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Spinal Co-Administration of Ginsenosides with Morphine Prevents the Development of Opioid Tolerance and Attenuates Opioid Dependence

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Abstract : The analgesic effect of ginsenosides or morphine was determined following intrathecal (i.t.) administration in rat tail-flick test. The effects of intrathecal co-administration of ginsenosides with morphine on the development of opioid tolerance and dependence were also examined using rat tail-flick test and naloxone-precipitated withdrawal, respectively. Administration of ginsenosides (i.t.) produced a weak antinociception in a dose-dependent manner. Administration of morphine (i.t.) also produced antinociception in a dose-dependent manner. The ED_{50} was 1.20 μg (1.14~1.29 μg). However, the acute co-administration of 200 μg ginsenosides with 0.1-1.0 μg morphine did not show additive effect on morphine induced analgesia in rat tail-flick test. I.t. co-administration of 200 μg ginsenosides with 10 μg morphine for 7 days inhibited development of tolerance induced by 10 μg morphine in rat tail-flick test, although i.t. co-administration of 50 or 100 μg ginsenosides with morphine was without effect. I.t. co-administration of 200 μg ginsenosides for 7 days also partially attenuated the development of morphine dependence as assessed by naloxone-precipitated withdrawal. In conclusion, these results suggest that i.t. administered ginsenosides produce a weak antinociception in rat tail-flick test and also prevent opioid tolerance and attenuate opioid dependence in chronic treatment with morphine at the spinal sites.

Key words : Ginseng, Ginsenosides, Opioid, Tolerance, Dependence, Spinal cord, Pain, Analgesia

Introduction

Ginseng, the root of *Panax ginseng* C.A. Meyer, is a traditional folk medicine that is reported to have many medicinal beneficial effects. A recent study demonstrated that ginsenosides, or ginseng saponin, are the main components responsible for the actions of ginseng.[1] The ginsenosides are well characterized and known to have a four-ring steroid-like structure with sugar moieties attached.[2] The ginsenosides show properties similar to, but not defined, acetylcholine, adrenaline, histamine, or opioids.[3]

As one of ginseng efficacies, ginseng alone or with other herbal medicines has been used to treat or attenuate opium addicts in traditional folk medicine. Recent reports might provide evidences for this traditional use of ginseng. For example, systemic treatment of standardized ginseng extract inhibits the development of tolerance to the analgesic effect of morphine in mice or rats.[4-6] Moreover, protopanaxatriol ginsenosides also attenuate the development of tolerance to the inhibitory effect of morphine on electrically evoked contraction of guinea-pig ileum.[7] On the other hand, the systemic treatment of ginsenosides also attenuates morphine-induced antinociception.[5,8] Moreover, i.t. administered ginsenosides antagonize supraspinal opioid-induced analgesia.[9]

In previous studies, we showed that ginsenosides inhibit voltage-dependent Ca^{2+} channels in sensory neurons as well as in identified nociceptor at the cellular level.[10-12] We also reported that systemic or intrathecal (i.t.) administration of ginsenosides relieves formalin induced-pain and that i.t. administered ginsenosides attenuate NMDA- and substance P-induced

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nociceptive behavior in mice.[13-15] Thus, ginsenosides could act on the spinal level to exert their physiological or pharmacological activity and also raise a possibility that ginsenosides could regulate or interact with pain modulation system at the spinal site like opioids.[9]

Recent studies showed that analgesia induced by the i.t. or epidural administration of opioids becomes important in the clinical pain management and that tolerance and dependence also develop to spinal opioid antinociception.[16,17] Although previous studies indicate that systemic treatment of ginsenosides attenuates morphine-induced tolerance as mentioned above[4,6], it is not well characterized whether ginsenosides inhibit the development of tolerance to analgesic effect of morphine at the spinal site. Therefore, the aim of this study was to investigate the effect of ginsenosides on the development of tolerance to analgesic effect of morphine at the spinal site using chronic co-administration model of ginsenosides and morphine in chronic i.t. catheterized rats. In addition, we also observed the effect of ginsenosides on morphine-induced dependence after i.t. co-administration with morphine. We found that i.t. co-administration of ginsenosides with morphine prevented the development of tolerance to analgesic effect of morphine and partially attenuated morphine-induced dependence at the spinal level.

Materials and Methods

1. Materials

Adult (300~350 g) male Sprague-Dawley rats obtained from Dae-Han animal breeding center were used. Animals were individually housed in cage with water and food pellets available *ad libitum*. The animal room was artificially illuminated from 07:00 to 19:00. Ginsenosides (ginseng total saponin) were kindly obtained from Korea Ginseng and Tobacco Research Institute. Ginsenosides were dissolved in saline. Morphine sulfate and naloxone hydrochloride (RBI, Natick) were also dissolved in saline.

2. Surgery and analgesia test

Each rat was implanted with an intrathecal catheter for drugs or vehicle delivery. The intrathecal implantation procedure was performed according to the method of Yaksh and Rudy (1976)[18] with slight modifications. Briefly, rats were anesthetized by intramuscular injection of ketamine (50 mg/kg) and xylazine (1 mg/kg) mixture. A gentamicin sulfate (0.4%)-flushed polyethylene (PE-10) tube was inserted into the rats subarachnoid space through an incision at the cisterna magna. The caudal end of the catheter was gently threaded to the site of spinal lumbosacral segments (about 8 cm from the incision). The rostral end was then secured with dental cement to a screw embeded in the skull. The skin wound was closed by suture. Those rats exhibiting postsurgical neurological disorders (e.g., limb paralysis) were excluded from the experiment. Tail-flick test was performed according to the method of D'Amour and Smith (1941) using a tail-flick meter (Model 7360, Ugo Basile).[19] Radiant heat was adjusted to attain a mean baseline of 5-6 s in controls and heat was automatically terminated at 15 s to avoid tissue damage. Baseline latencies were measured (mean of three trials with three min inter-trial interval) prior to drug administration. The analgesic response was accessed by the tail-flick test 20 min after saline alone or drug administration. At each time point, rats were tested three times (mean of three trials with three min inter-trial interval) and the latencies were averaged to improve accuracy.

3. Drug treatments for tolerance induction

Rats were assigned to 1 of 3 groups after a 7~9 day recovery period as shown in Table 1 according to the procedure of Kellstein and Mayer (1991).[20] For acute or chronic studies, i.t. administration were made in volumes of 20 μl (10 μl of drug followed by 10 μl of saline for flushing) delivered over approximately 30~35 sec with 50 μl Hamilton syringe. Rats were received injection once daily (17:00) for 7 days. In our preliminary experiments, we also found that 10 μg morphine exhibited consistently a greater degree of tolerance than 1 μg morphine treatment in tail-flick response after 7 days when the antinociception was evaluated with 1 μg morphine. For tolerance induction by morphine or for the determination of prevention of

Table 1. Treatment schedule for GS+Morphine

Group	Day 1 Test	Days 2-8 Tolerance induction	Day 9 Test
1	Morphine[a]	Saline+Saline	Morphine[a]
2	Morphine	Saline+Morphine[b]	Morphine
3	Morphine	GS[c]+Morphine[b]	Morphine

[a] Morphine sulfate, 1 μg i.t.
[b] Morphine sulfate, 10 μg i.t.
[c] GS (ginseng saponin), 50, 100, or 200 μg i.t.

morphine induced-tolerance by ginsenosides, we selected all rats showing equivalent analgesia by 1 μg morphine (i.t.) on day 1 test and divided into three groups as shown in Table 1. We also employed the three treatment groups on days 2 through 8 as shown in Table 1.

4. Measurement of withdrawal signs

After day 9 analgesic test with morphine as shown in Table 1, withdrawal was precipitated by administration of naloxone (1 mg/kg, i.p.).[21] We injected immediately rats with 1 mg /kg naloxone on day 9 after analgesia test and observed the appearance of various characteristic withdrawal symptoms noted during the next 60 min.[22] The frequency of expression of withdrawal symptoms and the occurrences (number of episodes) are counted with 15 min blocks.

5. Statistical analysis

For statistical evaluation, the total antinociceptive response of each animal was converted to area under the curve (AUC) using the trapezoidal rule. Statistical comparisons were made using one way ANOVA followed by Dunnetts test. Differences were considered to be statistically significant at $p<0.05$.

Results

1. Antinociception by acute i.t. administered ginsenosides or morphine in rat tail-flick test

We first tested the antinociceptive effect of i.t. administerd ginsenosides in rat tail-flick test. As shown in Fig. 1, ginsenosides did not produce analgesic activity at 50 and 100 μg/rat. However, ginsenosides produced analgesic effect by 30 % at dose of 200 μg/rat compared to saline treated group ($p<0.05$). Using same protocol, we found that i.t.

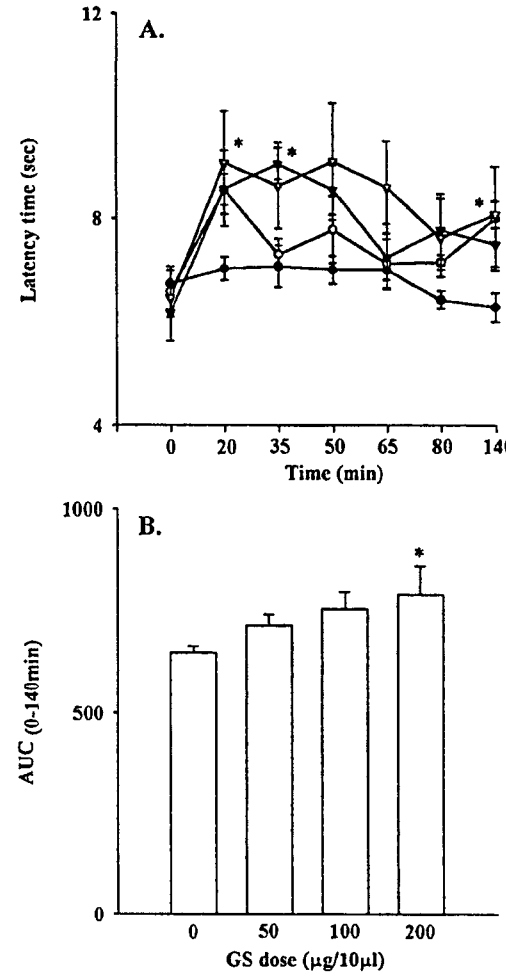

Fig. 1. Effect of i.t. injections of saline or ginsenosides (GS) on nociception in tail-flick test. (A). saline (●), 50 GS (○), 100 GS (▼), or 200 μg GS (▽). (n = 8~10 per dose). Rats responded to a focused-heat stimulus by flicking or removing their tail. Radiant heat was adjusted to attain a mean baseline of 5~6 s in controls and heat was automatically terminated at 15 s to avoid tissue damage. Baseline latencies were measured (mean of three trials with three min inter-trial interval) prior to drug administration. The analgesic response was accessed by the tail-flick test 20 min after saline alone or GS administration with 15 min blocks. Data shown represent mean±SEM. (B). The analgesic response was transformed tail-flick latancy time into area under curve (AUC)±SEM. *$p<0.05$ indicates a significant difference from corresponding value in saline-treated group using one way ANOVA followed by Dunnetts test.

administered morphine did not produce analgesic effect at dose of 0.1 μg/rat but started to produce

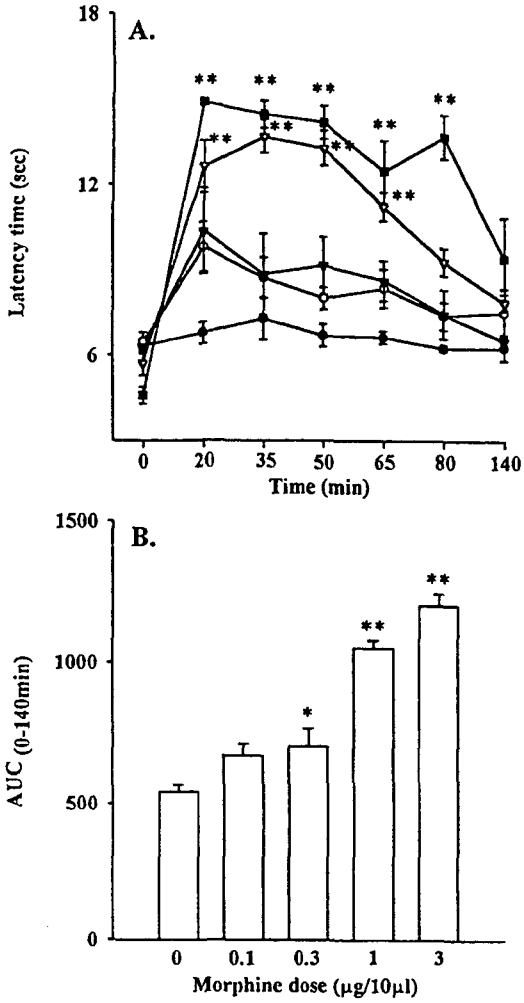

Fig. 2. Effect of i.t. injections of saline or morphine on nociception in tail-flick test. (A). saline (●), 0.1 (○), 0.3 (▼), 1.0 (▽), or 3.0 μg morphine (■). (n=6~8 per dose). Rats responded to a focused-heat stimulus by flicking or removing their tail. Radiant heat was adjusted to attain a mean baseline of 5~6 s in controls and heat was automatically terminated at 15 s to avoid tissue damage. Baseline latencies were measured (mean of three trials with three min inter-trial interval) prior to drug administration. The analgesic response was accessed by the tail-flick test 20 min after saline alone or morphine administration with 15 min blocks. Data shown represent mean±SEM. (B). The analgesic response was transformed tail-flick latancy time into area under curve (AUC)±SEM. *p< 0.05, **p<0.01 indicates a significant difference from corresponding value in saline-treated group using one way ANOVA followed by Dunnetts test.

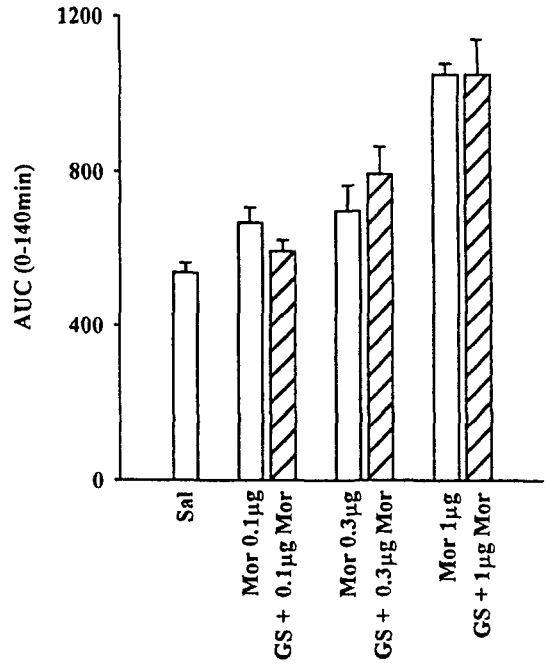

Fig. 3. Effect of i.t. co-administration of ginsenosides (GS) and morphine (Mor) on nociception in tail-flick test. Rats responded to a focused-heat stimulus by flicking or removing their tail. Radiant heat was adjusted to attain a mean baseline of 5~6 s in controls and heat was automatically terminated at 15 s to avoid tissue damage. Baseline latencies were measured (mean of three trials with three min inter-trial interval) prior to drug administration. The analgesic response was accessed by the tail-flick test 20 min after saline, morphine alone, or 200 μg GS+various doses of morphine. The analgesic response was transformed tail-flick latency time into area under curve (AUC)± SEM. (n=6 per group)

analgesic effect at dose over 0.3 μg/rat (Fig. 1A). The analgesic effect of morphine reached almost a plateau at dose of 1 μg/rat (Fig. 2B). Therefore, we used 1 μg morphine for analgesic evaluation to the tail-flick response after chronic morphine treatment. The ED_{50} was 1.20 μg/rat (1.14 ~1.29 μg/rat, 95% C. I.).

2. Antinociception by acute i.t. co-administered ginsenosides with morphine in rat tail-flick test

Because ginsenosides and morphine produce anti-noci-ception in rat tail-flick test as shown in Figs. 1 and 2, we tested whether i.t. co-administration of ginsenosides with morphine enhances the analgesic effect. As shown in Fig. 3, i.t. co-administration of 200 μg ginsenosides with various doses of morphine

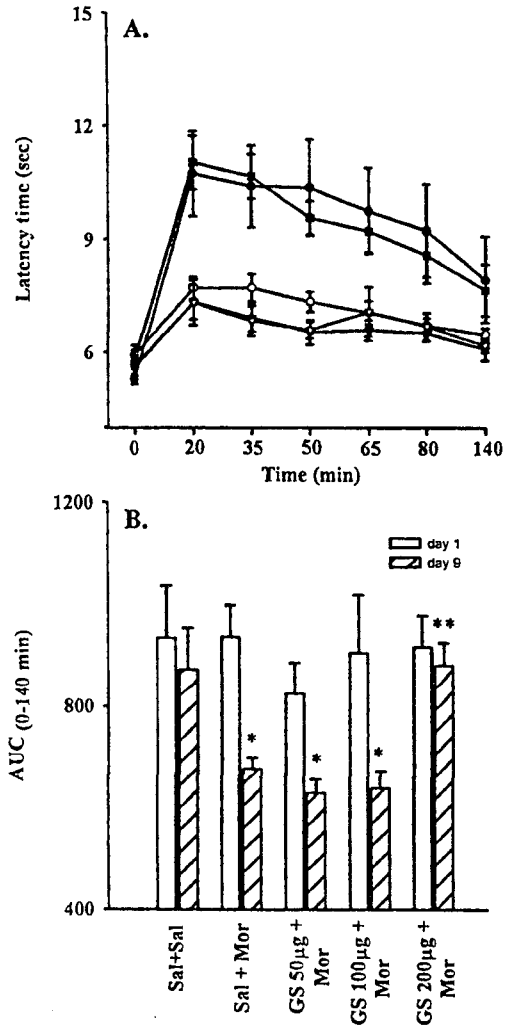

Fig. 4. Effect of ginsenosides (GS) on opioid tolerance by i.t. co-administration with morphine. (A). The evaluation of antinociceptive activity induced by 1 μg morphine on day 9 in animals receiving saline+saline (●), saline+ 10 μg morphine (○), 50 μg GS+10 μg morphine (▼), 100 μg GS+10 μg morphine (▽), or 200 μg GS+10 μg morphine (■) from days 2 to 8. The detailed method for tail-flick test was described in Fig. 1. (B). Within-group comparisons of antinociception induced by 1 μg morphine on day 1 (open bars) to day 9 (hatched bars). *p<0.01 indicates a significant difference from corresponding value in day 1 using one way ANOVA followed by Dunnetts test. **p<0.01 indicates a significant difference from corresponding value in day 9 saline+10 μg morphine group using one way ANOVA followed by Dunnetts test (n=8~11 per group).

did not produce additive analgesic effect compared to morphine alone.

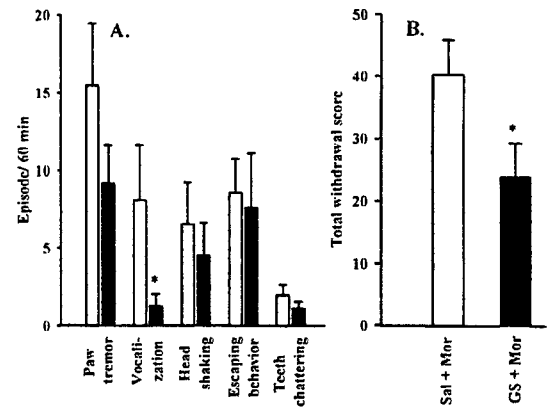

Fig. 5. Effect of ginsenosides (GS) on the precipitated withdrawal signs in morphine-dependent rats. (A). After chronic i.t. treatment with saline+morphine (10 μg, open bars) or GS (200 μg)+morphine (10 μg, closed bars) from days 2 to 8, rats were injected with naloxone (1 mg/kg, i.p.) on day 9 to precipitate withdrawal (n=12 per group). *p<0.05 indicates a significant difference from corresponding value in saline+morphine group using one way ANOVA followed by Dunnetts test. (B). The total withdrawal score for 60 min period of observation after naloxone challenge. *p<0.05 indicates a significant difference from corresponding value in saline+ morphine group using one way ANOVA followed by Dunnetts test.

3. Prevention of morphine-induced tolerance by ginsenosides co-administration

For tolerance induction by morphine or for the determination of prevention of morphine induced-tolerance by ginsenosides, we selected all rats showing equivalent analgesia by 1 μg morphine (i.t.) on day 1 test and divided into three groups as shown in Table 1. We also employed the three treatment groups on days 2 through 8 as shown in Table 1. On day 9 test, we administered all groups with 1 μg morphine for the antinociception evaluation. As shown in Fig. 4A and 4B, administration of 10 μg morphine (i.t.) from days 2 to 8 consistently produced tolerance, i.e., the analgesic effect of 1 μg morphine was significantly less ($p<0.01$) on day 9 than day 1 in rats and was also significantly less than that obtained on day 9 in saline-saline treated group. We investigated whether i.t. co-administration of ginsenosides with morphine prevents tolerance induced by morphine alone. As shown in Fig. 4A, co-administration of 50 or 100 μg ginsenosides with 10 μg morphine from days 2 to 8 did not prevent morphine-induced tolerance. However, co-administra-

tion of 200 μg ginsenosides with 10 μg morphine from days 2 to 8 prevented the development of tolerance induced by 10 μg morphine alone. As shown in Figure 4B, the co-administration of 200 μg ginsenosides but not 50 and 100 μg ginsenosides with 10 μg morphine maintained antinociception in day 9 analgesic test with 1 μg morphine as shown in day 1 analgesic test.

4. Attenuation of morphine-induced dependence by ginsenosides co-administration

We immediately administered all group of animals with 1 mg/kg naloxone (i.p.) prior to a 20 min test after day 9 analgesic test with 1 μg morphine. We checked various characteristic withdrawal symptoms for 60 min. When we measured the frequency of expression of withdrawal signs such as paw tremor, spontaneous vocalization, wet dog head shaking, escape behavior, and teeth chattering, we could observe that i.t. co-administration of 200 μg ginsenosides with morphine significantly attenuated the spontaneous vocalization compared to morphine-treated group (Fig. 5A) (Table 2). Other withdrawal signs were slightly reduced with 200 μg ginsenosides co-administration with morphine. However, in the total withdrawal score comparison, i.t. co-administration of 200 μg ginsenosides with morphine significantly attenuated the withdrawal symptoms (Fig. 5B). These results show that co-administration of ginsenosides partially blocks the development of opioid dependence at the spinal sites.

Table 2. Effects of GS on the naloxone-precipitated withdrawal syndrome in morphine-dependent rats

Withdrawal signs[a]	Saline+Morphine[b]	GS[c]+Morphine
Vacalization	8/11	4/13[d]
Abnormal posture	11/11	8/13
Ejeculation	0/11	1/13
Paw tremor	9/11	11/13
Head shaking	7/11	7/13
Escaping behaivor	9/11	10/13
Yawning	4/11	1/13
Teeth chattering	5/11	3/13
Sniffle	1/11	1/13
Hemorrhage in eyes	2/11	1/13

[a] In morphine-treated animals, withdrawal syndrome was precipitated with nalxone (1 mg/kg i.p.). Next, the presence of various withdraval signs was checked for 60 min.
[b] Morphine sulfate 10 μg i.t.
[c] GS (ginseng saponing) 200 μg i.t.
[d] $p<0.05$ from Morphine group by one-tailed Fisher's exact test.

Discussion

The present studies showed that i.t. administration of ginsenosides produced a weak antinociception compared to morphine in rat tail-flick test (Figs. 1 and 2), although we demonstrated, in previous study, that i.t. administration of ginsenosides relieves biphasic pain induced by formalin in mice.[13] Thus, we observed that the antinociceptive effect of ginsenosides is different depending on pain intensity. These results demonstrated that ginsenosides are more active to attenuate chemical-induced pains than noxious thermal pains.[12,15]

In present experiments, we also observed that acute i.t. morphine produce antinocieption over at dose of 0.3 μg and that the antinociceptive activity of morphine reaches near maximum level at dose of 1 μg (Fig. 2). Interestingly, acute i.t. co-treatment of 200 μg ginsenosides, at which dose ginsenosides produce a weak antinociception, with various doses of morphine did not produce additive antinociceptive activity compared to morphine alone treatment. The reason not exhibiting additive antinociceptive effect after co-administration of ginsenosides with morphine is currently unknown but one possibility is that the antinociceptive effect of ginsenosides might be too weak to affect morphine-induced antinociception in tail-flick test. Interestingly, the previous studies showed that the systemic or i.t. treatment of ginseng extract or ginsenosides antagonizes systemic or i.c.v. morphine-induced antinociception as mentioned above.[5,6,9] However, in the present study i.t. co-administration of ginsenosides with morphine did not antagonize morphine-induced antinociception. This discrepancy between their results and our results is currently unclear and it might require further investigation.

In experiments on the effect of ginsenosides in morphine-induced tolerance development, we showed that chronic i.t. co-administration of ginsenosides with morphine prevents the development of tolerance to the analgesic effects and that i.t. co-administration of gin-

senosides with morphine also partially attenuates withdrawal symptoms precipitated with naloxone. Interestingly, we found, in a dose response experiment, that at least 200 μg ginsenosides are required to block tolerance which develops in response to morphine (Fig. 4). These findings suggest that a larger dose of ginsenosides is required to block morphine-induced tolerance. These results are well consistent with previous reports that chronic systemic treatment of ginsenosides or ginseng extract with morphine attenuates the development of tolerance.[4,6] Moreover, the present study demonstrated that the anti-tolerance effect of systemically administered ginsenosides on systemic opioid could also be mediated via the spinal site.

On the other hand, it could be speculated that the prevention of opioid tolerance by ginsenosides is attributable to residual amounts of ginsenosides in the spinal cord that enhanced morphine analgesia on day 9. However, it seems not the case, since acute i.t. co-administration of ginsenosides (200 μg) and morphine (0.1 to 1 μg) did not enhance morphine induced-anti-nociception (Fig. 3) and even chronic ginsenosides treatment alone also did not significantly affect tail-flick response (data not shown).

Withdrawal symptoms after chronic spinal treatment of opioids are also induced by naloxone treatment.[16] The main withdrawal symptoms included abnormal posture, paw tremor, escaping behavior, spontaneous vocalization, head shaking, teeth chattering and yawning.[16] We could observe that spinal co-administration of ginsenosides with morphine caused slight reductions in the frequency or duration of those withdrawal symptoms but only the spontaneous vocalization was significantly reduc-ed after spinal chronic co-administration of ginsenosides with morphine compared to morphine alone treatment (Fig. 5 and Table 2). These results show the possibility that ginsenosides could modulate expression of tolerance as well as dependence induced after chronic opioid administration. However, chronic treatment with saline or ginsenosides alone did not show the various characteristic withdrawal symptoms induced by naloxone challege in rats (data not shown).

In summary, we found that spinal co-administration of ginsenosides with morphine prevents opioid tolerance and partially attenuates opioid dependence and these results provide additional evidence that ginsenosides mediates the development of opioid tolerance and dependence at the spinal level. Finally, these results show that ginsenosides may be useful adjunct to opioid analgesics in the management of chronic pain at the spinal site. However, future investigation is required for elucidation of spinal inhibition of opioid tolerance and on attenuation of opioid dependence by chronic ginsenosides co-administration with morphine before such clinical application becomes practical.

Acknowledgements

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요 약

백서를 이용하여 진세노사이드 혹은 모르핀을 척수강내 투여한 다음 tail-flick test를 통하여 진통 작용을 연구하였다. 또한, 진세노사이드를 모르핀과 함께 척수강내 장기 처리할 경우 모르핀에 의한 내성 및 의존성 유발에 미치는 영향을 연구하였다. 연구 결과, 척수강내 진세노사이드의 투여는 200 μg /rat에서 약한 진통 작용이 있는 것으로 나타났다. 모르핀은 투여 농도에 의존적으로 좋은 진통 효능을 보여주었으며, ED_{50}은 1.2 μg인 것으로 나타났다. 그러나 진세노사이드의 모르핀을 함께 척수강내 투여할 경우 모르핀의 진통 작용을 증가 시키지 않은 것으로 나타났다. 200 μg/rat 진세노사이드를 10 μg/rat 모르핀을 7일 동안 같이 투여할 경우 모르핀에 의한 통증 작용에 대한 내성을 억제하였으며, 모르핀에 의한 의존성을 부분적으로 억제하는 것으로 나타났다. 이러한 연구 결과는 척수 수준에서 진세노사이드가 모르핀의 장기 투여에 의하여 유도되는 모르핀에 대한 내성 및 의존성을 억제하는 것으로 사료된다.

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