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Antinociception induced by atorvastatin in different pain models

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ABSTRACT

Atorvastatin is a statin that inhibits the 3-hydroxy-methyl-glutaryl coenzyme A (HMG-CoA) reductase. Several landmark clinical trials have demonstrated the beneficial effects of statin therapy for primary and secondary prevention of cardiovascular disease. It is assumed that the beneficial effects of statin therapy are entirely due to cholesterol reduction. Statins have an additional activity (pleiotropic effect) that has been associated to their anti-inflammatory effects. The aim of the present study was to assess the antinociceptive activity of atorvastatin in five animal pain models. The daily administration of 3–100 mg/kg of atorvastatin by oral gavage induced a significant dose-dependent antinociception in the writhing, tail-flick, orofacial formalin and formalin hind paw tests. However, this antinociceptive activity of atorvastatin was detectable only at high concentrations in the hot plate assay. The data obtained in the present study demonstrates the effect of atorvastatin to reduce nociception and inflammation in different animal pain models.

1. Introduction

Statins are a group of drugs that inhibit 3-hydroxy-methyl-glutaryl coenzyme A (HMG-CoA) reductase, the enzyme responsible for the conversion of HMG-CoA to mevalonate, the rate-limiting step in *de novo* cholesterol synthesis (Schachter, 2005) and the treatment of dyslipidemia is the most common use for this type of medications. Several landmark clinical trials have demonstrated the beneficial effects of statins therapy for primary and secondary prevention of cardiovascular disease. Because serum cholesterol level is strongly associated with coronary heart disease, it has been generally presumed that the beneficial effects underlying statins therapy are entirely due to cholesterol reduction. However, the overall benefits observed with statins appear to be greater than merely the expected changes in lipid levels, suggesting effects beyond cholesterol lowering (Greenwood et al., 2006; Liao and Laufs, 2005; Schönbeck and Libby, 2004).

Most of the effects of statins, other than their lipid lowering activity have been correlated with their anti-inflammatory activity (Van der Most et al., 2009). Statins have been recognized as anti-inflammatory drugs since the first clinical observation of pravastatin decreasing the incidence of severe acute rejections, therefore, significantly improving the 1-year survival in heart transplant recipients (Kobashigawa et al., 1995). Statins may affect the function of the immune and inflammatory cells, including natural killer cells, monocytes, macrophages, microglia and T cells (Pannu et al., 2005; Kumar et al., 2010; Wahane and Kumar, 2010). Statins were found to inhibit C-reactive protein which is a major inflammation marker (Taubes, 2002). Moreover, statins inhibit the expression of adhesion molecules, monocytes chemotaxis, and matrix metalloproteinase activity (Ferro et al., 2000). Several reviews in recent years underline the evidence of immune and inflammatory effects of statins (Schönbeck and Libby, 2004; Greenwood et al., 2006; Ghittoni et al., 2007; Dinarello, 2010). Statins are also known to attenuate the secretion of pro-inflammatory cytokine interleukins (IL-1, 2, 4, 5, 10, 12), interferon-γ, and tumor necrosis factor-α (TNF-α), decrease the activity of cyclooxygenase-2 (COX-2), thromboxanes A2, and thromboxanes B2, and enhance the synthesis of prostacyclin which may contribute to decrease platelet activation (Schönbeck and Libby, 2004). Recently, Shi et al. (2011) found that systemic daily administration of statin from days 0 to 14 could completely prevent or reverse the mechanical allodynia and thermal hyperalgesia in neuropathic pain animal. The anti-inflammatory activity of statins is due to the reduction of IL-1β. These findings are very important if they could be translated to clinical studies, since these will open a new avenue for the use of statins in neuropathic pain management (Ray, 2011).

The aim of the present study was to assess the antinociceptive activity of atorvastatin in five animal pain models. Atorvastatin was selected because it is the most prescribed statin, with one of the most favorable safety profiles of these types of drugs available (Youssef et al., 2002).

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2. Material and methods

2.1. Animals

Male CF-1 mice (30 g), housed in a 12 h light–dark cycle at 22 ± 2 °C with *ad libitum* access to food and water were used. Experiments were performed in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigation of experimental pain, approved by the Animal Care and Use Committee of the Faculty of Medicine, University of Chile. Animals were acclimatized to the laboratory for at least 2 h before testing, each animal was used only once during the protocol and sacrificed immediately after the algesiometric test. The number of animals was kept at a minimum compatible with consistent effects of the drug treatments.

2.2. Writhing test

The procedure that was used has been previously described (Miranda et al., 2002). Mice were treated *per os* (p.o.) daily with saline or atorvastatin for 1 or 3 days before the assays and then injected intraperitoneally (i.p.) with 10 mL/kg of 0.6% acetic acid solution. A writhe is characterized by a wave of contraction of the abdominal musculature followed by the extension of the hind limbs. The number of writhes in a 5 min period was counted, starting 5 min after the acetic acid administration. Antinociception was expressed as an inhibition percentage of the number of writhes observed in control animals (20.4 ± 0.37, n = 22).

2.3. Tail flick test

This algesiometric test was similar to that previously described (Pinardi et al., 2002, 2003). A radiant heat, automatic tail flick algesiometer (U. Basile, Comerio, Italy) was used to measure response latencies. The light beam was focused on the animal's tail about 4 cm away from the tip and the intensity was adjusted so that baseline readings were between 2 and 3 s. An 8 s cut-off time was imposed to avoid damage to the tail. Control reaction time (latency of the response) was recorded twice, with an interval of 15 min between readings; the second reading was similar to the first. Only animals with baseline reaction times between 2 and 3 s were used for the experiments. Tail flick latencies were converted to the % of maximum possible effect (MPE) as follows:

$$\text{MPE\%} = [(\text{postdrug latency} - \text{predrug latency}) \div (\text{cut-off time} - \text{predrug latency})] \times 100.$$

Each animal was used as its own control and treated p.o. daily with saline or atorvastatin for 1 or 3 days before the assays. The dose that produced 50% of antinociception was expressed as MPE (ED_{50}) and was calculated from the linear regression analysis of the curve obtained by plotting log dose versus MPE%.

2.4. Formalin test in the hind paw

The method described by Miranda et al. (2007) was used. To perform the test, 20 μL of 2% formalin solution was injected into the dorsal surface of the mice's right hind paw with a 27-gauge needle attached to a 50 μL Hamilton syringe. Each mouse was immediately returned to the observation chamber. The degree of pain intensity was determined as the total time spent by the animal licking or biting the injected hind paw, measured by visual observation and a digital time-out stopwatch. The test shows two clear cut phases; phase I corresponds to the 5 min period starting immediately after the formalin injection and represents a tonic acute pain due to peripheral nociceptor sensitization and phase II was recorded as the 10 min period starting 20 min after the formalin injection and represents inflammatory pain. Mice were treated *per os* (p.o.) daily with saline (n = 25) or atorvastatin for 1 or 3 days before formalin injection. For each drug, analgesic effects were characterized after the administration of a minimum of four doses in logarithmic increments. The licking times observed were converted to a % of maximum possible effect (MPE) as follows:

$$\text{MPE\%} = 100 - [(100 \times \text{postdrug total licking time}) \div (\text{control total licking time})].$$

The dose that produced 50% of MPE (ED_{50}) was calculated from the linear regression analysis of the curve obtained by plotting log dose versus MPE%.

2.5. Orofacial formalin test

A modification of the method described by Luccarini et al. (2006), was used. To perform the test, 20 μL of 2% formalin solution was injected into the upper right lip of each mouse, with a 27 gauge needle. This formalin solution induced more consistent behavior and the possibility to produce less tissue damage. The mice were immediately returned to the observation chamber. The degree of pain intensity was determined as the total time period that the animal spent rubbing its lip with one of its extremities. Saline or atorvastatin was administered p.o. daily prior to the administration of formalin for 1 or 3 days before the assays. Two distinct phases were identified during the test; phase I corresponds to the 5 min period starting immediately after formalin injection and represents a tonic acute pain due to peripheral nociceptor sensitization. Phase II was recorded as the 10 min period starting 20 min after formalin injection and represents inflammatory pain. Each drug effect was characterized after the administration of at least four doses in logarithmic increments. Maximum possible effect (MPE), which represents antinociception, was calculated as follows:

$$\text{MPE\%} = 100 - [(100 \times \text{postdrug rubbing time}) / (\text{control rubbing time})].$$

The dose that produced 50% of MPE (ED_{50}) was calculated from the linear regression analysis of the curve obtained by plotting log dose vs. MPE%.

2.6. Hot plate

The hot plate test was performed using a modification of the method described by Melendez et al. (2002). In this case, the animals were free to move and the assay temperature was 45 ± 1 °C. The animal behavior considered as a sign of pain was the act of licking the forelegs or jumping off the hot plate. The base line latency for this behavior was recorded with a stop-watch. The cut-off time (T_{off}) was fixed at 30 s to avoid skin damage. Several measurements were performed with a 3 min interval: two at baseline (without any drug) and two after p.o. administration of the test drug.

Hot-plate latencies were converted to a maximum possible effect % (MPE) with the same equation used in the tail-flick assays.

2.7. Protocol

Dose–response curves for atorvastatin were obtained using at least six animals for each of at least four doses. A least squares linear regression analysis of the log dose response curve allowed the calculation of the doses that produced 50% of antinociception for each drug alone.