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In vivo and *in vitro* anti-inflammatory and anti-nociceptive activities of lovastatin in rodents

D.O. Gonçalves[1], I.B.F. Calou[1], R.P. Siqueira[1], A.A. Lopes[1], L.K.A. Leal[2], G.A.C. Brito[3], A.R. Tomé[4] and G.S.B. Viana[1,5]

[1]Departamento de Fisiologia e Farmacologia, Faculdade de Medicina,
[2]Departamento de Farmácia, Faculdade de Farmácia, Odontologia e Enfermagem,
[3]Departamento de Morfologia, Faculdade de Medicina,
Universidade Federal do Ceará, Fortaleza, CE, Brasil
[4]Faculdade de Veterinária, Universidade Estadual do Ceará, Fortaleza, CE, Brasil
[5]Departamento de Biofisiologia, Faculdade de Medicina de Juazeiro do Norte,
Juazeiro do Norte, CE, Brasil

Abstract

Statins are among the most prescribed drugs in recent clinical practice. They are also known for their pleiotropic actions, which are independent of their lipid-lowering properties. The effect of lovastatin was investigated against carrageenan-induced paw edema in male Wistar rats (200-250 g) and on leukocyte migration, as measured by carrageenan-induced peritonitis in male Swiss mice (20-25 g), which are models of acute inflammation. Lovastatin (administered 1 h prior to carrageenan), at oral doses of 2, 5, and 10 mg/kg, markedly attenuated paw edema formation in rats at the 4th hour after carrageenan injection (25, 43, and 37% inhibition, respectively). Inhibitions of 20, 45 and 80% were observed in the leukocyte migration, as evaluated by carrageenan-induced peritonitis in mice with lovastatin doses of 0.5, 1 and 5 mg/kg, as compared to controls. Furthermore, lovastatin (administered 1 h before initiation) reduced the nociceptive effect of the formalin test in mice, at both phases, at doses of 2, 5, and 10 mg/kg: first phase (51, 65, and 70%, respectively) and second phase (73, 57, and 66% inhibition of licking time, respectively). The anti-nociceptive activity of lovastatin was inhibited by naloxone (3 mg/kg, *sc*). Lovastatin (0.01, 0.1, and 1 µg/mL) inhibited by 23, 79, and 86%, respectively, the release of myeloperoxidase from human neutrophils. Leukocyte (predominantly neutrophils) infiltration was almost completely reduced by lovastatin treatment, as observed in the model of acute paw edema with hematoxylin and eosin staining. In addition, lovastatin decreased the number of cells expressing tumor necrosis factor-α (TNF-α) and the inducible form of nitric oxide synthase (iNOS) activity. Therefore, the alterations in leukocyte activity and cytokine release could contribute to the anti-inflammatory activity of lovastatin.

Key words: Statin; Inflammation; Anti-nociception; Myeloperoxidase; Cytokines

Introduction

In the early 80's, the statins, a new therapeutic class, were introduced as lipid-lowering agents. Since then, their role in the reduction of serum lipids has been extensively investigated in both experimental and clinical trials (1). Statins are among the most widely used prescription drugs, and exert their lipid-lowering actions by reversible and competitive inhibition of the enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), the rate-limiting step in the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate, a precursor of cholesterol (2). Statins also stimulate the hepatic expression of low-density lipoprotein (LDL) cholesterol receptors, which in turn increase LDL uptake from the circulation (3). Statins are also known for their pleiotropic effects, which are independent of their lipid-lowering properties (4). Among the effects of statins, the most relevant are anti-atherosclerotic (5) and anti-inflammatory actions (6), improvement of endothelial dysfunction (7), anti-thrombosis (8) and anti-oxidant (9) actions, prevention of Alzheimer's disease (10), and anti-neoplasic actions (3).

Correspondence: G.S.B. Viana, Departamento de Fisiologia e Farmacologia, Faculdade de Medicina, Universidade Federal do Ceará, 60020-181 Fortaleza, CE, Brasil. E-mail: gbviana@live.com

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Several studies have shown the ability of statins to inhibit the production of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) (11) and monocyte chemoattractant protein-1. Statins also seem to exert effects on the expression of nuclear factor kappaB (NF-κB), which plays a significant role in the production of pro-inflammatory mediators such as prostaglandins and adhesion molecules (12). These compounds also reduce C-reactive protein levels in clinical treatments (13). Furthermore, a recent study demonstrated the ability of statins to reduce polymorphonuclear leukocyte infiltration, one of the first steps in the development of inflammation (14).

Lovastatin is one of the most frequently investigated statins. It has been shown to exert anti-atherogenic, anti-thrombotic and anti-neoplasic effects, independent of its effects on serum lipids (15). Lovastatin can reduce cytokine production such as IL-2, IL-4, and interferon-gamma from activated human T cells, and can also down-regulate both activator protein-1 and NF-κB DNA-binding activities, as assessed by electrophoretic mobility shift assays (16).

The aims of the present study were to evaluate the anti-inflammatory properties of lovastatin on *in vivo* models of acute inflammation in rodents (carrageenan-induced paw edema in rats, and carrageenan-induced peritonitis in mice), and its anti-nociceptive effects (formalin test in mice). This is the first study showing that lovastatin, besides presenting a potent anti-inflammatory activity, produces anti-nociception and reduces pain of both neurogenic and inflammatory origin. Furthermore, we also investigated the effects of lovastatin on *in vitro* myeloperoxidase (MPO) release and on TNF-α and inducible nitric oxide synthase (iNOS)-expressing cells, and on rat paws after carrageenan-induced edema.

Material and Methods

Reagents

Lovastatin and dexamethasone were purchased from EMS Laboratory (Brazil) and dissolved in distilled water just before each experiment. Indomethacin, naloxone, carrageenan, and dextran were purchased from Sigma (USA) and formalin was purchased from Delta Laboratories (Brazil). Human leukocytes were kindly provided by the Ceará State Blood Center. All other reagents were of analytical grade.

Animals

Male Swiss mice (20-25 g) and male Wistar rats (200-250 g) were obtained from the Central Animal House of Universidade Federal do Ceará, Brazil. The animals were housed at 24 ± 2°C under a 12-h light/dark cycle, and had free access to a standard pellet diet (Purina chow, Brazil) and tap water. They were deprived of food, but not of drinking water, for 8 h before the experiments. The animals were treated according to current Brazilian law and to the NIH Guide for the Care and Use of Laboratory Animals. The project was approved by the Animal's Ethics Committee of the Faculty of Medicine, Federal University of Ceará. The doses of lovastatin were chosen on the basis of pilot studies.

Paw edema induced by carrageenan

Carrageenan-induced acute inflammation in the rat paw is used as a classical model of edema formation and hyperalgesia, for the study of non-steroidal anti-inflammatory drugs and selective cyclooxygenase-1 (COX-1) and COX-2 inhibitors. Five groups of 8 rats each were treated with lovastatin (LOV, 2, 5, and 10 mg/kg, *po*), indomethacin (INDO, 20 mg/kg, *po*), or vehicle (distilled water, *po*). One hour after receiving the drug(s), each animal received a subcutaneous injection of 0.1 mL 1% carrageenan in the right hind paw. The edema was measured immediately prior to the carrageenan injection and 1, 2, 3, 4, and 24 h later. The paw edema volume was determined in milliliters as the difference between the final and initial volumes, assessed with a plethysmometer from Ugo Basile, Italy.

Peritonitis induced by carrageenan

Mice were treated with LOV (0.5, 1, and 5 mg/kg, *po*), INDO (20 mg/kg, *po*) or vehicle (distilled water, *po*). One hour after treatment, each animal received an intraperitoneal injection of 0.25 mL 1% carrageenan to induce the inflammatory process (17) and, 4 h later, the animals were sacrificed by cervical dislocation. The peritoneal cavity was washed with 2 mL phosphate-buffered saline (PBS) containing heparin, and the exudate was then collected for analysis. An exudate sample of 20 µL was taken, and a 0.4-mL Turk's solution was added to it. The total number of neutrophils was then counted in a Neubauer chamber using light microscope. Data are reported as the mean (x 10^3/mm^3) number of leukocytes.

Nociception induced by formalin

The mouse formalin test is a model of tonic pain and localized inflammatory pain involving two response phases: the first (0-5 min) indicates neurogenic nociception, and the second (15-30 min) indicates inflammatory nociception (18). Groups of 8 mice each were treated with LOV (2, 5 and 10 mg/kg, *po*), morphine (10 mg/kg, *sc*) or naloxone (3 mg/kg, *sc*) 15 min prior to lovastatin or morphine administration. Thirty minutes later, each animal received a subcutaneous injection of 20 µL 1% formalin in the right paw. The time in seconds each mouse spent licking the injected paw was recorded.

Myeloperoxidase release from human neutrophils

The MPO release test from human neutrophils was performed according to Lucisano and Mantovani (19). MPO is widely used as a biomarker of inflammation. In the present study, 2.5 x 10^6 human leukocytes were suspended in

buffered Hank's balanced solution containing calcium and magnesium. The preparations contained predominantly neutrophils (85.0 ± 2.8%), and cell viability, determined by the Trypan blue test, was 97.7 ± 0.94%. Human neutrophils were incubated with LOV (0.01, 0.1 and 1 µg/mL) or INDO (35.7 µg/mL) as the reference drug, for 15 min at 37°C. Then, these cells (in the presence of LOV) were stimulated by the addition of phorbol myristate acetate (PMA, 0.1 µg/mL) for 15 min at 37°C and the suspension was centrifuged for 10 min at 2000 *g*, 4°C. Aliquots (50 µL) of the supernatants (without cells) were added to PBS (100 µL), phosphate buffer (50 µL, pH 7.0) and H_2O_2 (0.012%). After 5 min at 37°C, 20 µL 1.5 M 3,3',5,5'-tetramethylbenzidine (TMB) was added as a substrate, and the reaction stopped with 30 µL 1.5 M sodium acetate, pH 3.0. The absorbance was then determined at 620 nm.

HE staining and immunohistochemistry analyses for TNF-α and iNOS

The streptavidin-biotin-peroxidase method was used for immunohistochemistry assays of TNF-α and iNOS (14). Three groups of mice were treated with either distilled water (normal controls) or LOV (5 mg/kg, *po*). After 30 min, an intraplantar injection of 1% carrageenan was administered to the animals, except the normal controls, and 3 h later, all animals were sacrificed and 5 mm plantar region sections were immersed in buffered formalin solution for 24 h. The sections were then deparaffinized, dehydrated with xylol and ethanol, and immersed in 0.1 M citrate buffer, pH 6, under microwave heating, for 18 min, for antigen recovery. After cooling at room temperature for 20 min, the sections were washed with PBS, followed by a 15-min blockade of endogenous peroxidase with a 3% H_2O_2 solution. The sections were incubated overnight (4°C) with rabbit primary antibodies (anti-TNF-α or anti-iNOS, respectively) as 1:200 or 1:400 dilutions in PBS-BSA. On the next day, the sections were washed in PBS and incubated for 30 min with the secondary biotinylated rabbit antibody (anti-IgG) at 1:200 dilution in PBS-BSA. After washing in PBS, the sections were incubated for 30 min with the conjugated streptavidin peroxidase complex (ABC Vectastain® complex, Vector Laboratories, USA). After another washing with PBS, the sections were stained with 3,3'diaminobenzidine-peroxide (DAB) cromophore, counter-stained with Mayer hematoxylin, dehydrated, and mounted on microscope slides for analysis. Some sections from all groups were used for standard HE staining as well.

Statistical analysis

Data are reported as means ± SEM for 8 animals per group. Statistical analyses were carried out using one-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls *post hoc* test for multiple comparisons. P values < 0.05 were considered to be statistically significant.

Results

Effect of lovastatin on paw edema induced by carrageenan in rats

The effects of orally administered lovastatin on paw edema induced by carrageenan are shown in Figure 1. Subcutaneous administration of 1% carrageenan induced edema formation by the 3rd and 4th hours. Lovastatin at the tested doses of 2 and 5 mg/kg exhibited a dose-related effect against carrageenan-induced inflammation, as compared to the vehicle group (controls) during the same period of time. However, at the higher dose of 10 mg/kg, no further decrease in edema volume was observed, indicating that the maximum effect had been reached. At the 4th hour, inhibition was 25, 43, and 37% for the doses of 2, 5, and 10 mg/kg, respectively. Indomethacin (20 mg/kg), used as the positive control, inhibited (58%) carrageenan-induced edema during the same period of time, as compared to controls.

Effect of lovastatin on peritonitis induced by carrageenan in mice

The effects of orally administered lovastatin on carrageenan-induced peritonitis are shown in Figure 2. The intraperitoneal administration of 1% carrageenan provoked an intense leukocyte migration into the mouse peritoneal cavity, and lovastatin at the doses of 0.5, 1, and 5 mg/kg produced a dose-dependent inhibition of this effect of

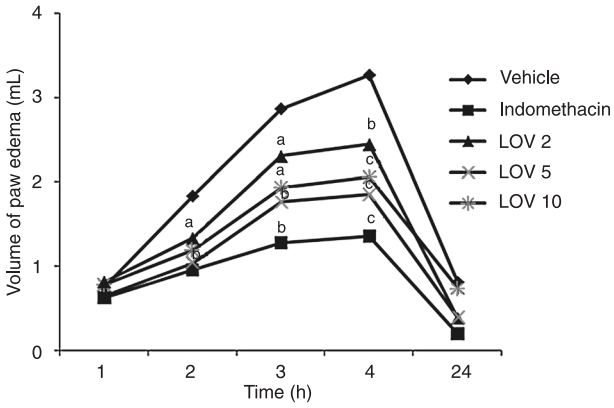

Figure 1. Effects of lovastatin (LOV) and indomethacin on paw edema induced by carrageenan in rats. The animals (8 per group) were treated with LOV (2, 5, and 10 mg/kg, *po*), indomethacin (20 mg/kg, *po*), or vehicle (distilled water, *po*). One hour after receiving these drugs, each animal was injected *sc* with 0.1 mL 1% carrageenan in the right hind paw. The edema was measured immediately prior to the carrageenan injection and 1, 2, 3, 4, and 24 h later. Its volume in mL was determined with a plethysmometer as the difference between the final and initial volumes. Data are reported as means ± SEM. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$ *vs* controls (ANOVA followed by the Student Newman-Keuls *post hoc* test).

the order of 20, 45, and 80%, respectively. At the higher dose (5 mg/kg) the effect was similar to that observed with INDO (20 mg/kg, *po*), used as the reference drug. No further increases were observed at the dose of 10 mg/kg, *po* (data not shown).

Effect of lovastatin on the formalin test in mice

The effects of intraperitoneal administration of lovastatin on formalin nociception are shown in Figure 3A. Subcutaneous administration of 1% formalin to the mouse paw induced a typical behavior of paw suspension and licking. Lovastatin at the doses of 2, 5, and 10 mg/kg, *po*, significantly reduced the duration of licking the injected paw in both phases of the formalin test: 1st phase (51, 65, and 70%, respectively) and 2nd phase (73, 57, and 66%). Morphine (10 mg/kg, *sc*), used as a positive control, also produced a significant reduction in both phases (76 and 69%).

Effect of naloxone on the anti-nociceptive activity of lovastatin and morphine in the formalin test in mice

The effects of the oral administration of lovastatin (5 mg/kg) and subcutaneous morphine (10 mg/kg) after pretreatment with the opioid antagonist naloxone (3 mg/kg, *sc*) are shown in Figure 3B. Naloxone significantly reversed the anti-nociceptive effects of morphine and lovastatin in the first phase of the test (related to nociception), indicating the

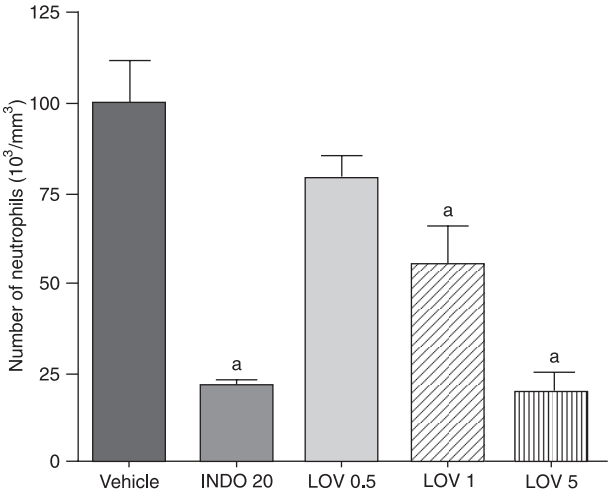

Figure 2. Effects of lovastatin (LOV) and indomethacin (INDO) on neutrophil migration induced by the intraperitoneal injection of carrageenan in mice. The animais (8 per group) were treated with LOV (0.5, 1, and 5 mg/kg, *po*), INDO (20 mg/kg, *po*) or vehicle (distilled water, *po*), and received 1% carrageenan (0.25 mL, *ip*) 1 h after the treatments. Four hours later, the animals were sacrificed, the peritoneal cavity washed with 2 mL phosphate-buffered saline containing heparin, and the exudate collected for neutrophil counting. Data are reported as means ± SEM. [a]P < 0.001 *vs* controls (ANOVA followed by the Student Newman-Keuls *post hoc* test).

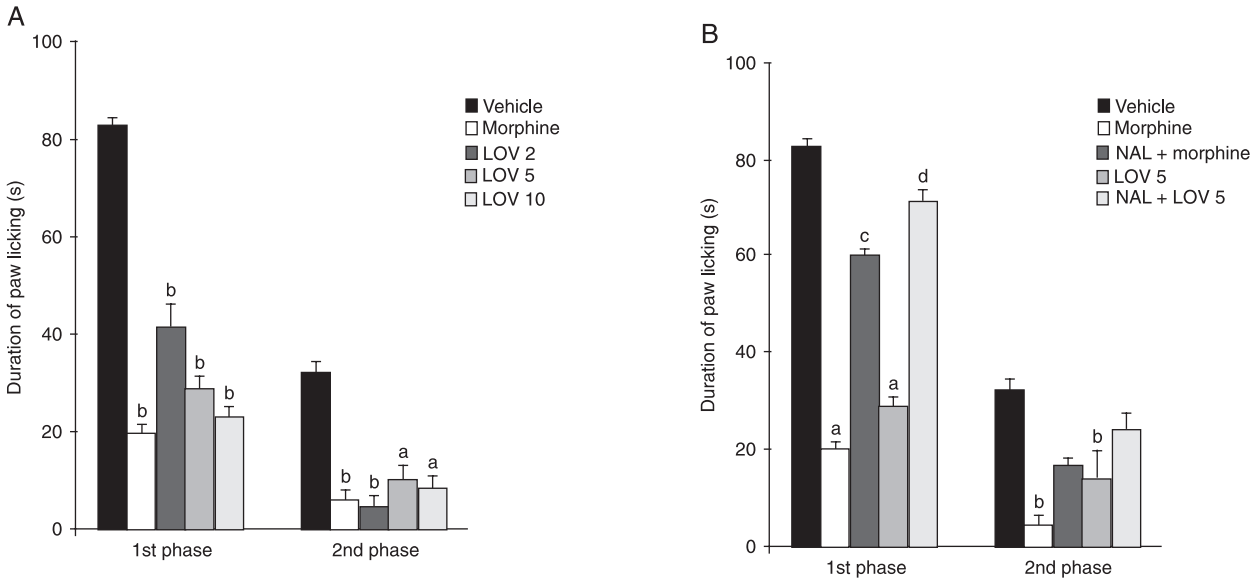

Figure 3. *A,* Effects of lovastatin (LOV) and morphine on the formalin test in mice. Mice (8 per group) were treated with LOV (2, 5, and 10 mg/kg, *po*) or morphine (10 mg/kg, *sc*), and 15 min later with naloxone (3 mg/kg, *sc*). Thirty minutes later, each animal received a subcutaneous injection of 20 µL 1% formalin in the right hind paw. The period of time (s) each mouse spent licking the injected paw was recorded. Data are reported as means ± SEM. [a]P < 0.01; [b]P < 0.001 *vs* controls (ANOVA followed by the Student Newman-Keuls *post hoc* test). *B,* Effects of naloxone (NAL) on the anti-nociceptive activity of lovastatin (LOV) and morphine, in the formalin test in mice. Data are reported as means ± SEM for 8 animals. [a]P < 0.001 *vs* controls; [b]P < 0.01 *vs* control; [c]P < 0.001 *vs* morphine; [d]P < 0.001 *vs* LOV 5 (ANOVA followed by the Student Newman-Keuls *post hoc* test).

involvement of the opioid system. The effect of naloxone + morphine in the second phase (related to inflammation) was not statistically significant.

Effects of lovastatin on the release of human neutrophil MPO stimulated with PMA

The effects of pre-incubation with lovastatin (0.01, 0.1 and 1 µg/mL) on MPO release from freshly isolated cells (2.5 x 10^6) stimulated with PMA (0.1 µg/mL) are shown in Figure 4. Lovastatin inhibited MPO release in a concentration-dependent manner by 23, 79, and 86% at the three concentrations tested, respectively. Indomethacin (35.7 µg/mL) used as a positive control inhibited MPO release by 93%.

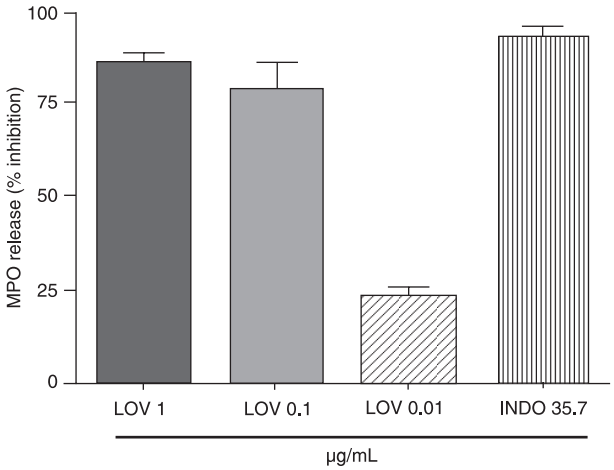

Figure 4. Effects of lovastatin (LOV) on the release of human neutrophil myeloperoxidase (MPO) stimulated with phorbol myristate acetate (PMA). The cells were pre-incubated with LOV (0.01, 0.1 and 1 µg/mL) or indomethacin (INDO, 35.7 µg/mL) as the reference drug, prior to the addition of PMA. The suspension was centrifuged (10 min at 2000 *g*, 4°C). Supernatant aliquots (50 µL) were added to PBS (100 µL), phosphate buffer (50 µL, pH 7.0) and H_2O_2 (0.012%). After 5 min at 37°C, the substrate 3,3',5,5'-tetramethylbenzidine (TMB, 1.5 mM, 20 µL) was added, the reaction stopped with 1.5 M sodium acetate, pH 3.0, and absorbance determined at 620 nm. Data are reported as percent inhibition of MPO release (mean ± SEM).

HE staining and immunohistochemistry analyses for TNF-α and iNOS

Figure 5A shows the paw from a rat that did not receive carrageenan (sham control). It indicates the preservation of tissue architecture with collagen fibers and the presence of fibroblasts. Intense edema formation can be seen in Figure 5B (carrageenan-injected group, inflammation control), indicated by large white spaces and the disappearance of collagen fibers. Also, carrageenan injection induced leukocyte migration, mainly neutrophils. Edema reduction and tissue preservation were observed in the group that received carrageenan and was pretreated with lovastatin (inflammation lovastatin) at the dose of 5 mg/kg, *po* (Figure 5C). Collagen fibers presented only a slight destruction after carrageenan injection. Neutrophil infiltration was almost completely reduced by lovastatin treatment. Fibroblasts were also present in significant amounts.

As shown in Figure 5D, no staining was observed in the sample that did not receive the primary antibody (sham control). Intense immunostaining was observed in Figure 5E, indicating a large amount of iNOS activity in rats injected with carrageenan (inflammation control). In the group treated with lovastatin (5 mg/kg, *po*), the immunostaining for iNOS and neutrophil infiltration were markedly reduced (Figure 5F, inflammation lovastatin). Thus, we could assume an involvement of iNOS inhibition in the anti-inflammatory actions of lovastatin.

Intense immunostaining was also observed in Figure 5H, indicating activity and expression of TNF-α in the rats injected with carrageenan (inflammation control). TNF-α is a well-known pro-inflammatory cytokine involved in the mechanisms of carrageenan. Lovastatin (5 mg/kg, *po*) also significantly reduced the immunostaining for TNF-α, associated with the presence of TNF-α in tissue (Figure 5I). The sham control (Figure 5G) was the same as in Figure 5D.

Discussion

Statins inhibit the conversion of HMG-CoA to mevalonic acid, and also down-regulate the production of bioactive sterols derived from the cholesterol synthesis pathway. *In vitro* studies indicate that the suppression of these mediators is responsible, at least in part, for the anti-inflammatory actions of statins (20). The present results indicate that lovastatin has a potent anti-inflammatory activity since it inhibits carrageenan-induced peritonitis and paw edema. Although these findings are consistent with studies showing these effects of statins in many inflammation models, most of them were carried out with other statins, mainly simvastatin (21-23).

Although all of these reports point to the anti-inflammatory effects of statins, most of them focused on cardiovascular diseases and atherosclerosis (24,25). In addition, we demonstrated for the first time that lovastatin produced anti-nociception and reduced pain of both neurogenic and inflammatory origin, as assessed by the formalin test. Surprisingly, this effect of lovastatin, similar to that of morphine, was also reversed by naloxone, suggesting the involvement of the opioid system.

Carrageenan-induced inflammation in the rat paw is a model of acute edema formation and hyperalgesia extensively used in the development of non-steroidal anti-inflammatory drugs and selective COX-1 and COX-2 inhibitors. Edema formation is the result of interaction among various inflammatory mediators that increase vascular permeability and/or blood flow (26). Carrageenan-induced edema has

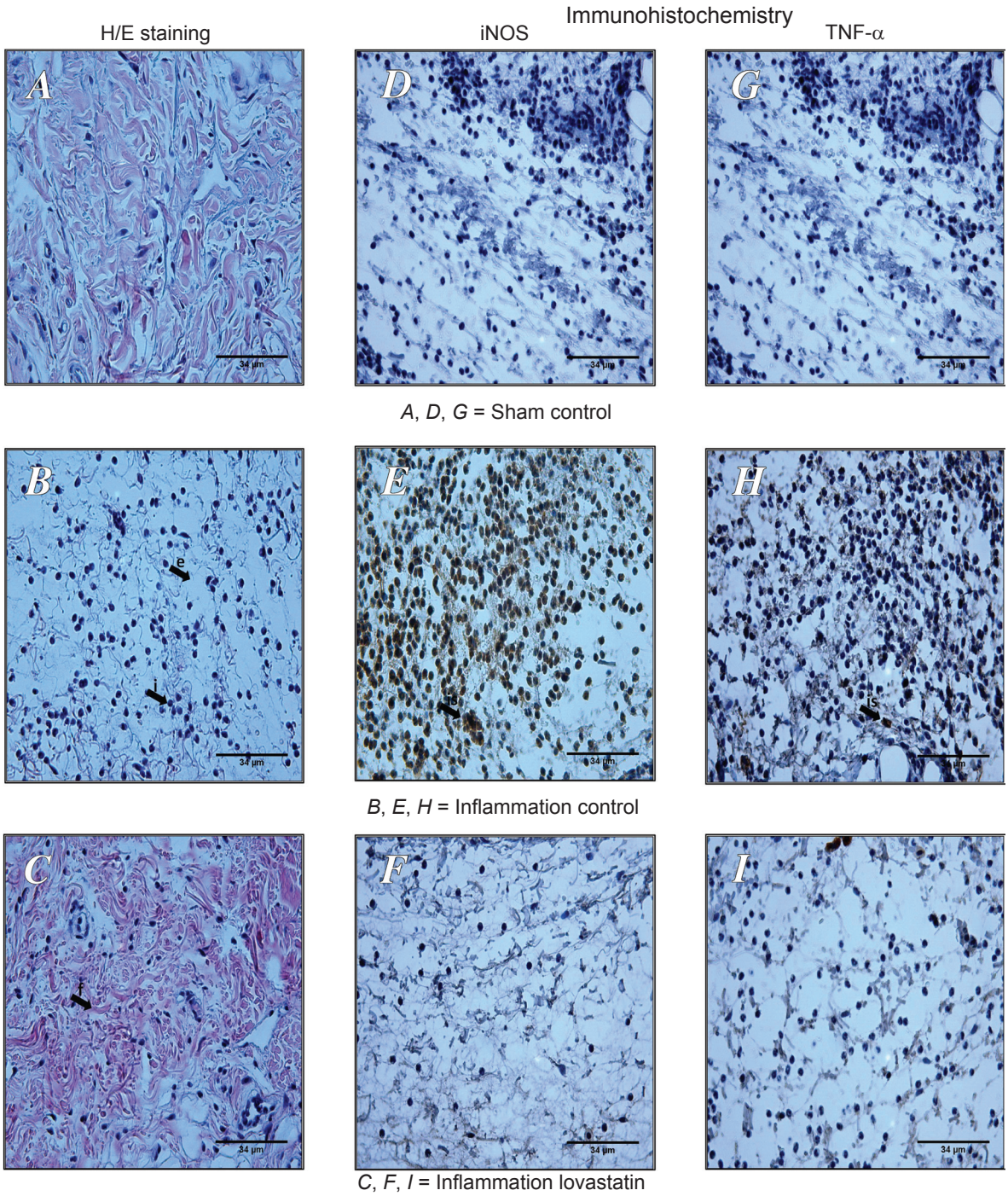

Figure 5. *A, B, C,* Hematoxylin and eosin (H/E) staining of rat paws in the model of carrageenan-induced edema. Micrographs (400X) of representative paw slices from each group are shown. *A,* Sham (untreated) control; *B,* inflammation control (with carrageenan treatment); *C,* inflammation lovastatin (LOV pretreatment, 5 mg/kg, *po*, followed by carrageenan administration 1 h later). Some of these changes are indicated by arrows as *e* (edema), *i* (inflammatory cell infiltration), and *f* (collagen fiber preservation). *D, E, F*, Immunohistochemistry for inducible nitric oxide synthase (iNOS) in the model of acute paw edema induced by carrageenan in rats. Micrographs (400X) of representative paw slices from each group are shown. *D,* Sham (untreated) control; *E,* inflammation control (treatment with carrageenan); *F,* inflammation lovastatin (LOV pretreatment, 5 mg/kg, *po*, followed by carrageenan administration 1 h later). The presence of immunostaining is indicated by an arrow as *is*. *G, H, I*, Immunohistochemistry for tumor necrosis factor-alpha (TNF-α) in the model of acute paw edema induced by carrageenan in rats. Micrographs (400X) of representative paw slices from each group are shown. *G,* Sham (untreated) control; *H,* inflammation control (treatment with carrageenan); *I,* inflammation lovastatin (LOV pretreatment, with 5 mg/kg, *po*, followed by carrageenan administration 1 h later). The presence of immunostaining is indicated by an arrow as *is*.

been described as a biphasic event. The early phase, observed about 1 h after carrageenan injection, is related to the production of serotonin, histamine, bradykinin, and cyclooxygenase products, while the late phase is due to neutrophil infiltration, as well as to the continuing production of arachidonic acid metabolites (27). Our data agree with others obtained in studies using another statin, simvastatin (28), which significantly reduced the edema extension when orally administered to mice 1 h before carrageenan injection, effects that were similar to those observed in the present study with indomethacin. Recently (14), histological examination of paw lesions confirmed that simvastatin inhibits the acute inflammation induced by carrageenan. These investigators concluded that simvastatin reduced the polymorphonuclear leukocyte infiltration, dose-dependently and similarly to indomethacin.

Furthermore, we performed an MPO test *in vitro*, which confirmed that lovastatin exerts a potent anti-inflammatory action. This statin significantly inhibited MPO release. This result was probably associated with the reduction of pro-inflammatory cytokines and the production of adhesive molecules, which is a common pleiotropic effect related to the use of statins, including lovastatin (29,30). It has been assumed (31) that the anti-inflammatory effects of statins may be due to inhibition of leukocyte adhesion and their migration to sites of inflammation what may also have occurred under our experimental conditions.

The degree of inhibition of leukocyte migration exerted by lovastatin in our study was similar to that seen with indomethacin, used as reference drug, suggesting that lovastatin may interfere with some molecular pathways of the inflammatory process. Vascular cell walls produce large amounts of cytokines, particularly IL-6, which plays a positive role in the local inflammatory reaction by amplifying leukocyte accumulation (32). Therefore, our results indicate that alteration in cytokine production could contribute to the decrease in leukocyte recruitment caused by lovastatin.

This result might be associated with the inhibition of prostanoid production related to COX-2 and lovastatin may block COX-2 production by a mechanism of gene expression inhibition related to NF-κB. This agrees, at least in part, with our finding that lovastatin decreases the number of TNF-α- and iNOS-expressing cells in the carrageenan-induced paw edema. In addition, lovastatin demonstrated an efficient action in both phases of the formalin test. In the first phase, lovastatin anti-nociception was reversed by the opioid antagonist naloxone, indicating a possible participation of opioid receptors in the anti-nociceptive action of this statin. In the second phase, lovastatin provoked a marked reduction of nociceptive behaviors, a result agreeing with its anti-inflammatory actions.

Our study also demonstrates that lovastatin significantly reduces the paw edema formation induced by carrageenan. It has been reported (33) that NO produced by cNOS is involved in the development of inflammation at early times after carrageenan administration, and that NO produced by iNOS is involved in the maintenance of the inflammatory response at later times. These carrageenan-activated inflammatory cascades are related not only to the innate immunity but also to the generation of reactive oxygen species (ROS) (34). Since inflammation is closely linked to the production of ROS, the molecular basis of the observed anti-inflammatory effects of statins may be related to their ability to block the production and/or activity of ROS (35). Therefore, the results of the present study indicate an anti-inflammatory effect of lovastatin on the paw edema and leukocyte migration in peritoneal exudate induced by carrageenan, inhibition of MPO release from *in vitro* human neutrophils, and an anti-nociceptive action in the formalin test. Other studies have shown that statins, such as atorvastatin, prevented hypernociception (36) dependent on cytokine inhibition and prostanoid production. However, the present study is the first report of the reversal of lovastatin anti-nociception by naloxone.

Furthermore, atorvastatin was recently shown to be even more effective than diclofenac in decreasing joint inflammation and hyperalgesia in a rat model of arthritis (37), and in reducing TNF-α production in lipopolysaccharide-activated monocytes from diabetic patients (38). Another statin, simvastatin, was reported to prevent the increase of iNOS concentration after ischemia/reperfusion injury in the rat liver (39). In our case as well as in those studies, these effects of lovastatin were mediated, at least in part, by the reduction of cytokines such as TNF-α, and by the decrease of iNOS production. Besides being involved in inflammatory processes, iNOS has also been implicated in neuropathic pain (39). Kappa-opioid agonists have been shown to be powerful anti-inflammatory drugs (40). Nevertheless, new studies must be carried out in order to determine in more detail the beneficial actions of lovastatin and other statins regarding the reduction of pro-inflammatory mediators.

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