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Antinociception induced by rosuvastatin in mice: Modulation by opioid receptor antagonists, risperidone and l-name

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Abstract

Statins are widely used in cardiovascular disease as cholesterol lowering drugs. However, they also have other actions (pleiotropic effects) including antiproliferative, antithrombotic, neuroprotective, immunomodulatory, anti-inflammatory and antinociceptive activity. The aim of this study was to determine the antinociception properties of rosuvastatin in two murine models of pain and the involvement of opioid antagonists (naltrexone, naltrindole, norbinaltorphimine), risperidone, and L-NAME (L-N^G-nitro arginine methyl ester) in this effect. Rosuvastatin was chosen among available statins because it is commonly prescribed and has high potency, efficacy, and an acceptable safety profile. Rosuvastatin antinociception was evaluated in the acetic acid writhing test and the formalin hind paw test by dose-response curves, before and after the i.p. administration of opioid antagonists, risperidone, and L-NAME. This work demonstrates that the assayed drugs modulate the antinociceptive effect of rosuvastatin in both experimental murine pain tests. The antinociception effect described for rosuvastatin may be due to a particular modulation induced by the opioid antagonists, risperidone and L-NAME. Given the broad effects of rosuvastatin, the results of this study may have novel clinical implications in the therapy of pain.

Keywords: Rosuvastatin; Analgesia; Opioid antagonists; Risperidone; L-NAME

1. Introduction

The pharmacological treatment of pain has been fundamentally led by two types of drugs: opioids and nonsteroidal anti-inflammatory drugs (NSAIDs). However, the side effects of these drugs, such as dependence and gastric problems, reveal that new drugs for pain treatment are needed. Thus, the study of statins, a group of drugs used in cardiovascular diseases, deserves to be considered. All statins are competitive antagonists of HMG-CoA (3-hydroxyl-3-methyl-glutarylcoenzyme A) reductase, and are commonly used as cholesterol lowering drugs. Statins also have several effects unrelated to lipid metabolism, called pleiotropic effects, among which antiproliferative, antithrombotic, antioxidant neuroprotective, and immunomodulatory actions have been described. In addition, anti-inflammatory and antinociceptive activities have been reported [1-3]. Other pleiotropic effects of statins include prevention in Alzheimer's disease, atherosclerosis and antineoplastic actions [4-6].

Statins show differences in their pharmacokinetic and pharmacodynamic properties. Among the statins currently available, rosuvastatin, a synthetic compound, is the most effective in lowering LDL-C (low density lipoprotein

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cholesterol) due to its potent inhibition of hepatic cholesterol synthesis. In addition, this statin has the longest terminal half-life, being marginally metabolized by cytochrome P450 and eliminated by the kidney and liver [8].

Several tests have been developed for the study of pain in rodents to provide tools for fundamental and translational research in this field. Thus, there are various experimental models available to evaluate pain using thermal, mechanical, and chemical stimuli. These resources have allowed to make progress in understanding the bases of pain. An example is the acetic acid writhing test where the irritating agent is administered intraperitoneally, inducing a stereotyped behavior characterized by contractions of the abdominal musculature which are quantified. This test is an assay of a reflexive response to a visceral and muscle irritant, and a model of nociception based on peripheral and central activation of nociceptors [7,8]. Another test is the formalin hind paw test, an assay in which pain is induced by the application of chemical stimuli in mice, which involves biphasic nocifensive behavior [3].

The drugs used in the management of pain include NSAIDs, opioids, antiepileptic drugs, adrenergic blockers, serotonin antagonists, antimuscarinic drugs, antidepressants, corticosteroids and others. Furthermore, several of them have been used alone or in association with other drugs. Although it has been shown that various drugs display antinociceptive activity in several algesimetry tests, there are few experimental reports for statins.

The aim of the present study was to evaluate the effect of rosuvastatin on antinociception in two murine pain tests, the acetic acid writhing test and the formalin hind paw test. In addition, the involvement of opioid antagonists, risperidone, and L-NAME in the antinociception induced by rosuvastatin was evaluated. Rosuvastatin was chosen because it has been barely studied in this topic, it is commonly prescribed, and has high potency, efficacy, and an acceptable safety profile among the statins available.

2. Materials and methods

2.1. Animals

Male CF-1 mice (25-30 g) housed on a 12-hr light–dark cycle at 22 ± 1 °C with free access to food and water were used. Experiments were performed by following the current Guidelines for the Care of Laboratory Animals and Ethical Guidelines for investigation of experimental pain approved by the Animal Care and Use Committee at the Faculty of Medicine, Universidad de Chile, protocol N°19278-MED-UCH, 13/06/2019. Animals were acclimatized to the laboratory for at least 1 hr before testing, and were used only once during the protocol. Investigators evaluating animal behavior were blinded to the treatment. Mice were euthanized by an overdose of anaesthetic immediately after the algesimetry test. The number of animals was kept to the minimum required to obtain reliable effects of the drug treatment.

2.2. Measurement of antinociception and anti-inflammation

Antinociception was assessed by performing the following murine assays.

- The acetic acid writhing test (WT) was used as described previously [8]. In this test, mice were injected i.p. with 10 ml of 0.6% acetic acid. The chemical stimulus induces a wave of contraction of the abdominal muscle followed by extension of the hind limbs (writhes) and reduction in motor activity. The number of writhes during the 5 min following the i.p. administration of chemical solution was counted. Antinociception is the percentage of inhibition of the number of writhes in control mice (18.7±1.2, n=24) and may be expressed as % MPE (percentage of the maximum possible effect).
- The formalin hind paw (FHP) test was used as described previously [3]. To perform the test 20 μL of 2 % formalin solution were injected into the dorsal surface of the right hind paw. The intensity of pain was assessed from the time, in seconds, that licking or biting of the injected paw was observed. The results of the test were recorded in two periods: phase I, corresponding to the 5 min starting immediately after formalin injection, and phase II, a period of 10 minutes starting 20 min after formalin injection. The control values were: 126.4 ± 8.4 sec for phase I (n =12), and 155.6 ± 10.2 sec for phase II (n=12). Licking time was converted to % MPE.

2.3. Experimental design

The antinociceptive activity of rosuvastatin was evaluated from dose-response curves for 3, 10, 30 and 100 mg/kg i.p. obtained in the algesimetry assays using six to eight animals for each dose. Dose-response curves were obtained before and after the i.p. administration of 5 mg/kg of naltrexone (NTX), 2 mg/kg of naltrindole (NTI), 2 mg/kg of norbinaltorphimine (norBNI), 0.001 mg/kg of risperidone (RISPER), or 1 mg/kg of L-NAME. The drugs were administered i.p. 30 min prior to each test. The dose that produces 50 % of the MPE (ED_{50}) was calculated from a linear

regression analysis of the dose-response curve. The different doses of rosuvastatin or the other agents did not induce significant behavioural changes or motor dysfunction in the mice.

2.4. Drugs

All drugs were freshly dissolved in sterile physiological salt solution in a constant volume at 10 ml/kg for i.p. administration. Rosuvastatin was a gift from Astra Zeneca Laboratories, Chile. Risperidone (RISPER) was provided by Royal Pharma S.A., Chile. L-NAME, naltrexone hydrochloride (NTX), naltrindole hydrochloride (NTI) and norbinaltorphimine dihydrochloride (norBNI) were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA.

2.5. Statistical analysis

Results are presented as means ± SEM and statistical differences between the results were analyzed by ANOVA followed by Tukey´s post-test; P values less than 0.05 ($P<0.05$) were considered statistically significant. All calculations were performed using the software Pharm Tools Pro, version 1.27, McCary Group Inc., PA, USA.

3. Results

3.1. Antinociception induced by rosuvastatin

The i.p. administration of rosuvastatin induced a dose dependent antinociceptive effect in mice in the acetic acid writhing test and in the formalin hind paw test (see Figure 1).

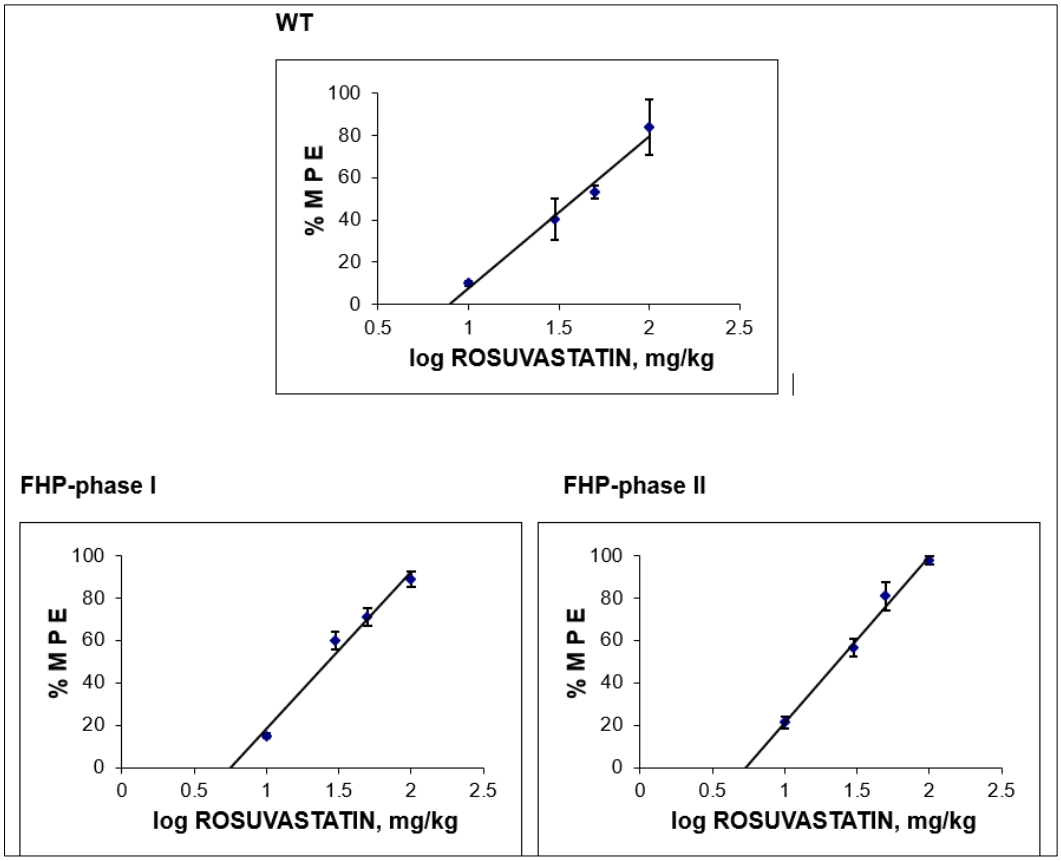


Figure 1 Dose-response curves for the i.p. administration of rosuvastatin in the acetic acid writhing test (WT) and the formalin hind paw (FHP) test in mice. Each point is the mean ± SEM of 6-8 mice. % MPE = antinociception expressed as percentage of the maximum possible effect

The relative potency of rosuvastatin was FHP-phase II > FHP-phase I > WT. The corresponding values expressed as ED_{50}, in mg/kg, were: 23.07 ± 1.48, 26.01 ± 2.41 and 32.80 ± 3.05, respectively. The analgesic ratio of the ED_{50} values for WT / FHP-II and WT / FHP-I was 1.42 and 1.26, respectively, and for FHP-I / FHP-II it was 1.12.

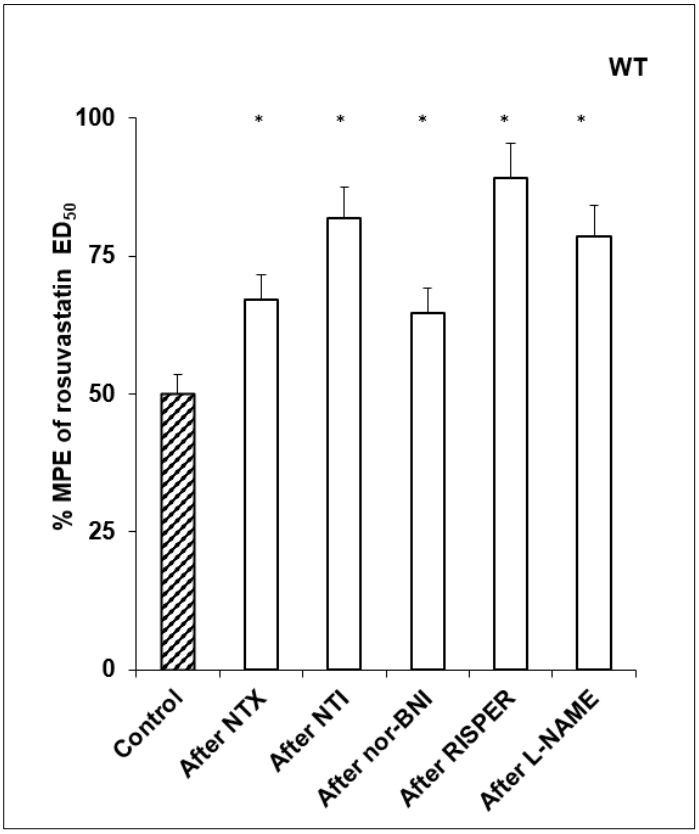
3.2. Effect of opioid antagonists, risperidone and L-NAME on the antinociception induced by rosuvastatin in the acetic acid writhing test (WT)

The i.p. treatment of mice with 5 mg/kg of naltrexone, 2 mg/kg of naltrindole or 2 mg/kg of norbinaltorphimine did not elicit significant effects compared to controls. Mice pretreated with the different opioid antagonists revealed a significant increase in the antinociception induced by rosuvastatin in the WT. The changes in the % MPE of the ED_{50} of the statin, expressed as the analgesic ratio (AR) between post and pretreatment, were 1.3 for NTX, 1.3 for norBNI and 1.6 for NTI. Pretreatment of mice with RISPER 0.001 mg/kg, i.p., and with L-NAME 1 mg/kg, i.p., increased significantly the efficacy of rosuvastatin with an AR of 1.8 and 1.6 respectively. These results are shown in Table 1 and Figure 2.

Table 1 Maximum possible effect (MPE) and analgesic ratio (AR) for the antinociceptive activity of ED_{50} of rosuvastatin in the acetic acid writhing test of mice, before and after treatment with i.p. 5 mg/kg of naltrexone (NTX), 2 mg/kg of naltrindole, (NTI), 2 mg/kg of Nor-binaltorphimine (Nor-BNI), 0,001 mg/kg of risperidone (RISPER) and 1 mg/kg of L-NAME

WT	% MPE PRE ROSUVASTATIN	% MPE POST ROSUVASTATIN	AR	P
NTX	50 ± 2.7	67.1 ± 4.6	1.3	0.005
NTI	50 ± 2.7	81.8 ± 5.3	1.6	0.005
Nor-BNI	50 ± 2.7	64.6 ± 6.8	1.3	0.005
RISPER	50 ± 2.7	89.2 ±12.2	1.8	0.005
L-NAME	50 ± 2.7	78.6 ± 3.4	1.6	0.005

WT: acetic acid writhing test. AR: ratio between% MPE post/pre-treatment of ED_{50} rosuvastatin. P values between pre and post treatment; p< 0.005, statistically significant.



The control and after pretreatment % MPE of the ED50 of rosuvastatin with the different drugs are shown in hatched and white columns. Columns represent the mean ± SEM of 6-8 mice. *: p<0.05, versus without pretreatment

Figure 2 Effect of pretreatment with naltrexone (NTX), naltrindole (NTI), norbinaltorphimine (norBNI), risperidone (RISPER), and L-NAME on the ED50 of rosuvastatin in the acetic acid writhing test (WT) in mice

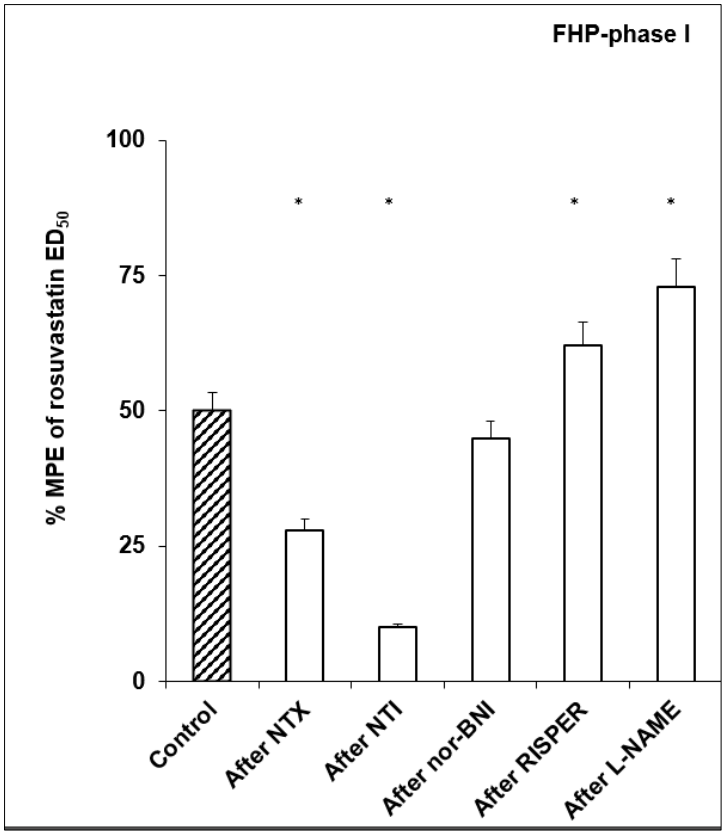
3.3. Effect of opioid antagonists, risperidone and L-NAME on the antinociception induced by rosuvastatin in phase I of the formalin hind paw (FHP) test

Mice pretreated with the different opioid antagonists revealed a significant variation in the antinociception induced by rosuvastatin in phase I of the FHP test. The reduction of the AR of rosuvastatin was 0.9 for norBNI, 0.6 for NTX, and 0.2 for NTI. Pretreatment of mice with RISPER 0.001 mg/kg, i.p., and with L-NAME 1 mg/kg, i.p., increased significantly the efficacy of rosuvastatin with an AR of 1.3 and 1.5 respectively. These results are shown in Table 2 and Figure 3.

Table 2 % MPE (maximum possible effect) and analgesic ratio (AR) for the antinociceptive activity of ED_{50} of rosuvastatin in the phase I of the formalin hind paw test of mice, before and after treatment with i.p. 5 mg/kg of naltrexone (NTX), 2 mg/kg of naltrindole, (NTI), 2 mg/kg of Nor-binaltorphimine (Nor-BNI), 0,001 mg/kg of risperidone (RISPER) and 1 mg/kg of L-NAME

WT	% MPE PRE ROSUVASTATIN	% MPE POST ROSUVASTATIN	AR	P
NTX	50 ± 2.4	28.1 ± 2.2	0.6	0.005
NTI	50 ± 2.4	10.2 ± 0.9	0.2	0.005
Nor-BNI	50 ± 2.4	44.5 ± 2.3	0.9	0.11
RISPER	50 ± 2.4	63.3 ± 4,2	1.3	0.005
L-NAME	50 ± 2.4	72.6 ± 6.3	1.5	0.005

WT: acetic acid writhing test. AR: ratio between % MPE post/pre-treatment of ED_{50} rosuvastatin. P values between pre and post treatment; p< 0.005, statistically significant.



The control and after pretreatment % MPE of the ED50 of rosuvastatin with the different drugs are shown in hatched and white columns, respectively. Columns represent the mean ± SEM of 6-8 mice. *: p<0.05, versus without risperidone pretreatment.

Figure 3 Effect of pretreatment with naltrexone (NTX), naltrindole (NTI), norbinaltorphimine (norBNI), risperidone (RISPER), and L-NAME on the ED50 of rosuvastatin in phase I of the formalin hind paw (FHP) test in mice

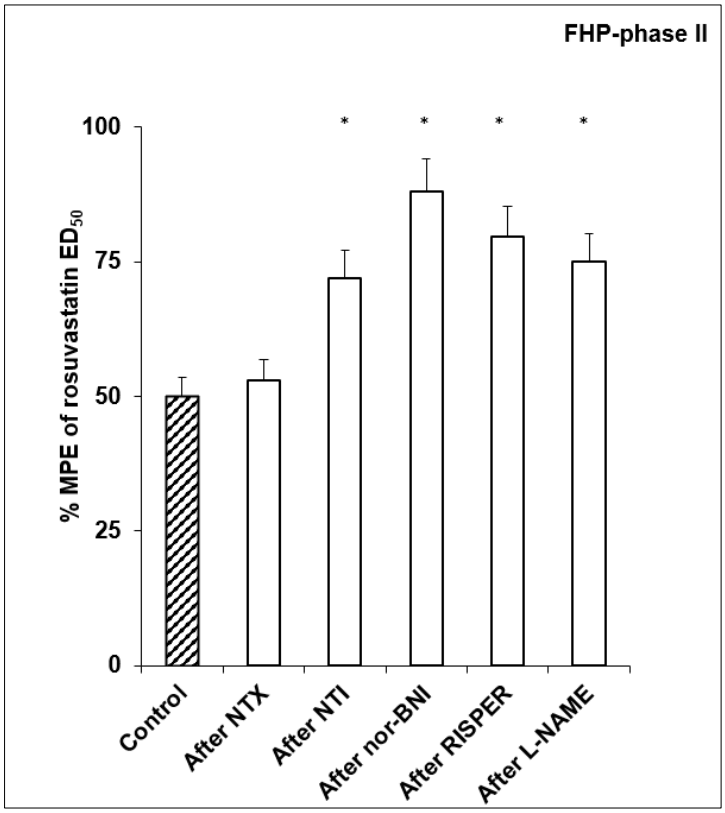
3.4. Effect of opioid antagonists, risperidone and L-NAME on the antinociception induced by rosuvastatin in phase II of the formalin hind paw (FHP) test

Mice pretreated with the different opioid antagonists (with the exception of naltrexone) revealed a variation in the antinociception induced by rosuvastatin in phase II of the FHP test. The changes in the AR of the statin were 1.8 for norBNI and 1.4 for NTI. Pretreatment of mice with RISPER 0.001 mg/kg, i.p., and with L-NAME 1 mg/kg, i.p., increased significantly the efficacy of rosuvastatin with an AR of 1.6 and 1.5 respectively. These results are shown in Table 3 and Figure 4.

Table 3 % MPE (maximum possible effect) and analgesic ratio (AR) for the antinociceptive activity of ED_{50} of rosuvastatin in the phase II of the formalin hind paw test of mice, before and after treatment with i.p. 5 mg/kg of naltrexone (NTX), 2 mg/kg of naltrindole, (NTI), 2 mg/kg of Nor-binaltorphimine (Nor-BNI), 0,001 mg/kg of risperidone (RISPER) and 1 mg/kg of L-NAME

WT	% MPE PRE ROSUVASTATIN	% MPE POST ROSUVASTATIN	AR	P
NTX	50 ± 2.4	53.5 ± 2.6	1.0	0.3
NTI	50 ± 2.4	72.2 ± 4.8	1.4	0.005
Nor-BNI	50 ± 2.4	87.7 ± 6.4	1.8	0.005
RISPER	50 ± 2.4	79.7 ± 8.2	1.6	0.005
L-NAME	50 ± 2.4	75.3 ± 4.2	1.5	0.005

WT: acetic acid writhing test. AR: ratio between % MPE post/pre-treatment of ED_{50} rosuvastatin. P values between pre and post treatment; p< 0.005, statistically significant.



The control and after pretreatment % MPE of the ED50 of rosuvastatin with the different drugs are shown in hatched and white columns, respectively. Columns represent the mean ± SEM of 6-8 mice. *: p<0.05, versus without risperidone pretreatment.

Figure 4 Effect of pretreatment with naltrexone (NTX), naltrindole (NTI), norbinaltorphimine (norBNI), risperidone (RISPER), and L-NAME on the ED50 of rosuvastatin in phase II of the formalin hind paw (FHP) test in mice

4. Discussion

The reduction of pain and inflammation are processes in which cyclooxygenases and thromboxanes have an important role. However, the development of other drugs that are more effective and have fewer side effects is still a challenge. Accordingly, the results of the present study show that rosuvastatin is a molecule capable of inhibiting pain and inflammation. Thus, the findings of this study are in agreement with previous results [2,3,9-13]. This antinociceptive activity of statins deserves further study since it has been reported that these drugs have contradictory effects: they reduce neuropathic pain in animals but increase it in men. These results could be attributable to the pleiotropic effects of statins [14].

The findings presented herein prove that rosuvastatin induces a dose-dependent antinociceptive effect which is in agreement with its ability to inhibit mediators of the inflammatory response through the inhibition of PPARs (peroxisome proliferator activated receptors) [4]. In addition, its inhibitory action on inflammatory biomarkers, such as TNF-α, IL-6, IL-1β, and MDA, and antioxidant properties have been described to explain the antinociceptive effects of rosuvastatin [11,15]. Also, studies have demonstrated that rosuvastatin attenuates the activation of microglial cells and astrocytes with a significant decrease in the level of IL-1β [12]. This effect could be due to the phosphorylation of extracellular signal-regulated kinase (ERK) [42/44]. These properties explain the action of rosuvastatin in reversing morphine tolerance to analgesia [11]. Likewise, other studies have demonstrated that rosuvastatin reduces the levels of proinflammatory cytokines such as IL-1ß, TNF-α, and IL-10, and increases anti-inflammatory mediators together with stimulating NO production by constitutive NOS [2,13,16].

NO is a gaseous molecule that acts both as a messenger and as a neurotransmitter, and is involved in various physiological actions, either as an excitatory or an inhibitory effector. NO has an important role in transmitting pain by producing hyperalgesia or antinociception, depending on its concentration. The changes in the activity of rosuvastatin reported here due to the action of the nitric oxide synthase (NOS) inhibitor L-NAME, seem to be unrelated to its lipid lowering action and may include the modulatory effect of NO on the efficacy of rosuvastatin. This may stem from the various effects of L-NAME on NOS since it has been reported to lead to an increase in the expression of e-NOS [17,19] and an inhibition of i-NOS [17, 18, 20], while lacking any effect on n-NOS [17].

In addition to the mechanisms described above, other targets could modulate antinociception induced by rosuvastatin, such as opioid receptors and other mediators. To study the involvement of MOR, DOR, and KOR in antinociception induced by rosuvastatin, naltrexone, naltrindole, and norbinaltorphimine have been used at doses that block MOR, DOR, and KOR, respectively [21,22]. It was reported early on that selective opioid receptor blockers induce analgesia in mice as well as in rats [23].

The reduction of the antinociceptive effect of rosuvastatin in phase I of the FHP test by naltrexone and naltrindole is in line with the antagonistic action of these drugs at MOR and DOR receptors. However, norbinaltorphimine, a known KOR receptor antagonist, had no effect. These findings seem to indicate that the activity of opioid drugs is related to the type of nociceptive stimulus and its location. Nonetheless, the participation of MOR, DOR, and KOR receptors in the WT and in phase II of the FHP test modulates the antinociception and anti-inflammatory effects of rosuvastatin.

Paradoxically, opioid antagonists have been reported to produce analgesia or enhance analgesia. Preclinical data have shown that the opioid antagonist naltrexone improves and prolongs analgesia by preventing abnormal G-protein coupling of opioid receptors [24]. Similarly, MOR and DOR receptor antagonists increase morphine analgesia, effect that could be related to full activity of the agonist [25]. From another point of view, antagonistic opioids have other properties in addition to their capacity to block MOR, DOR and KOR receptors. It has been demonstrated that naltrexone also exerts its effects as an antagonist of toll-like receptor 4 or TLR4 [26], which induce an increase in proinflammatory cytokines, substance P, nitric oxide and excitatory amino acids [27]. Moreover, a possible role of nitric oxide in the effects of naltrindole has been reported [28].

Risperidone is among the drugs used as coanalgesics or analgesic adjuvants which modulate pain by inducing changes in CNS neurotransmitters. Risperidone, a second generation antipsychotic, has been described as an antagonist of dopamine (D_2), serotonin ($5\text{-}HT_{2A}$), adrenergic (α_1 and α_2), and histamine (H_1) receptors [29]. This study shows that risperidone causes a variation in the antinociceptive efficacy of rosuvastatin. A possible explanation for this effect could be the modulatory action of risperidone on pharmacodynamic properties of the statin, including inflammatory mediators, cytokines, and NO, among others.

The findings of this research indicate that the changes in rosuvastatin antinociception induced by the opioid receptor antagonists, L-NAME, and risperidone, can be explained by the several mechanisms of action they share, two of which are the secretion of cytokines, such as IL-1β, IL-10, and TNF-α, and the modulation of NOS gene expression. Given the extensive effects of rosuvastatin, the results presented herein may have important clinical implications by suggesting that rosuvastatin may represent a new class of analgesic adjuvants.

5. Conclusion

In this study, the pleiotropic effects of rosuvastatin are highlighted with reference to its beneficial antinociceptive efficacy and its application as a novel way to approach pain management.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The experimental protocol was approved by Bioethics Committee on Animal Research, Protocol CICUA N° 19278-MED-UCH, Faculty of Medicine, University of Chile.

Author Contributions

All authors contributed to data analysis and interpretation, manuscript preparation and review. All authors read and approved the final manuscript.

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