Pharmacology, Biochemistry and Behavior 137 (2015) 38–43

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

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

Mohammad Taghi Mansouri [a,*], Mohammad Javad Khodayar [b], Amirhossein Tabatabaee [b,1], Behnam Ghorbanzadeh [c,1], Bahareh Naghizadeh [a]

[a] Department of Pharmacology, School of Medicine, Ahvaz Jundishapur Univ. of Med. Sciences, Ahvaz, Iran
[b] Department of Toxicology and Pharmacology, School of Pharmacy, Ahvaz Jundishapur Univ. of Med. Sciences, Ahvaz, Iran
[c] Department of Pharmacology, School of Medicine, Dezful Univ. of Med. Sciences, Dezful, Iran

ARTICLE INFO

Article history:
Received 25 May 2015
Received in revised form 23 July 2015
Accepted 3 August 2015
Available online 6 August 2015

Keywords:
Simvastatin
Antinociception
Tolerance
Physical dependence
Hot-plate test
Mice

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 adjutant 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., 2001), improvement of endothelial function (O'Driscoll et al., 1997) and regulation of neurotrophic levels (Wu et al., 2008). The neuroprotective

* Corresponding author at: Pharmacology Department, Medical School, Ahvaz Jundishapur Univ. of Med. Sciences, Ahvaz, Iran.
E-mail addresses: mansouri_smt@yahoo.com, mansouri-m@ajums.ac.ir (M.T. Mansouri).
[1] These authors are equally contributed to the work.

http://dx.doi.org/10.1016/j.pbb.2015.08.002
0091-3057/

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, Ohsawa 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 Plexiglas 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–Keul'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). ED_{50} 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 ED_{50} value of 88.01 (%95 CI, 52.12–149.97) mg/kg, as compared to vehicle-treated group [$F(4,136) = 34$, $P < 0.001$]. 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,