Modulation of morphine antinociceptive tolerance and physical dependence by co-administration of simvastatin

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Abstract

Statins, 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors, are widely used in the management of different diseases beyond their primary indication for lowering cholesterol. Previous studies have demonstrated the neuroprotective effects of simvastatin in different animal models. In the present study, we examined the effects of simvastatin (30, 60, 100 and 300 mg/kg, p.o.) on the development and expression of morphine-induced tolerance and dependence in mice. For the induction of morphine tolerance and dependence, mice were twice daily treated with morphine (10 mg/kg, s.c.) for 5 consecutive days. Tolerance was evaluated by the hot-plate test and physical dependence by naloxone challenge, on the sixth day. The results showed that oral administration of simvastatin produced antinociceptive activity in a dose-dependent way. Co-administration of simvastatin with morphine did not affect the acute morphine-induced analgesia (10 mg/kg, s.c.). However, repeated co-administration of simvastatin with morphine significantly attenuated the development of tolerance to the analgesic effect of morphine and inhibited the naloxone (5 mg/kg, s.c.)-precipitated withdrawal signs (jumping and body weight loss). Also, simvastatin at doses of 100 and 300 mg/kg attenuated the expression of morphine-induced tolerance and dependence. These data indicated that, while simvastatin can alleviate both development and expression of morphine-induced tolerance, it cannot enhance morphine-induced antinociception. Taken together, simvastatin may be used as an adjuvant therapeutic agent in combination with morphine and or other opioids in patients with severe chronic pain.

1. Introduction

Opioids are extensively used in the management of acute and chronic pain. However, chronic use of these drugs usually has undesirable side effects, in particular, tolerance to analgesia and dependence. Analgesic tolerance to opioid drugs is described by a reduced responsiveness to these compounds and is usually expressed by the need to use increasing doses to achieve the desired effect. In the other hand, abrupt cessation of chronic opioid use produces an intense but rarely life-threatening withdrawal syndrome in both animals and humans which is associated with physical dependence (Hernandez et al., 2009; Williams et al., 2001).

Opioid tolerance is a complex phenomenon that involves one or more of several mechanisms, including down-regulation of opioid receptors, regulation of G-protein-coupled receptor activation, alteration of the endogenous opioid peptides' affinity to their receptors, modulation of post-receptor processes, change in drug disposition to the receptor site (Liu and Anand, 2001; Taylor and Fleming, 2001), alteration of neurotransmission (Bolanos and Nestler, 2004), oxidative damage (Nakagawa et al., 2005; Starowicz et al., 2003) and also inflammation of the central nervous system (Hutchinson et al., 2011). In addition, other data indicate that particular G protein subunits and regulator of G protein signaling proteins participate in modulating opioid signaling, which may contribute to the development of opioid tolerance and dependence (Garzon et al., 2001).

Statin drugs, such as simvastatin are selective inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme for cholesterol biosynthesis in the liver (Lennernas and Fager, 1997). These compounds are widely used for the treatment of hypercholesterolemia (Grundy, 1988) and prevention of primary and secondary coronary heart disease (Liao and Laufs, 2005). Moreover, these drugs have benefits other than lowering lipids, such as anti-inflammatory effect (Yin et al., 2007), antioxidant (Di Napoli et al., 2004), improvement of endothelial function (O’Driscoll et al., 1997) and regulation of neurotrophic levels (Wu et al., 2008). The neuroprotective
effect of simvastatin in in vivo models of the brain (Balduini et al., 2003), peripheral nerve (Gholami et al., 2008), and spinal cord (Saito et al., 2011) injuries have also been reported. So, statins are being tested for their potential efficacy in treatment after brain injury (Chen et al., 2007).

Recently, the antinociceptive effect of simvastatin has been demonstrated in different animal models of pain (Shi et al., 2011; Miranda et al., 2011). Moreover, Ohshima et al. (2012) suggest that simvastatin-induced antinociception is mediated by attenuation of the sensitization of spinal nociceptive transmission. On the other hand, it is known that statins inhibit the synthesis of isoprenoids, including RhoA GTPase, which may contribute to the development of opioid tolerance and dependence (Goldstein and Brown, 1990). So, considering the mentioned evidences, the present study was aimed to investigate the effect of simvastatin on the development and expression of morphine-induced tolerance and dependence in mice.

2. Materials & methods

2.1. Animals

Experiments were conducted using adult male Swiss mice (25–30 g) obtained from the central animal house of Jundishapur University of Medical Sciences (Ahvaz-Iran). They were housed at 22 ± 2 °C and 12 h light/dark cycles (light from 7:00 to 19:00) with free access to food and water. All animals were randomly divided into groups of 6–8, acclimatized to the laboratory environment for at least one week before the experiments and used only once throughout the experiments. Animal care and experimental procedures were in accordance with the NIH Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985). All behavioral tests were performed by a blinded investigator.

2.2. Drugs

Morphine sulfate (Temad Co, Iran) and naloxone hydrochloride (Tolidaru Co, Iran) dissolved in physiological saline (0.9% NaCl) and simvastatin (Osveh Pharmaceutical Co, Iran) were suspended in a physiological saline solution containing 10% ethanol (Merck, Germany). Morphine was administered subcutaneously (s.c.), and simvastatin was administered by oral gavage (p.o.) in a volume of 10 ml/kg of body weight. Doses and drug administration schedules were based on the literature (Miranda et al., 2011; Ren et al., 2004; Way et al., 1969).

2.3. The mouse hot-plate test

Pain reflexes in response to thermal stimulus in the hot-plate test were assessed in accordance with Eddy and Leimbach's method (1953) as described previously. Each animal was placed on a 55 ± 1 °C hot plate which was surrounded by a clear acrylic cage, and latency time(s) to either hind paw licking or jumping (whichever came first) was recorded. The cut-off time was set as 15 s to avoid tissue damage. Before drug administration, the hot-plate latency was measured 3 times, and the average of the second and third trials was used as a baseline. The hot-plate latency was also measured following drug(s) administration. Antinociception was quantified by the percentage of maximum possible effect (% MPE), which was calculated as: %MPE = [(postdrug latency- baseline) / (cut-off time-baseline)] × 100.

2.4. The antinociceptive effects of simvastatin

Various single doses of simvastatin (30, 60, 100 and 300 mg/kg, p.o.) were administered 45 min before test and antinociceptive effects were assessed at 30-min time intervals for 120 min. The controls received only vehicle at the corresponding volume.

2.5. Effects of simvastatin on the acute antinociceptive effect of morphine

In this set of experiments, the animals received either various doses of simvastatin (30, 60, 100 and 300 mg/kg, p.o.), 45 min before morphine injection (10 mg/kg, s.c.). The antinociceptive effect was assessed at 15, 30 and 60 min following morphine injection. The controls received vehicle at the corresponding times.

2.6. Induction and assessment of morphine tolerance and dependence

Morphine tolerance and dependence was induced in mice by a repeated injection of morphine (10 mg/kg; s.c.) twice daily for 5 consecutive days as described by Ren et al. (2004). On the sixth day of experiment, the animals were assessed for both tolerance and dependence in accordance with the following method that previously described by Way et al. (1969). The decrease of morphine antinociception in hot-plate test was used to assess the degree of tolerance. Hot-plate latency was measured 15, 30 and 60 min after an injection of 10 mg/kg as a challenge dose (Ren et al., 2004). Moreover, physical dependence was evaluated by the incidence of jumping following administration of naloxone (5 mg/kg, i.p.) 2 h after challenge dose of morphine on sixth day. Immediately, after the naloxone injection, each mouse was placed in a Persilgalas box (40 cm long, 25 cm wide, 45 cm high) and frequency of jumps was recorded during 30 min. Also, changes in each mouse’s body weight were measured 1 h after the naloxone injection (Way et al., 1969).

For the assessment of the effects of simvastatin on the induction of morphine tolerance and dependence, simvastatin doses (30, 60, 100 and 300 mg/kg; p.o.) or its vehicle were given 45 min before each morphine injection throughout the induction, with none given on the test day. For the assessment of the effects of simvastatin on the expression of tolerance and dependence, animals that had received only morphine (10 mg/kg; s.c.) in the induction phase were used and the same doses of simvastatin mentioned were administered only on the test day, 45 min before acute morphine injection.

2.7. Statistical analysis

Data are presented as means ± SEM. A one-way analysis of variance (ANOVA) with Newman–Keuls's test was used to compare the effects of different treatment groups. A two-way ANOVA (treatment, time and their interaction) followed by a Bonferroni's test was used to compare the observed effects of the combination of simvastatin plus morphine and the expected sum of individual effects at each administration (Mansouri et al., 2014). ED50 Value and 95% confidence limits (CLs) for simvastatin dose–response curve were determined using least-squares linear regression. P-values less than 0.05 were considered to be statistically significant. All data calculations and statistical analysis were done with the GraphPad Prism Version 5.01 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Antinociceptive activity of simvastatin

As shown in Fig. 1A and B, oral administration of simvastatin (30, 60, 100, and 300 mg/kg) significantly increased hot-plate latency in a dose-dependent way with an ED50 value of 88.01 (95% CI, 52.12–149.97) mg/kg, as compared to vehicle-treated group [F(4,136) = 34, P < 0.0001]. However, low dose of simvastatin (30 mg/kg) failed to produce any analgesic effect (P > 0.05).

3.2. Effects of simvastatin on the acute morphine antinociception

To evaluate the effects of simvastatin on acute morphine-induced antinociception, the animals received various doses of simvastatin (30,
60, 100 and 300 mg/kg; p.o.), 45 min before morphine (10 mg/kg; s.c.) injection. Results revealed that the co-administration of various doses of simvastatin with morphine had no effect on the antinociceptive effect induced by morphine \[F(4,35) = 1.5, P = 0.22; \text{Fig. 2A, B}\].

3.3. Effects of chronic simvastatin on the tolerance to morphine analgesia

As shown in Fig. 3A, repeated injections of morphine twice daily for 5 consecutive days declined the antinociceptive effect of the drug indicating analgesic tolerance \[F(1,42) = 55.8, P < 0.001; \text{Fig. 3A}\]. In morphine-tolerant animals, when various doses of simvastatin (30, 60, 100 and 300 mg/kg, p.o.) were administered with morphine for 5 days, prevented the induction of morphine antinociceptive tolerance in a dose-dependent way \[F(4,89) = 14.97, P < 0.001; \text{Fig. 3B}\].

3.4. Effects of chronic simvastatin on morphine-induced dependence

To evaluate whether chronic simvastatin would alter the expression of behavioral withdrawal signs associated with morphine dependence, mice were treated twice daily for 5 consecutive days with s.c. administered vehicle, morphine alone, or morphine in combination with different doses of simvastatin (30, 60, 100 and 300 mg/kg, p.o.) as described earlier. On the test day, the mice were tested with naloxone (5 mg/kg, i.p.) 2 h after the last dose of morphine (10 mg/kg, s.c.). As shown in Fig. 4A, vehicle-treated animals exhibited no jumping behavior, whereas morphine-treated mice showed the behavior, indicating development of morphine dependence. Administration of different doses of simvastatin before morphine daily for 5 consecutive days significantly reduced the frequency of jumps in a dose-dependent way \[F(5,35) = 5.24, P < 0.05; \text{Fig. 4A}\]. However, the occurrence jumping was attenuated by the daily administration of simvastatin at doses 100 and 300 mg/kg. In addition, naloxone-induced weight loss was significantly reduced in the tolerant animals treated daily with simvastatin \[F(5,36) = 3.64, P < 0.05; \text{Fig. 4B}\].

3.5. Effects of acute simvastatin on the tolerance to morphine analgesia

The purpose of this study was to clarify the antinociceptive effect of simvastatin after the induction of morphine tolerance. Mice received vehicle or morphine twice daily for 5 consecutive days according to the abovementioned. On the test day (day 6), mice received various doses of simvastatin (30, 60, 100 and 300 mg/kg, p.o.) prior to the challenge dose of morphine and were tested for antinociception 30 min later in the hot-plate test. Results indicated that administration of simvastatin dose-dependently attenuated the expression of morphine tolerance \[F(5,102) = 29.79, P < 0.001; \text{Fig. 5}\].

3.6. Effects of acute simvastatin on the morphine-induced dependence

To evaluate whether simvastatin could prevent the expression of naloxone-precipitated morphine withdrawal signs in established
tolerance, we administered vehicle or various doses of simvastatin (30, 60, 100 and 300 mg/kg, p.o.) to morphine-tolerant mice prior to the challenge dose of morphine on day 6. An intraperitoneal dose of naloxone (5 mg/kg) was injected 2 h after the last dose of morphine (10 mg/kg; s.c.) for 5 days during the induction period. On day 6, hot-plate test was done after injection of challenge dose of morphine (10 mg/kg; s.c.). Each point represents the mean ± SEM for 6–8 mice. **P < 0.01 and ***P < 0.001 compared to animals treated with vehicle (two-way ANOVA with Bonferroni’s test).

4. Discussion

In the present study, we investigated the effect of simvastatin on morphine tolerance and dependence in mice. We found that co-administering the acute and chronic oral treatment with simvastatin significantly and dose-dependently protected mice from developing morphine-induced antinociceptive tolerance, and attenuated the naloxone-precipitated withdrawal signs of jumping and body weight loss.

The present results showed that different doses of simvastatin produced analgesic effect in the hot-plate test in mice. Our results are in agreement with earlier evidences that reported the analgesic effect of simvastatin in different animal models of pain (Miranda et al., 2011). However, simvastatin did not show any significant acute interaction with the antinociceptive activity of morphine in the hot-plate test.

One of the main problems associated with the chronic use of opioids such as morphine is tolerance. Repeated uses of morphine often cause patients to develop increasing resistance to the effects of the drugs, so that progressively higher doses are required achieving the same analgesic effects (Stoller et al., 2007; Zhang and Sweitzer, 2008). The mechanisms...
underlying the development of opioid tolerance and dependence are complex and not fully clarified. At the cellular and molecular level, chronic use of drug leads to alterations in receptor signaling, G protein function and/or signaling proteins (Gintzler and Chakrabarti, 2000). Moreover, growing evidences have shown that chronic morphine use may change synaptic plasticity and result in neuronal dystrophic remodeling and structural changes in the brain (Robinson and Kolb, 2004). On the other hand, it has been shown that simvastatin significantly improved functional recovery after experimental spinal cord injury by upregulating the expression of brain-derived neurotrophic factor (Han et al., 2011). Furthermore, in different studies neuroprotective effects of statins were reported (Van der Most et al., 2009). So, we hypothesized that simvastatin could prevent the pathological conditions associated long term morphine-induced antinociceptive tolerance and dependence.

NMDA receptor activation plays a role in the development of morphine tolerance and dependence. Chronic morphine administration may activate glutamate receptors, cause a subsequent calcium ion influx, and then trigger inflammatory cascades in the central nervous system (Mao, 1999). Moreover, it has been shown that MK-801 and dextromethorphan (as NMDA receptor antagonist) prevented morphine-induced tolerance and dependence. Also, Wang et al. (2009) indicated that simvastatin changes NMDA receptor binding density in the brain and shows possible NMDA antagonist-like activity. In the other hand, the anti-inflammatory effects of statins have been demonstrated in animal models of central nervous system disorders (Stive et al., 2003; Youssef et al., 2002). It has been reported that statins inhibited a number of inflammatory processes known to be important in brain damage and suppressed the secretion of potentially damaging cytokines such as IL-1β and TNF-α in spinal cord injury and ischemic stroke (Balduni et al., 2003; Chen et al., 2007). So, these effects could be possible mechanisms in the effects of simvastatin on morphine tolerance and dependence.

Other possibility in defining the role of simvastatin in prevention of morphine tolerance involves cytoskeleton systems. Small G-proteins are involved in many cellular functions including cytoskeletal rearrangement, intracellular trafficking, cell growth and development, cell motility, phagocytosis, and transcriptional regulation (Takai et al., 2001). The Rho subfamily of the small G-proteins modulates the actin-based cytoskeleton. Some evidences indicate that the small GTPase RhoA is involved in the regulation of various cellular functions, such as remodeling of the actin cytoskeleton and induction of transcriptional activity. Moreover, it has been shown that chronic morphine use alters the expression and function of the cytoskeletal proteins (García-Sevilla et al., 2004; Marie-Claire et al., 2004), and decreases the basal level of RhoA activity. Cordle et al. (2005) reported that simvastatin treatment effectively stimulated the GTP loading of RhoA. Furthermore, they demonstrated that this drug alters the actin-based cytoskeleton, which results in cellular morphological changes in vitro.

Our results showed that all simvastatin doses used have no significant effect on morphine-induced antinociception. However, the acute administration of simvastatin greatly potentiated the analgesic response in morphine-tolerant mice, indicating that these mice are more sensitive to the morphine-induced antinociception. Altogether, these findings may rule out the involvement of simvastatin in morphine tolerance prevention.

Combination therapy reduces not only morphine tolerance but dependence as well. Our results showed that simvastatin administered for five consecutive days with morphine reduced naloxone-precipitated signs. Morphine withdrawal is associated with decreased dopamine levels in various brain regions related to reward such as the ventral tegmental area and nucleus accumbens (Tokuyama et al., 2000; Walters et al., 2000). In previous studies, it was reported that chronic treatment with simvastatin profoundly increased dopamine D1 and D2 receptors in the prefrontal cortex while also altering dopamine level in various brain regions (Selley, 2005; Wang et al., 2005a, 2005b). So, simvastatin may be reducing the severity of morphine withdrawal by increasing dopamine levels and/or by affecting dopamine receptors in these areas.

Nitric oxide (NO) is a neurotoxin elevated in morphine tolerance and dependence (Dambisa and Lee, 1996). Moreover, it is well established that NO/PGC/cGMP pathway contributes to neuronal adaptations in response to repeated exposure and that NOS particularly nNOS inhibition ameliorates morphine withdrawal signs in different animal models (Cao et al., 2005; Machelska et al., 1997). Kureishi et al. (2000) indicated that simvastatin increases the initial, beneficial NO production by eNOS, while reducing NO overproduction by reducing iNOS. Furthermore, overproduction of NO by nNOS and iNOS is reduced by statins through down-regulating both enzymes (Moro et al., 2004).

In summary, the present results indicate that morphine antinociceptive tolerance and physical dependence can be prevented by co-administration of simvastatin. Given this information, it can be suggested the modulatory effect simvastatin on morphine tolerance and dependence. It is difficult to speculate on the exact mechanism of action at this time. Taken together, simvastatin may be used as an adjuvant therapeutic agent in combination with morphine and or other opioid drugs in patients with severe chronic pain.

Conflicts of interest

Nothing to declare.

Acknowledgments

This paper is issued from Pharm.D. thesis of (Seyed Amirhossein Tabatabae) and financial support was provided by the Vice Chancellor...
of Research, Ahvaz Jundishapur University of Medical Sciences (PRC-115), Ahvaz, Iran. Funding sources have no involvement in the study design, in the data collection, analysis and interpretation, in the manuscript writing and in the decision to submit the manuscript for publication.

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