Doi: 10.1111/j.1742-7843.2008.00302.x
*Journal compilation* © 2009 Nordic Pharmacological Society. *Basic & Clinical Pharmacology & Toxicology*, **104**, 185–191

# Simvastatin and Indomethacin Have Similar Anti-Inflammatory Activity in a Rat Model of Acute Local Inflammation

**Lana Nežić[1], Ranko Škrbić[1], Silva Dobrić[2], Miloš P. Stojiljković[3], Vesna Jaćević[2], Svjetlana Stoisavljević Šatara[1], Zoran A. Milovanović[2] and Nataša Stojaković[1]**

[1]Department of Pharmacology, Toxicology and Clinical Pharmacology, Medical Faculty, University of Banja Luka, Banja Luka, Republic of Srpska, Bosnia and Herzegovina, [2]National Poison Control Centre, Medical Military Academy, Belgrade, Serbia, and [3]Department of Pharmacology, Medical Faculty, University of East Sarajevo, Foča, Republic of Srpska, Bosnia and Herzegovina

(Received July 11, 2007; Accepted February 25, 2008)

*Abstract:* Statins, such as simvastatin, lower circulating cholesterol levels and are widely prescribed for the treatment of hypercholesterolaemia. Several studies have shown unexpected effects of statins on inflammation. We studied the anti-inflammatory effect of simvastatin using a standard model of an acute local inflammation, the carrageenan-induced footpad oedema. Experimental groups (n = 6–8) were given simvastatin in a dose range 5–30 mg/kg, indomethacin 1–8 mg/kg and methylcellulose (control) *per os*. Footpad volume was measured with a plethysmograph and compared with the pre-injection volume of the same paw. Swelling (in microlitres) was then calculated, and in drug-treated animals, per cent inhibition was derived through comparison with the control group. Histopathological examination of the skin biopsies was performed to examine severity of paw skin lesions and to confirm the simvastatin-induced inhibition of acute inflammation. Both simvastatin and indomethacin administered orally, 1 hr before carrageenan injection, significantly reduced the extent of footpad oedema. Indomethacin dose-dependently blocked the swelling; the maximal effect was obtained with 8 mg/kg by 48.3% ($P < 0.05$). Simvastatin produced a comparable anti-inflammatory activity at a dose of 5 mg/kg (32%), while 10 and 30 mg/kg caused a 47.6% and 51.7% reduction, respectively, with the maximal effect observed at 20 mg/kg by 57.2% ($P < 0.05$). The comparison of the $ED_{50}$ of these agents on molar basis showed equipotent anti-inflammatory activity. Histopathological examination of the footpad skin biopsies revealed that simvastatin, dose-dependently and comparablly to indomethacin, reduced polymorphonuclear leucocyte infiltration. These data support the hypothesis that simvastatin has an acute anti-inflammatory activity.

Lipid-lowering drugs represent one of the most effective therapeutic approaches used in clinical practice for the prevention and treatment of atherosclerosis. Statins competitively inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the first committed step of sterol synthesis, and lower plasma cholesterol levels. They have been widely investigated in clinical trials as cholesterol-lowering agents to reduce morbidity and mortality from coronary artery disease [1–3]. Recent clinical trials have showed that statins reduce the risk of cardiovascular events even in the absence of a significant decrease in blood cholesterol levels [4,5], suggesting that the benefits of statin therapy may also be ascribed to their action on non-lipid factors involved in endothelial dysfunction, nitric oxide bioavailability, inflammation–fibroproliferation and plaque stability, important features of atherosclerosis [6–9].

The effectiveness and speed by which statins decrease coronary events already led to the speculation that statins may favourably influence vascular biology *via* mechanisms other than lowering the plasma cholesterol levels. Statins might directly influence the cellular events other than cholesterol synthesis, because mevalonate, the product of HMG-CoA reductase, is the precursor of not only cholesterol, but also of many non-steroidal isoprenoid compounds. The isoprenoids farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) are known to play an important role in signal transduction pathways by their attachment to signalling proteins, such as Ras and Rho, which play crucial roles in the regulation of cell adhesion and migration, cell growth responses and gene expression [10].

There is growing evidence from clinical studies that statins have additional anti-inflammatory properties unrelated to their lipid-lowering activity by reducing inflammatory parameters, such as C-reactive protein, interleukin-6 (IL-6) and tumour necrosis factor (TNF)-α in patients with hypercholesterolaemia [11], as well as monocyte chemoattractant protein-1 (MCP-1) and tissue factor in experimental endotoxaemia [12]. Furthermore, high-dose simvastatin demonstrates potent vasoprotective properties during endotoxaemia that may be useful for patients with acute systemic inflammation and associated vascular hyporeactivity [13]. Moreover, Pruefer *et al*. demonstrated that simvastatin was a potent and effective endothelium-protective agent that reduced leucocyte-endothelial cell interactions *in vivo* [14]. *In vitro* studies of anti-inflammatory actions of statins on cell events have been conducted to explain the effects beyond

Author for correspondence: Lana Nežić, Department of Pharmacology, Toxicology and Clinical Pharmacology, Medical Faculty, University of Banja Luka, Save Mrkalja 14, Banja Luka 78000, Republic of Srpska, Bosnia and Herzegovina (fax +387 51 216 527, e-mail lanaphare@yahoo.com).

the lipid-lowering ones. Inhibition of HMG-CoA reductase activity in human monocytes treated with lipopolysaccharide (LPS) reduced the production of IL-8, IL-6 and MCP-1, chemotactic cytokines that are expressed in human atherosclerotic lesions and responsible for leucocyte recruitment at the infection site [15].

In this study, we aimed to test potential anti-inflammatory effects of simvastatin in acute local inflammation and also to compare with that of indomethacin, a well-known anti-inflammatory drug. We used the carrageenan-induced rat paw oedema model, a standard model of an acute local inflammation often used for anti-inflammatory agent screening [16–21]. The critical feature of this model is its short duration. Because of that, simvastatin does not have time to alter plasma lipid levels even at high doses and, therefore, the results may be interpreted without this confounding variable.

## Materials and Methods

*Animals.* Adult male Wistar rats (Military Medical Academy Research Laboratories, Belgrade, Serbia), weighing 180–220 g, were used in the carrageenan-induced rat paw oedema. Experimental groups consisted of six to eight animals each. The animals were deprived of food 18–20 hr before beginning of experiments with free access to tap water. All experimental procedures in animals were conducted according to the Guidelines on Human Care of Experimental Animals adopted by the Ethical Committee of Military Medical Academy (Belgrade, Serbia) and to other corresponding national legal codes.

*Drugs.* Simvastatin (Krka, Novo Mesto, Slovenia) was dissolved in 0.5% methylcellulose (Sigma, Taufkirchen, Germany), as 10 or 20 mg/ml stocks. Indomethacin (Galenika, Belgrade, Serbia) was dissolved in aqueous methylcellulose and served as a standard anti-inflammatory drug. Carrageenan (Sigma) was dissolved in saline to prepare 0.5% solution.

*Carrageenan-induced rat paw oedema test.* The carrageenan-induced rat paw oedema test has been used as an experimental model for screening the anti-inflammatory activity. Simvastatin was administered orally *via* oral gavage in doses of 5, 10, 20 and 30 mg/kg. The doses of simvastatin used were comparable to those used previously in rat/murine studies *in vivo* and were higher than those used in human beings because of a significant up-regulation of HMG-CoA reductase induced by statin treatment in rodents [22–24]. Indomethacin was used as a reference in doses of 1, 2, 4 and 8 mg/kg orally. The control animals received methylcellulose in a dose of 1 ml/kg orally. Carrageenan-saline solution (0.5% in a volume of 0.1 ml) was injected into the plantar surface of the right hind paw 1 hr after the oral administration of simvastatin or indomethacin. Three hours later, footpad volume was measured with a mercury plethysmograph and compared with the pre-injection volume of the same paw. Swelling (in microlitres) was then calculated, and in drug-treated animals, per cent inhibition was derived through comparison with the vehicle (methylcellulose) control group.

*Histopathological examination.* At necropsy, the paws from the control, simvastatin- and indomethacin-treated were carefully spread over a metal tray coated with wax and fixed with 10% neutral buffered formalin solution. Five days after fixation, all skin samples were divided into five portions for preparation of tissue sections. After process of fixation, all tissue samples were dehydrated in graded alcohol (100%, 96% and 70%), xylol and embedded in paraffin blocks. Finally, 2-μm thick paraffin sections were stained by haematoxylin and eosin method. From each specimen, whole visual fields magnified by 40× were analysed using Olympus-2 microscope (Tokyo, Japan).

*Semiquantitative analysis.* The type, degree and severity of tissue lesions and an amount of inflammatory cells in the tissue samples were counted in five accidentally selected visual fields from each animal, magnification ×40. The severity of paw skin lesions (tissue damage score – TDS) was graded on a scale of 0–4, based on the amount of inflammatory cells, haemorrhages and oedema, as well as the number of foci involved.

0 = normal findings.

1+ = mild damages; mild dilatation of the blood vessels with no changes in continuation of their walls. A few foci of inflammatory cell infiltrates.

2+ = moderate damages; discrete oedema and hyperaemia; various numbers of inflammatory cells infiltrates.

3+ = severe and focal damages; increased blood volume and vasodilatation associated with extensive hyperaemia and oedema; accumulation of inflammatory cells.

4+ = severe and diffuse damages; strong vasodilatation with erythrocytes accumulation (stasis) associated with massive hyperaemia and oedema; diffuse and intensive accumulation of inflammatory cells.

*Statistical analysis.* Analysis of variance (Turkey's test, *post hoc* analysis) was used for the assessment of anti-inflammatory activity of the drugs. Results were considered significant when $P < 0.05$. The 50% effective doses ($ED_{50}$) of drugs tested were calculated by the Lichfield and Wilcoxon procedure [25] and the 95% confidence intervals were calculated.

Statistical evaluation of paw skin lesions was performed using commercial statistical software (Stat for Windows, R.4.5, Stat Soft Inc., Tulsa, OK, USA, 1993). In the tables, all results are shown as the mean (X) ± S.D. The differences in tissue damage scores between groups were compared using the Kruskal–Wallis rank test. The differences with values of $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered significant.

## Results

### *Anti-inflammatory activity of simvastatin and indomethacin in the carrageenan-induced rat paw oedema test.*

Cyclooxygenase inhibitor indomethacin, administered in a dose range of 1–8 mg/kg orally 1 hr before carrageenan challenge, blocked the paw swelling in a dose-dependent manner. Indomethacin given in a dose of 1 mg/kg reduced footpad swelling by 18.4%, while in doses of 2 and 4 mg/kg, it caused 40.4% and 37.4% reduction, respectively, that was significant compared to the control ($P < 0.05$). The maximal effect was obtained with a dose of 8 mg/kg that reduced footpad swelling by 48.3% ($P < 0.05$ versus control; table 1).

Oral administration of simvastatin in a dose range of 5–30 mg/kg orally 1 hr before carrageenan challenge produced an inhibition of the footpad swelling comparable to that of indomethacin. Importantly, strong anti-inflammatory activity was observed with as little as 5 mg/kg of simvastatin (32%) ($P < 0.05$ versus control), while the doses of 10 and 30 mg/kg caused 47.6% and 51.7% reduction, respectively ($P < 0.05$ versus control). The maximal effect was observed at a dose of 20 mg/kg, with 57.2% of footpad swelling reduction after carrageenan injection ($P < 0.05$ versus control; table 1).

 

*Table 1.*

Anti-inflammatory activity of indomethacin and simvastatin on carrageenan-induced paw oedema in rats. Anti-inflammatory activity of different doses of indomethacin and simvastatin given orally (doses are expressed in mg/kg and μmol/kg) compared to control (vehicle) on carrageenan-induced paw oedema in rats was estimated 3 hr after the administration of carrageenan.

| Drugs | Dose (mg/kg) | Dose (μmol/kg) | Anti-inflammatory activity (%) |
|---|---|---|---|
| Control (vehicle)[1] | – | – | 0.0 ± 7.5 |
| Indomethacin | 1 | 2.8 | 18.4 ± 12.7 |
| Indomethacin | 2 | 5.6 | 40.4 ± 7.6[2] |
| Indomethacin | 4 | 11.2 | 37.4 ± 4.5[2] |
| Indomethacin | 8 | 22.4 | 48.3 ± 10.7[2] |
| Simvastatin | 5 | 11.9 | 32.0 ± 16.0[2] |
| Simvastatin | 10 | 23.9 | 47.6 ± 7.7[2] |
| Simvastatin | 20 | 47.8 | 57.2 ± 6.4[2] |
| Simvastatin | 30 | 71.7 | 51.7 ± 7.4[2] |

[1]Methylcellulose (1 ml/kg). The values are expressed as the mean ± S.D. (n = 6–8), [2]P < 0.05 versus control, ANOVA (Turkey's test, *post hoc* analysis).

Testing the anti-inflammatory activities of simvastatin and indomethacin, using their doses expressed in μmol/kg (5 mg/kg = 11.9 μmol/kg and 4 mg/kg = 11.2 μmol/kg, respectively), showed that the difference between them was not significant. A similar effect was also shown for doses of 10 mg/kg (23.9 μmol/kg) of simvastatin and 8 mg/kg (22.4 μmol/kg) of indomethacin (P = n.s.; table 1).

*Antiinflammatory $ED_{50}$ of simvastatin and indomethacin.*

By using the Lichfield and Wilcoxon procedure, we calculated values of $ED_{50}$ of simvastatin and indomethacin. They were expressed as a mean with 95% confidence limits given in parenthesis (table 2). Although the $ED_{50}$ of simvastatin in mg/kg is almost twice that of indomethacin, the comparison of their $ED_{50}$ in μmol/kg failed to show any significant difference. This result strongly suggests that simvastatin produced an acute anti-inflammatory activity comparable to that of indomethacin.

*Table 2.*

The anti-inflammatory $ED_{50}$ of indomethacin and simvastatin in carrageenan-induced rat paw oedema test. The anti-inflammatory $ED_{50}$s of drugs tested for oral use were calculated by the Lichfield and Wilcoxon procedure [25] and the 95% confidence intervals were calculated. The $ED_{50}$s are expressed in mg/kg and μmol/kg.

| Drugs | $ED_{50}$ (95% confidence limits)[1] mg/kg | $ED_{50}$ (95% confidence limits)[1] μmol/kg |
|---|---|---|
| Indomethacin | 7.8 (1.5–42.2) | 22.1 (4.3–112.5) |
| Simvastatin | 16.7 (2.6–106.1) | 39.9 (6.3–253.5) |

[1]Calculated by the Lichfield and Wilcoxon procedure [25].

*Effects of simvastatin and indomethacin on inflammatory changes in rat paw skin samples.*

The light microscopic findings of the control tissue slices (untreated rats given only 0.5% carrageenan by subplantar injection) revealed an acute oedema in the dermis with extensive extravasations, mainly polymorphonuclear leucocytes (PMN leucocytes), less eosinophiles, basophiles, lymphocytes, macrophages and some degranulated monocytes. There were also vasculitis and hyperaemia around the vessels in the dermis. The most pronounced PMN leucocytes infiltration was found at the borderline between the dermis and subcutis. The muscle fibres were moved apart and irregularly disposed. The hair follicles were unchanged (fig. 1A). As shown in table 3, in this group of animals, a mean TDS of 3.66 ± 0.47 was noticed.

On the other hand, indomethacin dose-dependently reduced histological tissue injury induced by carrageenan. In the group of rats treated with a dose of 4 mg/kg, an extensive oedema without hyperaemia was present. Oedema and PMN leucocytes infiltration were focal and located

*Table 3.*

Effects of indometacin and simvastatin on the tissue damage scores (TDS) on carrageenan-induced paw oedema in rats. Effects of different doses of indomethacin (4 and 8 mg/kg, orally) and simvastatin (5–30 mg/kg, orally) on TDS 3 hr after administration of carrageenan, were compared to those of the control group. The TDS in the tissue samples were counted in five accidentally selected visual fields from each animal, magnified by 40× and was graded on a scale of 0–4, based on the amount of inflammatory cells, haemorrhages and oedema as well as the number of foci involved.

| Drugs | TDS (6 paw skin samples × 6 sections) 0 | 1 | 2 | 3 | 4 | X ± S.D. |
|---|---|---|---|---|---|---|
| Control (vehicle)[1] | 0 | 0 | 0 | 12 | 24 | 3.66 ± 0.47 |
| Indomethacin, 4 mg/kg | 0 | 0 | 19 | 17 | 0 | 2.47 ± 0.51[2] |
| Indomethacin, 8 mg/kg | 0 | 18 | 18 | 0 | 0 | 1.50 ± 0.51[2,3] |
| Simvastatin, 5 mg/kg | 0 | 0 | 0 | 19 | 17 | 3.47 ± 0.51 |
| Simvastatin, 10 mg/kg | 0 | 0 | 10 | 26 | 0 | 2.72 ± 0.43[2,4] |
| Simvastatin, 20 mg/kg | 0 | 22 | 14 | 0 | 0 | 1.36 ± 0.47[2,4] |
| Simvastatin, 30 mg/kg | 0 | 16 | 20 | 0 | 0 | 1.55 ± 0.50[2,4] |

[1]Methylcellulose (1 ml/kg). The differences in TDS between groups were statistically analysed using the Kruskal–Wallis rank test and results were expressed in X ± S.D. [2]P < 0.001 versus control, [3]P < 0.001 versus indomethacin 4 mg/kg, [4]P < 0.001 versus simvastatin 5 mg/kg.

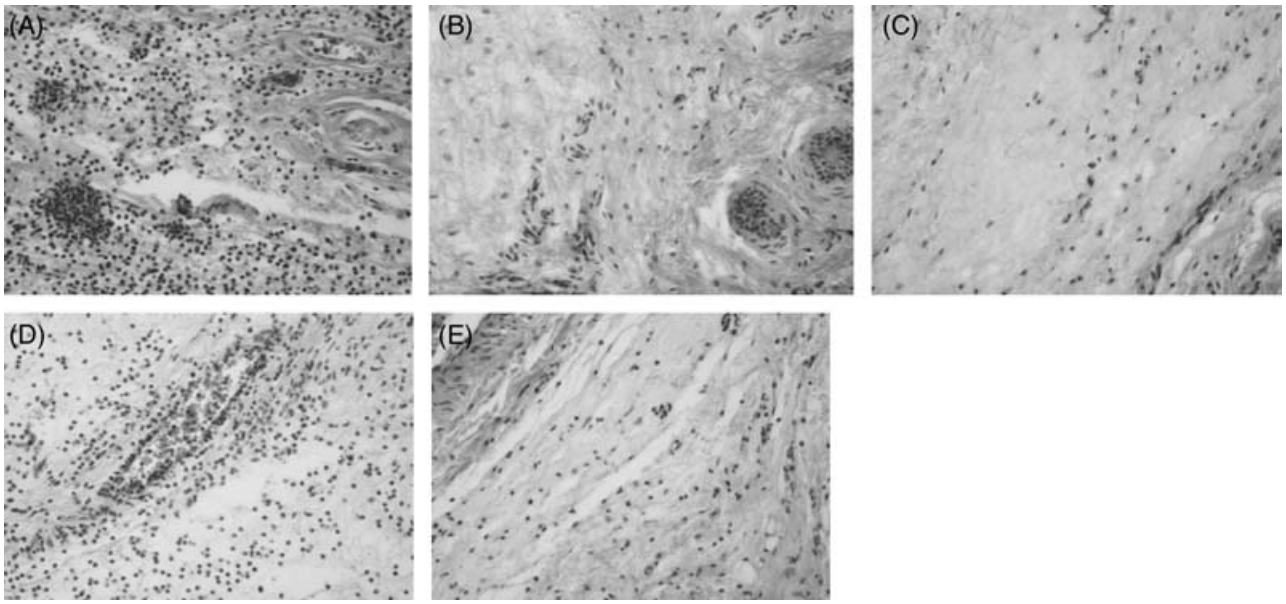


Fig. 1. Histological evidence of decreased inflammation in paw skin samples of rats treated with indomethacin and simvastatin. A representative section from each group of six rats is shown. (A) Control (untreated) rats given carrageenan, acute oedema, hyperaemia, extensive PNM leucocytes infiltration with eosinophiles, basophiles and lymphocytes are seen, with disorganisation of muscle fibres. (B) Carrageenan-challenged rats treated with indomethacin (4 mg/kg, orally); (C) Carrageenan-challenged rats treated with indomethacin (8 mg/kg, orally); (D) Carrageenan-challenged rats plus treatment with simvastatin (5 mg/kg, orally); (E) Carrageenan-challenged rats plus treatment with simvastatin (20 mg/kg, orally); a dose-dependent reduction in inflammatory infiltrates is seen compared to (A); haematoxylin and eosin staining, magnification ×40.

mainly at the borderline of the dermis and subcutis, as well as in the deeper layers of the subcutaneous tissue. The endothelial cells were activated and hair follicles were unchanged (fig. 1B). A mean TDS of $2.47 \pm 0.51$ was established in this group, which was significantly reduced compared to the carrageenan-only treated group ($P < 0.001$; table 3). These changes were still more reduced in the group of rats treated by higher dose of indomethacin (8 mg/kg) (TDS was $1.50 \pm 0.51$; $P < 0.001$ versus control and the group received 4 mg/kg of indomethacin; table 3). The endothelial cells were activated without proliferation and the hair follicles were unchanged (fig. 1C).

Simvastatin dose-dependently reduced histological tissue injury induced by carrageenan. In the group treated with simvastatin 5 mg/kg, the histopathological changes at the inflamed site were similar to those in the control group, but by 5% reduced (fig. 1D). In rats treated with simvastatin in a dose of 10 mg/kg, oedema and hyperaemia in particular parts of the dermis were significantly less pronounced in comparison with the control group. Although the PMN leucocytes infiltration was reduced in this group, it still remained diffused. The endothelial cells were activated and hair follicles were unchanged (TDS was $2.72 \pm 0.43$; $P < 0.001$ versus control and the group received 5 mg/kg of simvastatin; table 3).

In the groups of rats treated with simvastatin in doses of 20 and 30 mg/kg, most of the histological changes caused by carrageenan were significantly minimized (TDS were $1.36 \pm 0.47$ and $1.55 \pm 0.50$, respectively; $P < 0.001$ versus control and the groups received 5 mg/kg or 10 mg/kg of simvastatin; table 3). In the dermis and subcutis, oedema was mild without hyperaemia. PMN leucocytes infiltration was markedly reduced and focally disposed only at the borderline between the dermis and subcutis, as well as in the deeper layers of the subcutaneous tissue. The endothelial cells were activated and small numbers were proliferated (fig. 1E).

## Discussion

Subplantar injection of carrageenan is a common model for studying an acute inflammation, followed by swelling of the footpad that can be reproducibly measured after 3 hr [19,26–30]. Inhibition of footpad swelling provides a well-characterized gauge of anti-inflammatory activity. In this experiment, it was shown that simvastatin produced strong anti-inflammatory activity comparable to that of indomethacin in the carrageenan-induced rat paw oedema. The potency and effectiveness of simvastatin in this model were dose-dependant, with a maximal effect achieved by the highest doses used. The comparison of the $ED_{50}$ of these agents on a molar basis showed equipotent anti-inflammatory activity. Both simvastatin and indomethacin were effective acutely (i.e. when administered 1 hr before carrageenan injection), and neither compound was effective when administered 24 hr before injection as it was published earlier [22]. This anti-inflammatory effect could not be caused by plasma lipid-lowering mechanism, because simvastatin does not change plasma lipid levels in rats in a short treatment

 

course. Importantly, the strong anti-inflammatory effect of simvastatin in this experiment occurred within 3 hr, well before any lipid changes could possibly occur [22]. These observations suggest that although plasma lipids may affect inflammation, they cannot account for all of the anti-inflammatory effects of statins observed. These data strongly support the hypothesis that simvastatin has anti-inflammatory activity beyond its cholesterol-lowering activity.

The footpad swelling affected by carrageenan represents an acute inflammatory response that has been described as a biphasic event: the early phase, observed around 1 hr, is attributed to the release of histamine, serotonin and bradykinin, and, to less extent, prostaglandins, while the late phase (over 1 hr) has been linked to PMN leucocytes infiltration, as well as to the continuing of the prostaglandin production [26]. Carrageenan activates macrophages and PMN leucocytes, which secrete numerous pro-inflammatory mediators. In this delayed phase of carrageenan-induced inflammation, there is a production of PMN leucocytes derived reactive oxygen species and free radicals [21,31,32], as well as nitric oxide, cytokines such as TNF-α [33,34] and IL-6 [35] that have been shown to play an important role in various forms of inflammation.

Bearing in mind the mediators involved in the late or second phase of carrageenan-induced paw oedema, it could mean that anti-inflammatory effects of simvastatin, similarly to indomethacin, are probably related to inhibition of the main pro-inflammatory mediators production or PMN leucocytes infiltration as well as to inhibition of release of PMN leucocyte-derived mediators including free radicals. The hypothesis of simvastatin blocking activity on PMN leucocytes infiltration is in accordance with our histological examination demonstrating reduced PMN leucocytes infiltration and tissue injury in carrageenan-treated paw when rats were pre-treated with high doses of simvastatin. This effect was comparable to that of indomethacin. There are many articles which have shown the influence of statins on leucocytes recruitment in the inflammation site. Statins significantly attenuate leucocytes rolling, adherence and transmigration through inhibition of adhesion molecules expression [14,36], up-regulation of endothelial nitric oxide synthase [37] and stimulated nitric oxide production in endothelial cells [38,39]. Furthermore, it has been shown that statins exert anti-inflammatory effects in an acute local inflammation or *in vitro* by inhibition of pro-inflammatory mediators production such as IL-6, MPC-1 and RANTES (regulated on activation, normal T cell expressed and secreted) [35,40], as well as TNF-α, IL-1β [41] and macrophage cyclooxygenase-2 [42]. Statins might also mediate anti-inflammatory effects in part through their actions on decreased reactive oxygen species production [13,43]. The studies of Weitz-Schmit *et al*. have shown that some statins seem to have allosteric properties that allow them to block cell–cell interactions directly. They demonstrated in an *in vitro* study and in carrageenan-induced rat paw oedema that lovastatin and simvastatin bind to an allosteric site (L-site, named for lovastatin) of the β2-integrin leucocyte function antigen-1 (LFA-1) [44,45] which plays an important role in leucocyte adhesion and migration and T-cell activation, and consequently reduce the interaction mediated by intercellular adhesion molecule-1/LFA-1.

The study by Diomde *et al*. [35] sheds light on the mechanism responsible for the inhibitory effects of statins on leucocyte recruitment. They compared the effects of sterol and non-sterol derivatives arising from mevalonate biosynthesis in an *in vivo* model of local acute inflammation, in mice receiving within 24 hr three repetitive doses of one of the three different statins (lovastatin, simvastatin or pravastatin) or squalestatin, a selective inhibitor of the synthesis of sterol derivatives only. The short-term treatment with statins inhibited leucocyte recruitment and the exudate production of IL-6, MCP-1 and RANTES. Co-administration of mevalonate reversed the effect of statin on leucocyte recruitment. The inhibition of sterol synthesis by squalestin did not have any anti-inflammatory effect, indicating that biosynthesis of non-sterol compounds arising from mevalonate is crucial for the *in vivo* regulation of pro-inflammatory cytokine and chemokine production by statins and therefore leucocyte migration. In a recent study, rapid anti-inflammatory effects of high doses of simvastatin have been demonstrated in the murine model of acute allergic asthma. Simvastatin reduced the total inflammatory cell infiltrate and eosinophilia, as well as IL-4 and IL-5 levels in bronchoalveolar lavage fluid [46]. Together, these studies have shown direct effects of statins on leucocyte recruitment that are unrelated to plasma cholesterol-lowering activity.

It is difficult to define *in vivo* the specific molecular mechanism by which statins affect the cell migration, because of the complexity of the cholesterol biosynthesis. Statins, via inhibition of mevalonate pathway, block the synthesis of isoprenoids, GGPP and FPP, and facilitate accumulation of inactive Rho and Ras in the cytoplasm. Furthermore, statins have also been reported to inhibit nuclear factor kappa B (NF-kB) activation, a transcription factor that is activated in response to inflammatory stimuli, such as cytokines (e.g. IL1-β, TNF-α) or trough activated glucose transporter protein (GTP)-binding proteins (Ras–Rho). In fact, on stimulation NF-κB enters the nucleus where it can induce the transcription of inflammatory genes and numerous pro-inflammatory cytokines production. The most frequently proposed model is that statins interrupt the pro-inflammatory signalling by down-regulation of Rho-related protein activation, which, in turn, requires post-translational modification involving non-sterol mevalonate-derived compounds to be active. Increasing evidence suggests that statins are able to down-regulate IL-6 and MCP-1 transcription as a consequence of interference with the schistosome GTP-binding proteins/NF-κB transduction pathway [10,47]. Moreover, the study by Paumelle *et al*. has been reported that simvastatin requires peroxisome proliferator-activated receptor (PPAR) α expression to exert its anti-inflammatory effects *in vivo* in models of acute local inflammation and *in vitro* in macrophages and neutrophils. Simvastatin, used *in vivo* in subcutaneous air pouches and carrageenan footpad oedema in similar doses

 

as those used in our study, dose-dependently and significantly decreased neutrophil recruitment. In addition, in *in vitro* conditions, it reduced activity of inducible nitric oxide synthase, as well as IL-6 and TNF-α levels, via PPARα-dependent mechanism. It has been confirmed that PPARα exerts anti-inflammatory activities by negatively interfering with pro-inflammatory signalling pathways including NF-κB [48].

Leucocyte infiltration plays the major role of the early inflammatory response in the inflammation and tissue damage associated with both infectious and non-infectious diseases. Our results support the hypothesis that simvastatin has a potent anti-inflammatory effect in acute local inflammation, which might be a consequence of its inhibitory effect on PMN leucocyte infiltration. It is important to emphasize that simvastatin, as shown in our study, could have an anti-inflammatory effect when administered orally, which is the route of administration of the drug in clinical practice. However, for detailed elucidation of effects of simvastatin on carrageenan-induced acute inflammatory response, further studies are required. They might lead to new understanding of the actions of statins, and possibly, to new therapeutic indications for these drugs.

## References


1 Scandinavian Simvastatin Survival Study. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the survival study group. Lancet 1994;**344**:1383–9.

2 Zhou Z, Rahme E, Pilote L. Are statins created equal? Evidence from randomized trials of pravastatin, simvastatin, and atorvastatin for cardiovascular disease prevention. Am Heart J 2006;**151**:273–81.

3 Ford I, Murray H, Packard CJ, Shepherd J, Macfarlane PW, Cobbe SM, the West of Scotland Coronary Prevention Study Group. Long-term follow-up of the West of Scotland Coronary Prevention Study. N Engl J Med 2007;**357**:1477–86.

4 John S, Schneider MP, Delles C, Jacobi J, Schmieder RE. Lipid-independent effects of statins on endothelial function and bioavailability of nitric oxide in hypercholesterolemic patients. Am Heart J 2005;**149**:473.

5 Sanguigni V, Pignatelli P, Lenti L, Ferro D, Bellia A, Carnevale R *et al*. Short-term treatment with atorvastatin reduces platelet CD40 ligand and thrombin generation in hypercholesterolemic patients. Circulation 2005;**111**:412–9.

6 Rutishauser J. The role of statins in clinical medicine-LDL-cholesterol lowering and beyond. Swiss Med Wkly 2006;**136**:41–9.

7 Son JW, Koh KK, Ahn JY, Jin DK, Park GS, Kim DS *et al*. Effects of statin on plaque stability and thrombogenicity in hypercholesterolemic patients with coronary artery disease. Int J Cardiol 2003;**88**:77–82.

8 März W, Köenig W. HMG-CoA reductase inhibition: anti-inflammatory effects beyond lipid lowering? J Cardiovasc Risk 2003;**10**:169–79.

9 Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999;**340**:115–26.

10 Seasholtz TM, Brown JH. RHO signaling in vascular diseases. Mol Interv 2004;**4**:348–57.

11 Devaraj S, Chan E, Jialal I. Direct demonstration of an anti-inflammatory effect of simvastatin in subjects with the metabolic syndrome. J Clin Endocrinol Metab 2006;**91**:4489–96.

12 Steiner S, Speidl WS, Pleiner J, Seidinger D, Zorn G, Kaun C *et al*. Simvastatin blunts endotoxin-induced tissue factor *in vivo*. Circulation 2005;**111**:1841–6.

13 Pleiner J, Schaller G, Mittermayer F, Zorn S, Marsik C, Polterauer S *et al*. Simvastatin prevents vascular hyporeactivity during inflammation. Circulation 2004;**110**:3349–54.

14 Pruefer D, Makowski J, Schnell M, Buerke U, Dahm M, Oelert H *et al*. Simvastatin inhibits inflammatory properties of Staphylococcus aureus alpha-toxin. Circulation 2002;**106**:2104–10.

15 Rezaie-Majd A, Maca T, Bucek RA, Valent P, Müller MR, Husslein P *et al*. Simvastatin reduces expression of cytokines interleukin-6, interleukin-8, and monocyte chemoattractant protein-1 in circulating monocytes from hypercholesterolemic patients. Arterioscler Thromb Vasc Biol 2002;**22**:1194–9.

16 Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962;**11**:544–7.

17 Pinheiro RM, Calixto JB. Effect of the selective COX-2 inhibitors, celecoxib and rofecoxib in rat acute models of inflammation. Inflamm Res 2002;**51**:603–10.

18 Petrović SD, Dobrić S, Bokonjić D, Niketić M, Garcia-Pineres A, Merfort I. Evaluation of Tanacetum larvatum for an anti-inflammatory activity and for the protection against indomethacin-induced ulcerogenesis in rats. J Ethnopharmacol 2003;**87**:109–13.

19 Kundaković T, Fokialakis N, Dobrić S, Pratsinis H, Kletsas D, Kovačević N *et al*. Evaluation of the anti-inflammatory and cytotoxic activities of naphthazarine derivatives from *Onosma leptantha*. Phytomedicine 2006;**13**:290–4.

20 Abdel-Salam OM, Baiuomy AR, Arbid MS. Studies on the anti-inflammatory effect of fluoxetine in the rat. Pharmacol Res 2004;**49**:119–31.

21 Halici Z, Dengiz GO, Odabasoglu F, Suleyman H, Cadirci E, Halici M. Amiodarone has anti-inflammatory and anti-oxidative properties: an experimental study in rats with carrageenan-induced paw edema. Eur J Pharmacol 2007;**566**:215–21.

22 Sparrow CP, Burton CA, Hernandez M, Mundt S, Hassing H, Patel S *et al*. Simvastatin has anti-inflammatory and antiatherosclerotic activities independent of plasma cholesterol lowering. Arterioscler Thromb Vasc Biol 2001;**21**:115–21.

23 Ni W, Egashira K, Kataoka C, Kitamoto S, Koyanagi M, Inoue S *et al*. Anti-inflammatory and antiarteriosclerotic actions of HMG-CoA reductase inhibitors in a rat model of chronic inhibition of nitric oxide synthesis. Circ Res 2001;**89**:415–21.

24 Leung, BP, Sattar N, Crilly A, Prach M, McCarey DW, Payne H *et al*. A novel anti-inflammatory role for simvastatin in inflammatory arthritis. J Immunol 2003;**170**:1524–30.

25 Litchfield JT, Wilcoxon FA. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 1949;**96**:99–113.

26 Di Rosa M, Giroud JP, Willoughby DA. Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J Pathol 1971;**104**:15–29.

27 Pinheiro RM, Calixto JB. Effect of the selective COX-2 inhibitors, celecoxib and rofecoxib in rat acute models of inflammation. Inflamm Res 2002;**51**:603–10.

28 Francischi JN, Chaves CT, Moura AC, Lima AS, Rocha OA, Ferreira-Alves DL *et al*. Selective inhibitors of cyclo-oxygenase-2 (COX-2) induce hypoalgesia in a rat paw model of inflammation. Br J Pharmacol 2002;**137**:837–44.

29 Miceli N, Taviano MF, Giuffrida D, Trovato A, Tzakou O, Galati EM. Anti-inflammatory activity of extract and fractions from Nepeta sibthorpii Bentham. J Ethnopharmacol 2005;**97**:261–6.

30 Suleyman H, Gul HI, Gul M, Alkan M, Gocer F. Anti-inflammatory activity of bis(3-aryl-3-oxo-popyl) methylamine hydrochloride in rat. Biol Pharm Bull 2007;**30**:63–7.

31 Salvemini D, Wang ZQ, Bourdon DM, Stern MK, Currie MG, Manning PT. Evidence of peroxynitrite involvement in the


 

carrageenan-induced rat paw oedema. Eur J Pharmacol 1996;**303**:217–20.
32 Boughton-Smith NK, Deakin AM, Follenfant RL, Whittle BJR, Coarland LG. Role of oxygen radicals and arachidonic acid metabolites in the reverse passive arthus reaction and carrageenan paw oedema in the rat. Br J Pharmacol 1999;**110**:896–902.
33 Nacife VP, Soeiro MN, Gomes RN, D'Avila H, Castro-Faria NHC, Meirelles MN. Morphological and biochemical characterization of macrophages activated by carrageenan and lipopolysaccharide *in vivo*. Cell Struct Funct 2004;**29**:27–34.
34 Marzocco S, Di Paola R, Serraino I, Sorrentino R, Meli R, Mattaceraso G *et al*. Effect of methylguanidine in carrageenan-induced acute inflammation in the rats. Eur J Pharmacol 2004;**484**:341–50.
35 Diomede L, Albani D, Sottocorno M, Polentarutti N, Donati MB, Bianchi M *et al*. The *in vivo* anti-inflammatory effect of statins is mediated by nonsterol mevalonate products. Arterioscler Thromb Vasc Biol 2001;**21**:1327–32.
36 Landsberger M, Wolff B, Jantzen F, Rosenstengel C, Vogelgesang D, Staudt A *et al*. Cerivastatin reduces cytokine-induced surface expression of ICAM-1 via increased shedding in human endothelial cells. Atherosclerosis 2007;**190**:43–52.
37 Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of endothelial nitric oxide synthase by HMG-CoA reductase inhibitors. Circulation 1998;1129–35.
38 Scalia R, Gooszen ME, Jones SP, Hoffmeyer M, Rimmer DM, Trocha SD *et al*. Simvastatin exerts both anti-inflammatory and cardioprotective effects in apolipoprotein E-deficient mice. Circulation 2001;**103**:2598–603.
39 Stalker TJ, Lefer AM, Scalia R. A new HMG-CoA reductase inhibitor, rosuvastatin, exerts anti-inflammatory effects on the microvascular endothelium: the role of mevalonic acid. Br J Pharmacol 2001;**133**:406–12.
40 Veillard NR, Braunersreuther V, Arnaud C, Burger F, Pelli G, Steffens S *et al*. Simvastatin modulates chemokine and chemokine receptor expression by geranylgeranyl isoprenoid pathway in human endothelial cells and macrophages. Atherosclerosis 2006;**188**:51–8.
41 Xu H, Liu P, Liang L, Danesh FR, Yang X, Ye Y *et al*. RhoA-mediated, tumor necrosis factor alpha-induced activation of NF-kappaB in rheumatoid synoviocytes: inhibitory effect of simvastatin. Arthritis Rheum 2006;**54**:3441–51.
42 Habib A, Shamseddeen I, Nasrallah MS, Antoun TA, Nemer G, Bertoglio J *et al*. Modulation of COX-2 expression by statins in human monocytic cells. FASEB J 2007;**21**:1665–74.
43 Bandoh T, Sato EF, Mitani H, Nakashima A, Hoshi K, Inoue M. Antioxidative potential of fluvastatin via the inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity. Biol Pharm Bull 2003;**26**:818–22.
44 Weitz-Schmidt G, Welzenbach K, Brinkmann V, Kamata T, Kallen J, Bruns C *et al*. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. Nat Med 2001;**7**:687–92.
45 Weitz-Schmidt G, Welzenbach K, Dawson J, Kallen J. Improved lymphocyte function-associated antigen-1 (LFA-1) inhibition by statin derivatives: molecular basis determined by x-ray analysis and monitoring of LFA-1 conformational changes *in vitro* and *ex vivo*. Biol Chem 2004;**279**:46764–71.
46 McKay A, Leung BP, McInnes IB, Thomson NC, Liew FY. A novel anti-inflammatory role of simvastatin in a murine model of allergic asthma. J Immunol 2004;**172**:2903–8.
47 Abeles AM, Pillinger MH. Statins as antiinflammatory and immunomodulatory agents: a future in rheumatologic therapy? Arthritis Rheum 2006;**54**:393–407.
48 Paumelle R, Blanquart C, Briand O, Barbier O, Duhem C, Woerly G *et al*. Acute antiinflammatory properties of statins involve peroxisome proliferator-activated receptor-alpha via inhibition of the protein kinase C signaling pathway. Circ Res 2006;**98**:361–9.

 