitory effect on tolerance to U50-induced analgesia in mice (Fig. 1 and Table 3).

Table 3
U50 dose–response parameters in U50-naive and -tolerant mice chronically administered with vehicle or GTS (100 mg/kg ip)

Administration[a]	Mean ED_{50} (mg/kg)	95% CI (mg/kg)	Ratio of mean ED_{50} to naive control
Vehicle + vehicle	17.90	15.20–19.60	0.9
U50 + vehicle	47.34	38.79–55.89*	2.6
Vehicle + GTS	11.01	7.51–14.51*	0.6
U50 + GTS	17.48	15.13–19.83#	0.9

[a] Mice were administered with vehicle or U50 (40 mg/kg ip) twice daily for 6 days. GTS was administered once a day on Days 4–6. Analgesic response to U50 on Day 7 was measured at 30 min after the administration in mice from all groups. The data were fitted to sigmoidal E_{max} model and ED_{50} was estimated.

* $P < .05$ vs. vehicle + vehicle.

# $P < .05$ vs. U50 + vehicle.

Table 4
Effect of acute treatment of GTS on U50-induced analgesia in U50-naive and -tolerant mice

Treatment[a]	Analgesic response to U50 AUC $_{0-180\ min}$
U50-naive + vehicle	2291.7 ± 147.0
U50-naive + GTS	9896.9 ± 680.6*
U50-tolerant + vehicle	351.6 ± 59.7
U50-tolerant + GTS	6633.4 ± 297.6#

All the values are expressed as mean ± S.E.M., $n = 5$.

[a] Mice were rendered tolerant to U50 by twice daily administration of U50 (40 mg/kg ip) for 6 days. Similar treatment was done in another group of animals with vehicle (10 ml/kg ip). GTS (100 mg/kg ip) was administered along with U50 on Day 7 in U50-naive and -tolerant mice and the analgesic response was measured.

* $P < .05$ vs. U50-naive + vehicle.

# $P < .05$ vs. U50-tolerant + vehicle.

3.4. Effect of acute treatment of GTS on U50-induced analgesia in U50-naive and -tolerant mice

As shown in Table 4, coadministration of GTS (100 mg/kg ip) potentiated the U50 (40 mg/kg ip)-induced analgesia in U50-naive and -tolerant mice. The potentiation of U50-induced analgesia by the administration of GTS (100 mg/kg ip) was about five folds more in U50-tolerant mice compared to that of U50-naive mice (Table 4).

4. Discussion

The present study clearly demonstrates that acute and chronic administration of GTS (100 mg/kg) potentiates the U50-induced analgesia in U50-naive mice. The present studies also demonstrate that GTS inhibits the tolerance to U50-induced analgesia in mice. GTS in the doses used in the study did not produce analgesia (Nemmani and Ramarao, 2000). It is interesting to note that GTS (100 mg/kg), which inhibited completely the tolerance to U50-induced analgesia, has potentiation effect on U50-induced analgesia on chronic administration. Similar to the present study, COX inhibitors and NMDA receptor antagonist, MK-801, were reported to potentiate the morphine-induced analgesia on chronic administration. The potentiation effect of these agents may possibly the reason for the restoration of morphine potency in morphine-tolerant animals (Dunbar and Yaksh, 1996; Powell et al., 1999).

Hence, further studies were conducted to verify whether GTS (100 mg/kg) potentiation effect is contributing in inhibition of tolerance to U50-induced analgesia. The extent of tolerance to U50-induced analgesia with and without GTS administration was assessed by U50 dose–response curves (Fig. 1). GTS (100 mg/kg ip od, Days 4–6) alone shifted the U50 dose–response curve to leftward indicating

its potentiating effect of U50-induced analgesia. As expected, U50 on chronic administration shifted the dose–response curve of U50-induced analgesia by 2.6-fold rightward. In comparison, GTS (100 mg/kg ip od, Day 4–6) prevented the rightward shift of the dose–response curve of U50-induced analgesia in U50-tolerant mice. These results suggest that chronic administration of GTS reversed the tolerance to U50-induced analgesia. Hence, potentiating effect of GTS (100 mg/kg) may be partially contributing in the inhibition of tolerance to U50-induced analgesia. These findings suggest that GTS inhibit the tolerance to U50-induced analgesia by interfering with mechanisms involved in the development of tolerance to U50-induced analgesia in mice.

The mechanisms involved in the potentiation of U50-induced analgesia and inhibition of tolerance to U50-induced analgesia by GTS are not known. Recently, it was reported that ginseng saponins did not alter the metabolism of coadministered drugs (Henderson et al., 1999). Further, it was reported that that the development of tolerance to kappa opioid-induced analgesia is not a pharmacokinetic phenomenon (Von Voigtlander et al., 1981). Thus, it is unlikely that the observed potentiation of U50-induced analgesia and inhibition of tolerance by GTS is a pharmacokinetic phenomenon. Interestingly, cotreatment of GTS also potentiated the U50-induced analgesia in U50-tolerant mice. These observations suggest that the mechanisms that are playing a role in the potentiation of U50-induced analgesia are present in U50-tolerant condition.

It was reported that ginseng-induced analgesia were not blocked by naltrexone in rat (Bhargava and Ramarao, 1991). Recently, ginseng saponins were reported to mimic the actions of opioids without activating opioid receptors on electrically evoked contractions of guinea pig ileum, and mouse vas deferens (Watanabe et al., 1988). In addition, ginseng extract was reported to inhibit Ca^{2+} channels in rat sensory neurons, which was not blocked by naloxone indicating the involvement of nonopioid mediated effects (Nah and McCleskey, 1994). Hence, GTS does not appear to act at the opioid receptor/endogenous opioid system.

The involvement of serotonergic system in the U50-induced analgesia and tolerance to U50-induced analgesia was reported earlier (Ho and Takemori, 1989; Von Voigtlander et al., 1984). In addition, GTS has been reported to alter U50-induced analgesia through serotonergic mechanisms (Kim et al., 1992). Clomipramine, a selective serotonin uptake blocker was reported to potentiate the U50-induced analgesia (Kunihara et al., 1992). Hence, potentiation of U50-induced analgesia by GTS was supported by the fact that GTS inhibits the uptake of 5-HT (Tsang et al., 1985) and alters the levels of serotonin in various regions of brain (Petkov, 1978). Hence, GTS may appear to modulate the U50-induced analgesia and its tolerance by serotonergic mechanisms.

Other possible mechanisms of interaction of GTS and U50 may occur at the signal transduction pathway involving cAMP or influx of Ca^{2+}. It is a well-known fact that the second messenger system, AC–cAMP, plays role in opioid action (Sarma et al., 1977). Using spinal cord and dorsal root ganglion cocultures, it was shown that kappa opioid agonists inhibit the voltage-dependent Ca^{2+} uptake as well as adenyl cyclase activity via pertussis toxin-sensitive GTP-binding proteins (Attali et al., 1989; Vogel et al., 1989). Moreover, chronic administration of kappa opioid agonists was reported to induce qualitative and quantitative changes in GTP-binding proteins (Attali and Vogel, 1989). Hence, U50-induced analgesia and tolerance to its analgesia involves adenyl cyclase and/ calcium mediated mechanisms. Inhibition of adenyl cyclase and calcium channels by *P. ginseng* was reported in the rat cortex and sensory neurons, respectively (Nah and McCleskey, 1994; Petkov, 1978). It has been reported that inhibition of voltage-gated Ca^{2+} channels in sensory neurons by opioids may contribute to the analgesic action (Johnson and Flemming, 1989). On the other hand, calcium channel blockers were reported to block tolerance to U50-induced analgesia (Contreras et al., 1993). Thus, GTS may possibly inhibit the tolerance to U50-induced analgesia through adenyl cyclase and/ calcium mediated mechanisms.

In conclusion, the present studies demonstrated that acute and chronic administration of GTS potentiates U50-induced analgesia in U50-naive mice. In addition, chronic administration of GTS inhibits the tolerance to U50-induced analgesia. Potentiation effect of GTS on U50-induced analgesia partially accounts for the inhibition of tolerance to U50-induced analgesia. Given the safety and utility of *P. ginseng* (Gillis, 1997), the present findings suggest that GTS may be useful as an adjuvant for the inhibition of tolerance to analgesic response of kappa opioid selective agonists such as U50 that are reported to have less or no addiction liability.

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