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Research report

# Atorvastatin attenuates the antinociceptive tolerance of morphine via nitric oxide dependent pathway in male mice

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ABSTRACT

The development of morphine-induced antinociceptive tolerance limits its therapeutic efficacy in pain management. Atorvastatin, or competitive inhibitor of 3-hydroxy-methyl-glutaryl coenzyme A (HMG-CoA) reductase, is mainstay agent in hypercholesterolemia treatment. Beyond the cholesterol-lowering activity, exploration of neuroprotective properties of this statin indicates its potential benefit in central nervous disorders. The aim of the present study was to assess the effects of atorvastatin in development and expression of morphine-induced analgesic tolerance in male mice and probable involvement of nitric oxide. Chronic and acute treatment with atorvastatin 10 and 20 mg/kg, respectively, could alleviate morphine tolerance in development and expression phases. Chronic co-administration of nitric oxide synthase (NOS) inhibitors including L-NAME (non selective NOS inhibitor; 2 mg/kg), aminoguanidine (selective inducible NOS inhibitor; 50 mg/kg) and 7-NI (selective neuronal NOS inhibitor; 15 mg/kg) with atorvastatin blocked the protective effect of atorvastatin in tolerance reversal. Moreover, reversing the atorvastatin effect was also observed in acute simultaneous treatment of L-NAME (5 mg/kg) and aminoguanidine (100 mg/kg) with atorvastatin. Co-treatment of guanylyl cyclase inhibitor, ODQ (chronic dose: 10 mg/kg and acute dose: 20 mg/kg) was associated with prevention of atorvastatin anti-tolerance properties. Our results revealed that the atorvastatin modulating role in morphine antinociceptive tolerance is mediated at least in part via nitric oxide in animal pain models of hot plate and tail flick.


## 1. Introduction

Morphine and related opioids, the most potent analgesic class, have been widely utilized for acute and chronic pain management. The clinical utility of opioids is a huge challenge for management of chronic pain such as cancer pain and neuropathic pain due to the rapid development of tolerance and hyperalgesia (Marek et al., 1991; Mao et al., 1995; Mayer et al., 1995; Trujillo and Akil, 1991). The loss of effectiveness after continued exposure or the requirement for dose increasing to maintain the same therapeutic effect is the definition of tolerance which occurs in two independent phases (induction and expression) (Bhargava, 1994; Johnson and Fleming, 1989; Raghavendra and Kulkarni, 1999).Therefore, restoring the efficacy of these major analgesics is of great importance in clinical setting.

Statins identified in 1976 by Endo and colleagues (Endo et al., 1976). These medications are competitive inhibitors of 3-hydroxy-methyl-glutaryl coenzyme A (HMG-CoA) reductase which catalyzes the conversion of HMG-CoA to mevalonate (an early, rate-limiting step in cholesterol biosynthesis) (Schachter, 2005). Statins are the best-tolerated and most effective agents for treating dyslipidemia (Zhou and Liao, 2009). Statins exert pleiotropic effects which could be protective in many conditions including neuro-

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logical disorders, inflammation, pain and cardiovascular diseases (Bifulco et al., 2008; Ludman et al., 2009; Reiss and Wirkowski, 2009; Rosenson, 2001; Shi et al., 2011; Weitz-Schmidt, 2002; Zhou and Liao, 2010). Recently, several landmark experimental trials demonstrated the beneficial effects of statin therapy for morphine-induced analgesic tolerance and dependence. Mansouri et al. (2015) reported that simvastatin modulates morphine antinociceptive tolerance and physical dependence. There is evidence indicating that rosuvastatin attenuates the morphine analgesic tolerance (Li et al., 2015a,b).

Nitric oxide (NO), a potent guanylyl cyclase stimulator, is synthesized by nitric oxide synthase (NOS) from amino acid L-arginine via a NADPH-dependent pathway. The recognized isoforms of NOS are: inducible (iNOS), neuronal (nNOS) and endothelial (eNOS), the last two enzymes are constitutively expressed (Förstermann and Sessa, 2012; Shafaroodi et al., 2012, 2015). Based upon the previous reports NO and cGMP (*cyclic guanosine monophosphate*) systems mediate opioid antinociception and tolerace/dependence phenomena (Babey et al., 1994; Elliott et al., 1994; Vaupel et al., 1995).

Nitric oxide may play dual role in both phases of morphine tolerance. Studies suggest that NOS inhibition prevents naloxone-precipitated withdrawal signs and development and expression of analgesic tolerance while some reports indicate that inhibition of NOS accelerates the development of tolerance. (Dambisya and Lee, 1996; Kolesnikov et al., 1993). It is well-determined that blockade of NO overproduction through selective iNOS inhibitors attenuates the development of morphine tolerance and dependence and administration of NO donors leads to exacerbation of opioid withdrawal signs in dependent animals (Abdel-Zaher et al., 2006; Dambisya and Lee, 1996). Data about role of NO in tolerance and dependence phenomena are controversial.

It has been presumed that atorvastatin neuroprotection is not entirely due to cholesterol reduction but several mechanisms such as NO/cGMP pathway are responsible in part for the salutary effects. Statins rapidly enhance the NO bioavailability which augments cerebral perfusion, and up-regulates the eNOS expression via inhibition of isoprenylation of RhoA GTPase. Statins prolong the half-life of NOS, increase the expression of iNOS and nNOS, activate the eNOS via PI3K/protein kinase Akt pathway and lead to transcriptional alteration (Moezi et al., 2012; Stepień et al., 2005; van der Most et al., 2009; Wang et al., 2010).

The current investigation aimed to assess the role of atorvastatin in development and expression phases of morphine-induced analgesic tolerance by two pain models: hot plate and tail flick. We also examined the possible contribution of NO in atorvastatin effects on morphine analgesic threshold.

## 2. Materials and methods

### 2.1. Subjects

This study was performed on male NMRI (Naval Medical Research Institute) mice weighing 23–30 g (Tehran University of Medical Sciences, Iran). Animals were housed in the standard cages and maintained under controlled laboratory conditions (temperature: 24 ± 1 °C, humidity: 55 ± 10%, lighting: 12-h light/dark cycle) with free access to both standard laboratory pellet chow and tap water. All procedures were conducted in compliance with institutional Guideline for the Care and Use of Laboratory Animals with the approval of Tehran University Research and Medical Ethics Committees. All behavioral experiments carried out at the same time of the day. Each mouse was used only once in this study and each group consisted of 6-8 animals.

### 2.2. Chemicals

The following drugs were used throughout this study: atorvastatin, a HMG-CoA inhibitor (Sobhan, Iran); morphine, an opioid agonist (Temad, Iran); L-NAME [L-$N^G$-Nitro-L-arginine methyl ester hydrochloride], a non-specific inhibitor of NOS (Sigma, USA); aminoguanidine, a selective inhibitor of iNOS (Sigma, USA); 7-NI (7-nitro indazole), a selective inhibitor of nNOS (Sigma, USA); L-arginine, a NO donor (Sigma, USA) and ODQ [1H-[1,2,4] Oxadiazolo[4,3-a]quinoxalin-1-one], a selective and potent sGC (soluble guanylyl cyclase) inhibitor (Sigma, USA). Atorvastatin suspension was prepared in carboxymethyl cellulose (CMC, 0.5%) and was administered trice daily by oral gavage. Morphine, L-NAME, aminoguanidine, 7-NI and ODQ were given intraperitoneally (i.p.) in a volume of 10 ml/kg of the mice body weight based on two protocols which are defined in the treatment section. Morphine, L-NAME and aminoguanidine were dissolved in sterile isotonic saline solution. 7-NI and ODQ were prepared as fine suspensions in sterile isotonic saline using polysorbate 80 and DMSO 1% (w/v) as co-solvents.

### 2.3. Induction and assessment of morphine tolerance

Morphine analgesic tolerance was induced via repeated injection of morphine trice a day for 5 consecutive days: 50 mg/kg (8:00 a.m), 50 mg/kg (11:00 a.m) and 75 mg/kg (4:00 p.m, higher dose prevents the withdrawal signs over-night). On the 5th day animals received only a single dose of morphine 50 mg/kg (Dambisya et al., 1991; Homayoun et al., 2002; Javadi et al., 2013). Loss of antinociceptive property of morphine in hot plate and tail flick tests was used to assess the degree of tolerance. Hot plate test: animals were placed separately on an electrically-heated surface (55 ± 1 °C) (Tahghigh-Gostaran-Teb, Iran). To confine the mouse on the heated surface, an open plexiglas tube (18 cm high × 22 cm diameter) was used. The time interval (sec) between placement of animal and licking the hind paws or jumping with all four feet was recorded by a stopwatch as the end point. The increase in hot plate threshold was considered as a measure of analgesic activity. In order to avoid tissue damage animals were removed from the hot plate surface if they could not respond within 90 s. Animals showing a reaction time greater than 90 s were excluded from the subsequent experiment. In a trial, prior to drug administration animals were tested for 5 days in order to obtain a standard control reaction time level. The analgesic effect of morphine was determined 60 min after the first morphine injection on the first, third and fifth days. Tail flick assay: the apparatus (Ugo Basile, Italy) was used to measure response latencies. Animals were restrained with their tail positioned in tail flick apparatus. The light beam was focused on the dorsal surface of animal's tail. The time interval between initiation of radiant heat stimulation and abrupt removal of tail from this noxious stimulus was considered as tail flick threshold (cut-off time = 10 s) (D'amour and Smith, 1941). We applied the tail flick model 45 min after the first treatment on the first, third and fifth days. The two tests were performed on the same group of animals.

### 2.4. Treatments

To assess the role of atorvastatin in the induction and expression phases of morphine analgesic tolerance, we designed two protocols. In the protocol one: different doses of atorvastatin (0.01, 0.1, 5, 10, 20 mg/kg) were administered perorally (p.o) trice a day for 5 days 45 min prior to injection of every dose of morphine and hot plate/tail flick tests were performed on first, third and fifth days. In the second protocol: different doses of atorvastatin (0.01, 0.1,1, 5, 10, 20 mg/kg) were administered by gavage as single doses only on the 5th day 45 min before the last dose of morphine (50 mg/kg)