Original Article

Studies on a novel regimen for management of orofacial pain and morphine tolerance

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Abstract *Background/purpose:* The prevalence of orofacial pain is high but the etiology of or-ofacial pain is not well understood. Because of clinical treatment is not so effective, it is urgent to explore novel regimens with more effective and less side effects for clinical application.
Materials and methods: Male mice (ICR strain) were injected with capsaicin ($10 \mu g / 5 \mu l$) in vibrissa pad. Spontaneous orofacial pain in 20 min was recorded after receiving capsaicin to quantify the nociceptive level. Green tea polyphenols (GTP 60 mg/kg), memantine (Mem 10 mg/kg), and GTPm (GTP 30 mg/kg plus Mem 3 mg/kg) were dissolved in 2% carboxymethyl cellulose, which was orally administered to mice twice per day and five times per week consecutively for 2 weeks. TruScan photobeam tracking was used to record changes of behavior and locomotor activities.
Results: GTPm by itself attenuated orofacial pain induced by capsaicin. Moreover, GTPm enhanced morphine analgesic effects, reduced morphine depressant side effects and delayed morphine tolerance. Along with this experiment, GTPm was tested on the hot plate (52 °C)-induced peripheral thermal pain. It was found that both memantine and GTPm reduced morphine-analgesia in hind paw thermal pain.
Conclusion: In this study, GTP (60 mg/kg/day) orally administrated produced a significant analgesic effect on capsaicin—induced orofacial pain. Memantine combined with GTP synergistically not only reduced orofacial pain but also enhanced morphine analgesic effects. Thus, a new regimen of GTPm orally administered twice per day attenuated orofacial pain after consecutive 5 days.

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Introduction

It is estimated that the prevalence of orofacial pain is high, up to 26% of the general population in the world suffered from this unbearable disease.[1,2] Anxiolytics (opioids and tricyclic antidepressants) and anti-inflammatory agents (NSAIDs and corticosteroids) are the most commonly prescribed medications for orofacial pain, and opioids are the most common-prescribed drug from dentistry. Both groups of medications show a little effective in reducing client's pain; However, those medications have shown several side effects, such as sedation, dizziness, nausea, vomiting, constipation, physical dependence, tolerance, and respiratory depression, which may cause additional health problem. Therefore, medication with effective symptom relief and minimal side effects will be needed.

Recent studies revealed that injury and inflammation increase the release of allogeneic substances which include cytokines (e.g. prostaglandin, interleukins 1β(IL-1β), IL-6, IL-8, tumor necrosis factor alpha), bradykinin, serotonin, norepinephrine, neuropeptides (substance P, calcitonin gene-related peptide (CGRP)), and protons that lowers the pH in orofacial area.[3] The orofacial areas are innervated by trigeminal primary afferents fibers which project to trigeminal subnucleus caudalis via trigeminal ganglion. These afferent fibers with free nerve endings, are termed nociceptors, respond to noxious mechanical and/or chemical stimuli. These nociceptors have receptors such as serotonin (5-HT3 receptor), glutamate (NMDA receptor), capsacin (transient receptor potential vanilloid 1 (TRPV1)), adenosine triphosphate (P2X receptor), and neuropeptides.[4-6] The activation of these receptors gives rise to peripheral sensitization and orofacial pain.[7] Lastly, persistent nociceptive input from peripheral afferent nerve can contribute to the development of central sensitization, which is the result of chronic orofacial pain.[4]

Thus, it is apparent that inflammation, oxidative stress, and glutamate especially NMDA receptors play an important role in orofacial pain. Therefore, we postulate that the strategy for better management of orofacial pain must be multifunctional encompassing anti-inflammation, anti-oxidation and NMDA receptor antagonists. Green tea polyphenols (GTP) possess antioxidant, anti-inflammation, and neuroprotective functions through signal transduction modulation such as inhibition of NFκB signaling, suppressing nitric oxide (NO) production and free radical scavenge.[8-12] Memantine is an uncompetitive antagonist of NMDA receptor which modulated trigeminal neuropathic pain by functional coupling with TRPV1 at trigeminal ganglion of spinal cord.[13] Although memantine has benefits in orofacial pain treatment, but severe side effect such as hallucination and delirium limit the use in clinic.[14,15] Hence, we use combination of different mechanisms of drugs to effect symptom relief and minimize side effects. We proposed that combination of GTP with memantine (GTPm) might have analgesic effect on orofacial pain. In this study, we also investigated the analgesic effect of memantine used alone and GTPm with or without morphine, which is commonly used for treatment of orofacial pain, but morphine induced unbearable side effects (depression, constipation, respiratory inhibition) and

morphine tolerance.[16-18] Thus, we tried in this study to test whether GTPm was capable of attenuating morphine tolerance which could be precipitated by the μ-opioid receptor antagonist naloxone.[19] Additionally, we wanted to know whether the anti-nociception effects of orofacial region and peripheral tissue of body are the same. Thus, we may identify different pain etiology and regulation between trigeminal distribution area and peripheral nervous system of the body.

Materials and methods

Animals

Adult male ICR (Institute for Cancer Research) mice 8-weeks old supplied by local LASCo were used in this study. All mice were housed in groups of 5–7 in a cage with the same strain mates, in the animal faculty of the Chung Shan Medical University. Mice were allowed free access to food and water in a temperature-controlled (22 ± 1.5 °C) and relative humidity 50–70% environment maintained on a 12/12 h light/dark cycle (light on 07:00 to 19:00). The experiment protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Chung Shan Medical University Experimental Animal Center Approval No.1777.

Drugs

Memantine, capsaicin, morphine HCL, naloxone HCL were purchased from Sigma–Aldrich Co. (St. Louis, Mo, USA).

Preparation of green tea polyphenols (GTP)

One hundred grams of Chinese green tea, Longjing tea (produced by Wangs' Tea Enterprise Co., Ltd., Taipei, Taiwan), was suspended in 1 L of distilled water at 75 °C for 30 min; then the supernatant was collected. This step was repeated three times. The supernatant was filtered to eliminate chlorophylls and undissolved particles. The total aqueous layers were concentrated to 0.5 L under reduced pressure using a rotary vacuum evaporator. The concentrated solution was extracted with an equal volume of chloroform three times to eliminate caffeine and pigments. The remaining aqueous phase was then extracted with an equal volume of ethyl acetate three times to extract tea polyphenols. The ethyl acetate was combined and evaporated in vacuum. The residue was dissolved in a small volume of distilled water and freeze-dried. This golden brown solid matter was called green tea polyphenols.

Preliminary study

Green tea polyphenols (GTP 60 mg/kg), memantine (Mem 10 mg/kg), GTPm (GTP 30 mg/kg plus Mem 3 mg/kg) was dissolved in redistilled water, which was orally administered to mice once per day and five times per week consecutively for 4 weeks as described in our previous report.[20] Capsaicin (10 μg/5 μl) was subcutaneously

injected in vibrissa pad of mice and induces spontaneous orofacial pain response, including 3 distinct patterns of acute grooming behaviors: fore-paw rubbing, lower lip skin/cheek rubbing against enclosure floor and hind paw scratching.[1] We recorded the total times of orofacial pain responses in 20 min after receiving capsaicin to quantify orofacial nociceptive level.

Drug treatment of mice

The mice were divided into 6 groups: (1) the control group (n = 6); (2) the memantine group (n = 5); (3) the GTPm group (n = 5); (4) the morphine group (n = 7); (5) the morphine plus memantine group (n = 7); (6) the morphine plus GTPm group (n = 6). The dosage and protocols of drug administrations were shown on Table 1.

Nociceptive assay

Orofacial pain response induced by capsaicin and peripheral hot plate thermal pain test were carried out to assess the analgesic effect of the drugs in mice. Capsaicin selectively activates TRPV1 receptor of the trigeminal nerves, which plays a key role in regulating nociception.[23] In this study, we recorded the total times of orofacial pain response in 25 min after receiving capsaicin to quantify orofacial nociceptive level on days one, five and ten. The hot plate test was used to assess the effects of memantine, GTPm, morphine, and naloxone on the thermal nociceptive threshold of mice. Each mouse was placed on a 52 ± 0.5 °C hot plate to induce thermal pain. The response to either a lick of hind paw or a jump was recorded. In the absence of a response within 50 s, the animals were removed from the hot plate to avoid tissue damage.[24] The hind paw withdrawal latency was measured on days two, four and nine (50 min after the injection of morphine). On day eleven, hot plate thermal pain test was measured before and after the injection of naloxone.

TruScan photobeam tracking

TruScan photobeam Tracking are used to record behavior (walking distance in margin and center area, number of times for jumping, rest time and total time of walking) of mice to compute emotional alteration. The tracking activity of depressed mice exhibits limited center area walking distance; while normal mice distributes equally in margin and center area walking distance. Additionally, the tally of jumping and standing show the exploration and curious behavior of normal mice. One of the side effects of morphine is depressed mood, which causes reduction in locomotor activity and exploratory behavior. If memantine and GTPm can improve this side effect, locomotor activity and exploratory behavior will increase and the tracking in margin and center area will be equally distributed. TruScan photobeam Tracking will be measured 20 min after the injection of morphine on day nine. On day eleven, TruScan photobeam Tracking will be measured 5 min after the injection of naloxone.

Statistics

Results for each experiment were represented as mean $\pm$ SEM. One way ANOVA followed by a post-hoc t test was used to evaluate differences between the groups. The level of significance was defined as $p < 0.05$.

Results

In the preliminary study, both GTP alone and GTPm but not memantine were almost eqi-effectively to attenuate orofacial pain as shown on Fig. 1. It was noted that the dose of GTP in GTPm was about half of that used alone and that of memantine in GTPm was one third of that used alone.
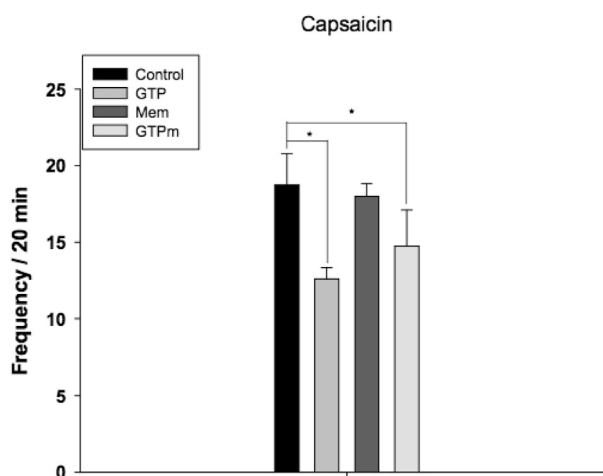
Table 1 The dosage and protocols of drug administrations.

Group	Drug	Day										
		1	2	3	4	5	6	7	8	9	10	11
1,4	Vehicle	+	+	+	+	+	+	+	+	+	+	+
2,5	Memantine[a]	+	+	+	+	+	+	+	+	+	+	+
3,6	GTPm[a]	+	+	+	+	+	+	+	+	+	+	+
4,5,6	Morphine[b]	−	−	−	−	−	−	−	−	+	+	+
1−6	Naloxone[c]	−	−	−	−	−	−	−	−	−	−	+

[a] Memantine (10 mg/kg) or GTPm (combination of GTP 30 mg/kg with memantine 3 mg/kg) are dissolved in 2% carboxymethyl cellulose (CMC) and applied on bilateral buccal mucosa of mice twice daily (morning and afternoon) for consecutive eleven days.
[b] Morphine was intraperitoneally injected twice per day (9 AM and 4 PM) for consecutive three days from day 9 (7.5 and 15 mg/kg), day 10 (30 and 30 mg/kg), day 11 (7.5 and 22.5 mg/kg) respectively.[21]
[c] Naloxone (1 mg/kg) was intraperitoneally injected 1 h after injection of morphine.[22]

Figure 1 Anti-nociceptive effects of green tea polyphenols, memantine either alone or in combination on capsaicin-induced orofacial pain in mice. GTP, memantine, either alone or in combination GTPm was orally administered to mice once per day and five times per week consecutively for 4 weeks. Capsaicin (10 μg/5 μl) was subcutaneously injected at vibrissa pad to induce orofacial pain. Note that both GTP alone and GTPm were effectively to attenuate orofacial pain.

Therefore it is expected that GTPm would produce fewer side effects than GTP alone. In order to induce an analgesic effect in a shorter period, we prepared memantine and GTPm in 2% CMC and administered in bilateral buccal mucosa twice per day. The results shown on Fig. 2A and B revealed that Memantine and GTPm exerted differential effects on capsaicin-induced orofacial pain (Fig. 2A) and peripheral thermal pain of hind paw induced by hot-plate (52 ± 0.5 °C, Fig. 2B).Both memantine and GTPm exhibited analgesic effect by decreasing orofacial pain responses, frequencies of rubbing and scratching induced by capsaicin on day 5 (Fig. 2A).By contrast, neither memantine nor GTPm had effects on hot plate thermal pain of hind paw on day 4 (Fig. 2B).Furthermore, both memantine and GTPm alleviated the analgesic effect of morphine by shortening the paw withdrawal latency on day 9 (Fig. 2B).Morphine
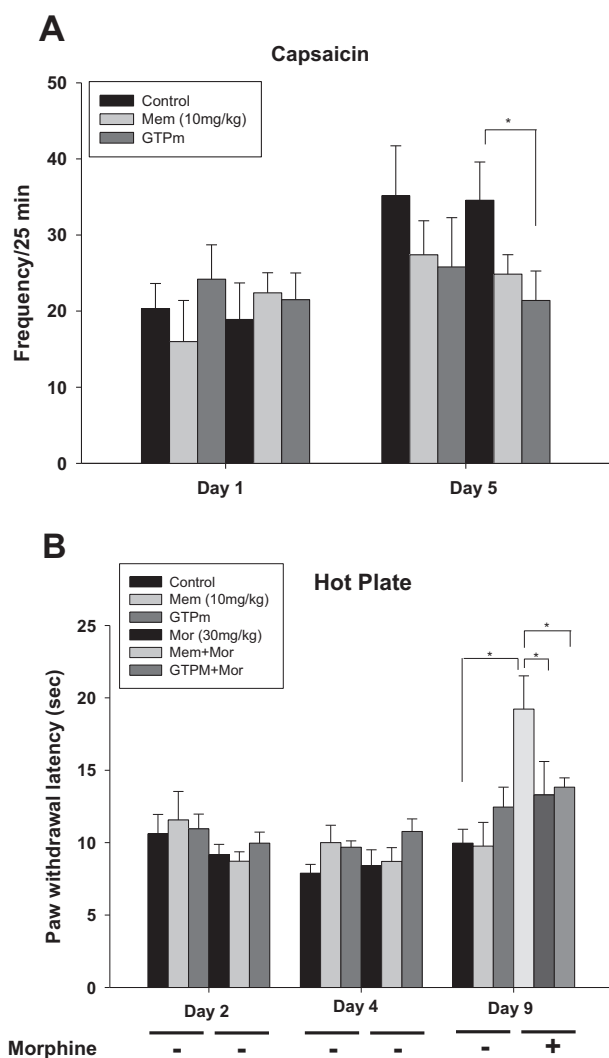
significantly prolonged hind paw withdrawal latency which was reduced by both memantine and GTPm (Fig. 2B). After ten days treatment with memantine or GTPm, capsaicin-induced orofacial pain was reduced by GTPm (Fig. 3A) and the anti-nociceptive effect of morphine was significantly enhanced by memantine and GTPm (Fig. 3B). By contrast, both memantine and GTPm still had no effect on thermal pain even after consecutive eleven days administration but both significantly reduced the analgesic effect of morphine on hind paw test (Fig. 4A). Naloxone was intraperitoneally injected which effectively abolished analgesic effect of morphine but only partially reduced the analgesic effect induced by morphine plus GTPm in group 6 (Fig. 4B). Naloxone-induced hyperactivities were significantly higher in morphine-treated mice which were markedly reduced by GTPm but not by memantine (Fig. 5), suggesting a possibility that GTPm attenuated morphine tolerance. After consecutive nine days and eleven days respectively, drug treatments of either memantine or
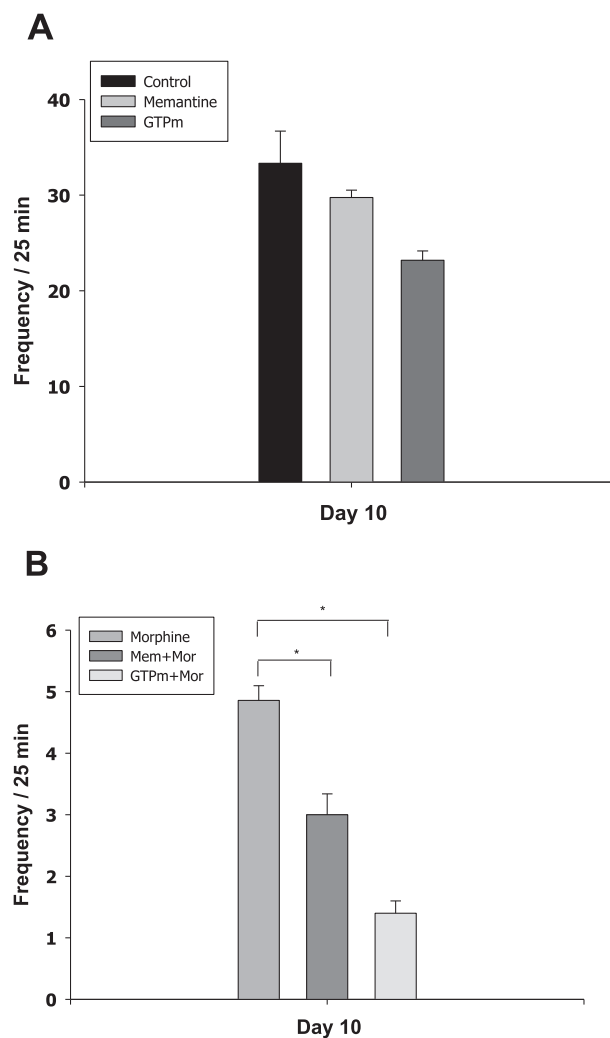

Figure 2 Effects of memantine and GTPm on orofacial pain and hind paw thermal pain in mice. Note that GTPm significantly attenuated the capsaicin-induced orofacial pain after treatment for five days (A) but had no effect on hind paw thermal pain after nine days treatment. Morphine administered on day 9 significantly prolonged hind paw withdrawal latency (B) which was attenuated by both memantine and GTPm.

Figure 3 Enhancement by GTPm of morphine-analgesic effect on capsaicin-induced orofacial pain in mice. After ten days treatment with memantine or GTPm, capsaicin-induced orofacial pain was significantly alleviated by GTPm (A) and anti-nociceptive effect of morphine was significantly enhanced by memantine and GTPm (B).
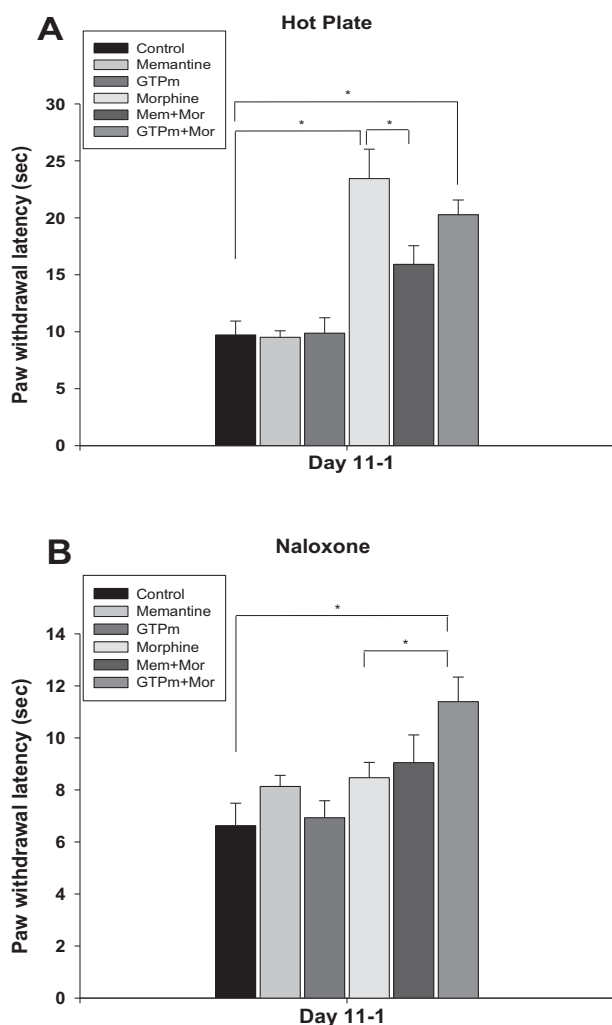
A Hot Plate

B Naloxone

Figure 4 Effects of memantine and GTPm either alone or in combination with morphine on thermal pain in mice without (A) or with naloxone treatment (B). Mem and GTPm significantly attenuated analgesic effect of morphine (A). Naloxone (1 mg/kg) effectively abolished analgesic effect of morphine but only partially reduced that indued by combined morphine plus GTPm (B).

GTPm did not significantly altered the locomotor activities of the mice including total distance (Fig. 6A), total jump (Fig. 6B) and rest time (Fig. 6C). In addition, except that the initial dose of morphine (7.5 mg/kg, M1 on day nine) had no effect on total distance (Fig. 6A) and rest time (Fig. 6C) but significantly decreased total jump frequencies (Fig. 6B). Furthermore, three consecutive treatments of morphine (30 mg/kg) significantly decreased total distance and jump frequencies but increased the rest time which were exaggerated by memantine but attenuated by GTPm, suggesting that GTPm suppressed naloxone-induced morphine withdrawal symptom.

Discussion

The high incidence and poor treatment of orofacial pain still awaited for our effort to elucidate the mechanisms and
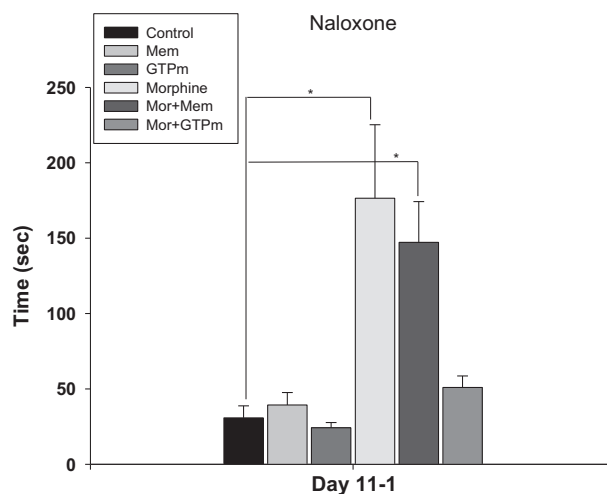

Figure 5 Effects of memantine and GTPm on naloxone-induced hyperactivities of morphine-treated mice. Naloxone markedly induced hyperactivity of morphine-treated mice. GTPm but not memantine alleviated hyperactivities of the morphine-treated mice.

develop a newer effective drug.[2] Recent studies pointed out multifactorial etiology of orofacial pain including neuroinflammation and activation of NMDA receptors.[3,7] In the present study, we provided evidence for the synergistic antinociceptive efficacy of a novel regimen (GTPm) that combined the anti-inflammatory GTP and an uncompetitive NMDA receptor antagonist memantine in alleviating capsaicin-induced orofacial pain in mice. This finding suggests that the natural phytopolyphenols combined with the NMDA receptor antagonists can be a potential therapeutic agent for orofacial pain. Although GTP alone exhibited antinociceptive effect as well as that of GTPm (Fig. 1). However, dose of GTP was twice as that in GTPm. Morever, Memantine dose (3 mg/kg) in GTPm is one third in memantine alone (10 mg/kg). Therefore, the advantage of lower doses of GTPm would be expected to be safer as we pointed in our previous report.[20]

One of the important findings of this study is that GTPm is not only effective in reducing capsaicin-induced orofacial pain but also enhanced analgesic effect of morphine (Fig. 3). Morphine is an effective analgesic frequently used in dentistry. However, the side effects of morphine (tolerance, addiction, depression, constipation) often cause discontinuous application of morphine. In this study, we have found that GTPm not only enhance analgesic effect of morphine on orofacial pain but also reduced morphine-decreased locomotor activities and increased rest time (Fig. 6A and C), suggesting that GTPm attenuated morphine tolerance liability. Furthermore, in our unpublished data, GTPm exhibited biphasic effects which synergistically reduced hind paw thermal pain when using low-dose (3 mg/kg) of morphine, but antagonized morphine analgesic effect during effective dose of morphine (10 and 30 mg/kg). The mode of action of such modulatory effect of GTPm on morphine analgesia is continuously investigated in our laboratory. However, in the peripheral hot plate thermal pain (52 °C) model test, GTPm had no analgesic effect but suppressed morphine analgesic effect (Fig. 2B). This finding is in agreement with the report that
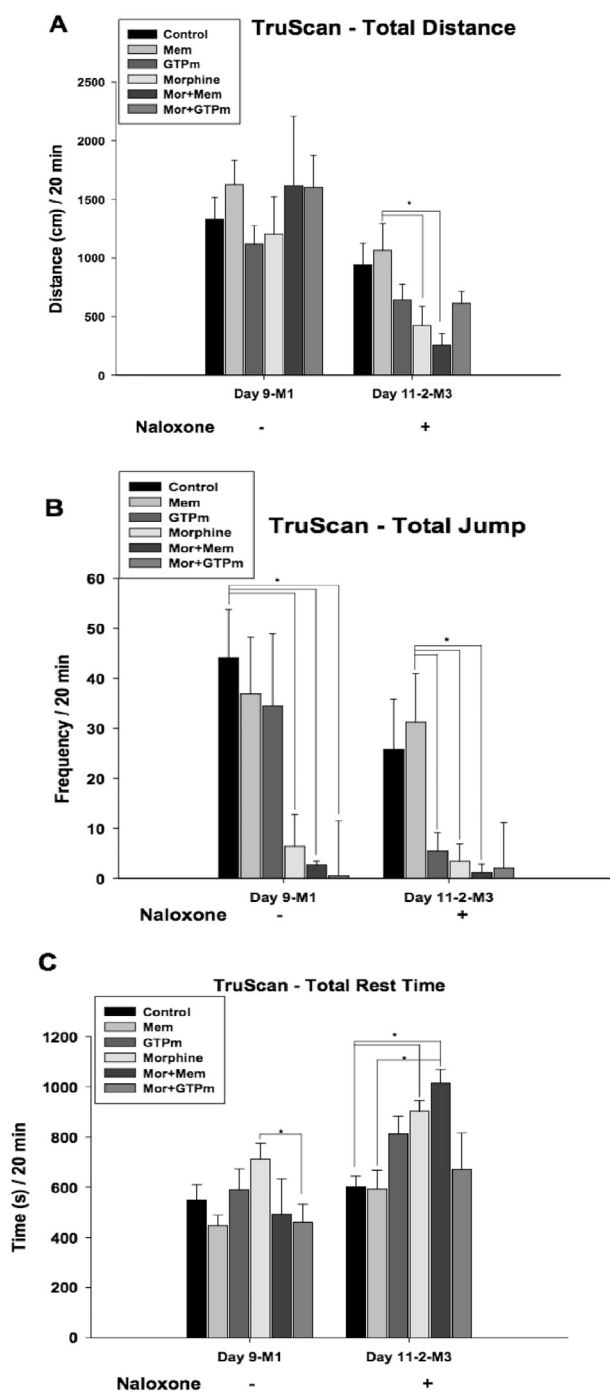
Figure 6 Effects of memantine, GTPm either alone or in combination with morphine on locomotor activities of mice. Neither memantine (Mem) nor GTPm has effect on the locomotor activities including total distance (A), total jump (B) and rest time (C). GTPm apparently inhibited naloxone-induced morphine withdrawal symptoms in the decrease of total ambulatory distance (A) and the increase in rest time (C).

NMDA receptor antagonist at glycine site, HA-966 markedly potentiated anti-allodynic effect of 5HT1B/1D receptor agonist dihydroergotamine and zolmitriptan on orofacial pain induced by chronic constriction injury to infraorbital nerve but not that allodynia at hind paw induced by peripheral

sciatic nerve injury.[25,26] These findings in animal studies are in accordance with the reports of clinical patients that classical analgesic drugs for peripheral neuropathic pain are usually poorly effective against orofacial pain, suggesting that the mechanisms underlying peripheral neuropathic pain may be different from those in orofacial pain. We proposed that activation of NMDA receptors of trigeminal nerve directly enhanced orofacial pain but the peripheral sciatic nerve projection to spinal cord where the activation of NMDA receptors perhaps facilitated the release of GABA at interneurons as suggested by Li et al. (2015)[27] and Petrus et al. (2009).[28] Further studies are needed to elucidate this important issue.

Recent studies on the possible mechanisms of morphine analgesic tolerance indicated that increased production of nitric oxide and free radicals played important roles.[16—18] In this study, GTPm markedly suppressed naloxone-induced hyperactivities (Fig. 5) and then lowering the locomotor activities (Fig. 6) of morphine-treated mice. This result obtained is in accordance with our proposal that GTP alleviated the induction of morphine tolerance mediated by its pleiotropic properties of anti-oxidation, decreased NO production and suppression of NFκB signaling.[8—12,20]

There are many kinds of animal model of orofacial pain. Capsaicin-induced orofacial pain is frequently adopted for studying the possible etiology and development of newer drugs for orofacial pain. Capsaicin is known to be an antagonist of transient receptor potential vanilloid (TRPV1), which plays an important role in trigeminal neuropathic pain. TRPV1 has been found to be functionally interacted with NMDA receptors.[13,15,29] Therefore, memantine is effective to alleviate capsaicin-induced orofacial pain. Other models of orofacial pain such as chronic constriction injury of infraorbital nerve and inflammatory-chemicals (formaldehyde, carrageenan and Complete Freund Adjuvant) injection on vibrissae pad have been reported. It needs to test the efficacy of our newer combination regimen in other models of orofacial pain.

In conclusion, we have demonstrated that the novel regimen of combined GTP with low dose of memantine (3 mg/kg) not only synergistically attenuated capsaicin-induced orofacial pain in mice but also enhanced analgesic effect of morphine and reduced morphine side effects in orofacial pain. It is expected that such novel regimen of natural phytopolyphenols with low dose of memantine may be a potential effective analgesic for orofacial pain therapy.

Conflicts of interest statement

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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