Oxymatrine Inhibits Development of Morphine-Induced Tolerance Associated With Decreased Expression of P-glycoprotein in Rats

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Abstract
The effect of oxymatrine on the development of tolerance to the antinociceptive effects of morphine was investigated in rats. The degree of tolerance was assessed using the tail-flick test before and after 6 days of twice daily administration of oxymatrine premorphine (10/20/30 mg/kg). High doses of oxymatrine inhibited the development of morphine tolerance (resembling the effect of 7.5 mg/kg of the NMDA receptor antagonist memantine) while also increasing the antinociceptive effects. A high dose of oxymatrine (30 mg/kg) also significantly inhibited the dramatic increase in expression of morphine-induced P-glycoprotein (P-gp), an ATP-dependent efflux pump acting at the blood–brain barrier, by Western blot analysis. Furthermore, these studies suggest that P-gp modulates the development of morphine tolerance while not affecting the magnitude of the analgesic effect of morphine. These results imply that oxymatrine prevention of the development of tolerance of morphine may be related to a considerable inhibition of P-gp expression. In contrast, the authors’ data suggest that the mechanism of oxymatrine enhancement of morphine’s analgesic effects is not associated with increase in the level of expression of P-gp. However, they believe that their findings can be used by researchers to develop therapies that will allow patients to take morphine without becoming tolerant of its benefits.

Keywords
Sophora japonica, oxymatrine, morphine, tolerance, P-gp

Introduction
Morphine has been used for cancer pain control, especially in patients who have little or no pain relief, who suffer more intolerable side effects, or who are in advanced stages of disease.¹ Morphine doses can be increased when previous doses are no longer as effective; however, this is associated with many side effects, such as pruritus, nausea, vomiting, urinary retention, respiration depression, and tolerance.² Morphine tolerance is a significant obstacle to effective pain relief in chronic pain in cancer patients.³

Recent studies revealed that inherited differences in drug transporters (eg, P-glycoprotein [P-gp]) play a critical role in the development of morphine tolerance.⁴ P-gp is an ATP-dependent efflux pump acting at the blood–brain barrier, which is a major determinant of morphine bioavailability and influences the clinical efficacy of morphine.⁵ Repeated doses of morphine cause the expression of P-gp to be significantly increased in the brain tissue and capillary endothelial cell membranes, which partly results in an attenuated analgesic effect, as found using P-gp knockout mice, indicating that the development of morphine tolerance may be prevented by changing the levels of P-gp expression.⁵⁻⁸

Oxymatrine is an alkaloid extracted from the Chinese herb Sophora japonica L. (Fabaceae; syn. Sophora flavescens Ait.) with activities of anti-inflammation, inhibition of immune reaction, and antiviral effects, and it especially has the capacity to inhibit P-gp expression.⁹,¹⁰ Our clinical studies have shown potentially quite large effects—in terms of maintaining the pain-relieving effect—of a combination of oxymatrine and morphine during prolonged use while also reducing their side effects.

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effects, suggesting that oxymatrine involves opioid interaction (see discussion below). In the present work, the ability of oxymatrine to affect the development of morphine tolerance through changing morphine induced upregulation of P-gp expression was investigated.

To achieve this goal, we have explored the effect of administration of oxymatrine premorphine in the development of morphine tolerance as well as the effect of oxymatrine on the expression of P-gp in naive and dependent animals.

Materials and Methods

Animals

Adult male Wistar strain rats (150-180 g) were obtained from Laboratory Animal Center (Tianjin, China). The animals were housed at a temperature of 23°C to 24°C with a 12-hour light–dark cycle (lights on 8:00 AM to 8:00 PM). Food and water were available ad libitum. The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, adopted by The Chinese Pharmacological Society.

Administration

Morphine sulfate (Sigma, St Louis, MO) and oxymatrine (Green Valley Pharmaceutical Co Ltd, Shanghai, China) were injected subcutaneously 30 minutes before the tail-flick assays in doses of 10 mg/kg and 10, 30, and 50 mg/kg, respectively. Systemic injection was attempted. For morphine-induced tolerance experiments, morphine was administered by an intramuscular injection. Memantine HCl (Merz & Co, Germany) was dissolved in sterile physiological saline that served as a placebo. All drugs were made fresh the day before the experiment and stored at −4°C in the refrigerator. The dose of morphine is expressed as the base and the doses of other compounds as their respective salts.

Morphine Analgesia

For the morphine tolerance and analgesic effect studies, we performed the tail-flick assay using the 52°C water tail-immersion approach. All tail-flick results were expressed as the percentage of maximum possible effect, that is %MPE, equal to (postdrug latency−predrug latency)/100/cutoff time−predrug latency. The tail flick was applied prior to and every 15 minutes following morphine administration over a 120 minute observation period. Time−antinociception (%MPE) curve was constructed, and the area under the curve (AUC) was calculated from time versus %MPE. The morphine antinociceptive analysis was performed close to the same time of day for all respective groupings. MPE values were also used to construct morphine cumulative dose–response curves by nonlinear regression; these curves were used to calculate antinociceptive ED50 values using GraphPad Prism version 3.00 (GraphPad Software, CA) software.

Western Blot Analysis for P-gp Expression

After analgesic analysis, rats were decapitated, and their brains were removed. A part of the forebrain was dissected and immediately lysed in SDS-sample buffer (50 mM Tris–HCl, pH 6.8, 2% SDS, 10% glycerol), boiled, and reduced with β-mercaptoethanol. Samples (20 μg) and molecular weight standards (BioRad) were electrophoresed in 7.5% SDS-PAGE acryl amide gels and transferred to a Polyvinylidene fluoride (PVDF) membrane (Bio-Rad, Hercules, CA). The membranes were blocked for 2 hours at 25°C with 5% skim milk in TBS (TBS buffer: 20 mM Tris-HCl, pH 7.5; 150 mM NaCl), incubated with a rabbit anti-P-gp antibody (1:100; Invitrogen Carlsbad, CA) and a rabbit anti-β-actin antibody (1:1000; Pierce Chemical, Rockford, IL) overnight at 4°C and then reacted with horseradish peroxidase–conjugated antirabbit IgG (Sigma) for 2 hours at 25°C. Immunoreactive proteins were detected by chemoluminescence (ECL kit; Amersham, UK). On immunoblots, the antibodies recognize a 170 kD protein in the particulate fraction.

Statistical Analysis

The results are expressed as the mean ± standard error. Differences between the individual mean values in different groups were analyzed by 1-way analysis of variance (ANOVA) with Tukey’s test as a post hoc analysis. Differences of P < .05 were considered significant.

Results

Effects of Oxymatrine on the Development of Morphine Tolerance

There were no differences in premorphine ED50 values among groups (Table 1). Treatment with 10 mg/kg twice daily of morphine produced a 7.73-fold increase in the ED50 values as determined postmorphine. In contrast, pretreatment with 20 or 30 (but not 10) mg/kg of oxymatrine given prior to each dose of morphine attenuated the development of morphine tolerance. The effects of oxymatrine were related to the dose. This was evidenced by a significant decrease in both postmorphine ED50 values (statistically significant for the dose 30 mg/kg) and antinociceptive morphine fold shifts of oxymatrine for the doses of 30 mg/kg, as compared with the control group that received placebo + morphine (Table 1). Administration of oxymatrine premorphine decreased the tolerance of morphine significantly (P < .05) as compared with rats treated with and morphine + saline. Similarly, memantine (7.5 mg/kg) produced an inhibition of morphine tolerance.
Effects of Oxymatrine on Reverse of Morphine Tolerance

To determine whether the oxymatrine could reverse morphine tolerance, we also assessed morphine analgesia after tolerance was developed. Group (A) rats from Table 1, which had developed morphine tolerance, were randomly assigned to 2 groups of 8 animals each. For another 5 days, they were treated at a dose of 10 mg/kg twice daily morphine or 30 mg/kg of oxymatrine given prior to each dose of morphine. The analgesic effect, presented as the %MPE after vehicle administration, was similar between group (A1) and group (A2). See Figure 1A. The least-squares slope of the %MPE across the later 5 days showed a significant difference between the 2 groups (Figure 1B). The effect of administration of oxymatrine in tolerant rats indicated the ability of oxymatrine to reverse the development of morphine tolerance. Thus, oxymatrine could lead to new therapies that allow morphine to be administered without patients becoming tolerant of it.

Oxymatrine Effect on the Tail-Flick Response and Analgesic Effect of Morphine

To determine whether oxymatrine might itself produce an analgesic effect and/or affect the antinociceptive effects of morphine, we treated animals at a dose of 30 mg/kg twice daily of oxymatrine with saline for 7 days and compared them with group B. Analysis of AUC revealed that treatment with oxymatrine (30 mg/kg) + placebo produced longer tail-flick responses compared with placebo + placebo treatment (Figure 2A). Moreover, the results indicate that oxymatrine, at the dose effectively inhibiting development of morphine tolerance, produced an antinociceptive effect in the tail-flick test, which is in agreement with our clinical study (Figure 2B). Furthermore, morphine + oxymatrine showed marginally higher analgesic effect than morphine alone (statistically significant for 120 and 150 minutes). However, the AUCs showed no statistical significance between them, indicating that this dose of oxymatrine enhanced the antinociceptive effects of morphine compared with treatment with morphine alone.

Expression of Cortical P-gp: Correlation Between Morphine Analgesia and Morphine Tolerance

To assess whether oxymatrine administration may result in an alteration of P-gp expression in the brain that may subsequently
analgesic effect than morphine alone (P < .05). Figure 3A shows the time courses of tail-flick responses to morphine alone and in combination with oxymatrine. Morphine + oxymatrine showed significantly higher analgesic effect than morphine alone (P < .05) at 120 and 150 minutes. The number is given in parentheses. Inset: presented are mean SEM AUC values calculated with the same data. One-way ANOVA and post hoc Newman–Keul’s test revealed that the treatment with oxymatrine (30 mg/kg) + placebo differed significantly (P < .05) from placebo + placebo treatment.

Abbreviations: SEM, standard error of the mean; AUC, area under the curve; %MPE, percentage of maximum possible effect.

Discussion

Oxymatrine is receiving increasing interest in the West, with studies focusing on cancer, ischemia-reperfusion, and hepatitis B in laboratory animals, culture studies, and so on. The matrine alkaloids—matrine and oxymatrine—have been a focal point of Chinese medical research into the treatment of cancer and were reported to be quite helpful for eliminating multidrug resistance in cancer therapy. The inhibitory effect of oxymatrine on P-gp expression may support the body’s natural cellular sensitivity to therapeutic agents, thus, preventing excessive P-gp pump activity and also improving the success rate of patients undergoing chemotherapy. Based on our clinical evidence, we hypothesized that oxymatrine may attenuate the morphine tolerance by abolishing the upregulation of the morphine-induced P-gp expression and the intrinsic individual differences of brain P-gp expression levels.

In this study, we found that preadministration of oxymatrine with morphine greatly attenuated the development of morphine tolerance. Moreover, at the dose effectively inhibiting development of morphine tolerance, oxymatrine produced antinociceptive effects compared with saline, which indicates that oxymatrine has the ability to both reduce the development of tolerance and enhance morphine analgesia.

Recent investigations have demonstrated that morphine’s analgesic effects are considerably dependent on P-gp expression, suggesting that induction of P-gp is one mechanism involved in the development of morphine tolerance. Chronic morphine exposure could increase P-gp in the rat brain, reducing morphine’s pharmacological activity, which can be altered in the presence of P-gp inhibitors. In other words, it is considered that pretreatment with a P-gp inhibitor enhances morphine antinociception in rats.

In this study, we examined the effect of oxymatrine on morphine-induced P-gp expression in rats. The data obtained show that P-gp was markedly decreased by preadministration of oxymatrine in morphine-treated rats. Furthermore, P-gp affects the bioavailability of many oral chemotherapy agents and can induce multidrug resistance. These results are consistent with the observations that oxymatrine has P-gp inhibitory action resulting in partial reversal of multidrug resistance.

Because the administration of oxymatrine alone failed to reduce significantly the P-gp expression in rats, it is probable that the inhibitory effect of oxymatrine on the expression of P-gp may result from the advanced induction of morphine. It has been shown that matrine produces an antinociceptive effect mainly through the activation of κ-opioid receptors and partially through μ-opioid receptors. It is possible that the analgesic property of oxymatrine is related to the other activity of some of its components. Several reports suggested that alkaloids produced analgesic effects by blocking the decrease of the met-enkephalin levels observed in morphine-tolerant rats.

Contribute to the change of analgesia in morphine-tolerant rats, we compared P-gp expression among the groups by Western blot studies. This is consistent with previous evidence indicating that high levels of P-gp expression may be associated with attenuated analgesia in morphine treated rats. Western blot analysis showed comparable P-gp protein levels in various groups, including morphine-treated rats, to which P-gp heavily projected. Brain P-gp was approximately 3-fold higher in morphine-treated rats as compared with saline controls. At the same time, in oxymatrine-treated premorphine compared with morphine-treated rats, the P-gp levels that were increased by morphine were significantly diminished, based on Western blot analysis (Figure 3A). Moreover, the alteration of P-gp expression levels showed negative correlation with the analgesic effect of morphine (Figure 3B), whereas there was no relationship between β-actin expression levels and the analgesic effects of morphine (data not shown). Thus, our results suggest that with respect to oxymatrine, its analgesic effects were negatively correlated with the level of expression of P-gp.
rats and by preventing the expressions of G-proteins and protein components of the cAMP system.20-22

On the other hand, in this study, we observed that individual differences in P-gp expression are highly negatively correlated with individual differences in morphine antinociceptive effects in rats (data not shown). In addition, in the clinic, it is known that the doses of morphine needed for pain relief vary between individuals.14 Although it is empirically known that there is interindividual variability in morphine analgesia, the mechanism of these differences is not clear. The present results suggest that the variation in the P-gp expression levels or its function in the blood–brain barrier may contribute to the interindividual variability of analgesic effects of morphine.23 Furthermore, we have shown that chronic administration of oxymatrine with morphine and administration of oxymatrine alone enhance antinociceptive effects of morphine in rats; this was not attributed to its P-gp inhibitory action.24 However, the AUC of morphine analgesia was not related to the P-gp expression levels as described above. The hypothesis was therefore advanced that oxymatrine may produce analgesic potencies by activation of opioid receptors,25 which suggests that the role of the P-gp in morphine analgesia can be dissociated from its role in morphine tolerance. Moreover, this may help in the development of an effective strategy for customization of morphine therapy in cancer patients.

In conclusion, administration of oxymatrine with morphine not only inhibited tolerance to morphine but also increased the antinociceptive effects of morphine. Furthermore, morphine induces the increased expression of P-gp, which was abolished by oxymatrine, indicating that oxymatrine has an inhibitory effect on the expression of P-gp. The present findings support the clinical observation of individual differences in morphine effects in cancer pain therapy.

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References

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