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Short Communication

Topical anti-inflammatory effect of hypocholesterolaemic drugs

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Abstract

Objectives The topical anti-inflammatory effect of simvastatin, atorvastatin, pravastatin, ezetimibe and combined ezetimibe + simvastatin was investigated, using the croton oil model of ear oedema in mice.

Methods Simvastatin, atorvastatin, pravastatin, ezetimibe and ezetimibe + simvastatin combination (dissolved in 20 µl of 70% acetone) were topically applied simultaneously with croton oil (200 µg/ear, dissolved in 20 µl of 70% acetone) at the inner surface of each ear. Ear oedema and myeloperoxidase activity, indicative of polymorphonuclear cell migration, were assessed 6 h after inflammatory stimuli.

Key findings It was found that statins can act as topical anti-inflammatories, but the pharmacological effect is dependent on statin polarity. At 0.3 mg/ear inhibition of ear oedema was 79%, 67% and 40% for simvastatin, atorvastatin and pravastatin, respectively. Simvastatin and atorvastatin also remarkably diminished myeloperoxidase activity, even at low concentrations (0.03 mg/ear). Pravastatin, the most polar statin, however, did not cause any reduction in ear oedema or myeloperoxidase activity at low doses. The order of topical anti-inflammatory activity was pravastatin $<<<$ atorvastatin $\leq$ simvastatin. Ezetimibe, another hypocholesterolaemic drug, also presented anti-inflammatory effects, inhibiting ear oedema by 64% at 0.3 mg/ear. However, when used in combination with simvastatin, no further beneficial effect was observed.

Conclusions These results consistently support current evidence showing that statins can be used for treatment of dermatological disorders. Polarity of the molecule, however, is a factor that should be considered before recommending use.

Keywords atorvastatin; croton oil ear oedema; ezetimibe; pravastatin; simvastatin

Introduction

The beneficial effects of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) in cardiovascular disease have generally been attributed to their cholesterol-lowering properties. However, many in-vitro and in-vivo studies have indicated that statins have direct anti-inflammatory effects that are not mediated by their hypocholesterolaemic activity.[1]

Recently, it has been suggested that statins could be invaluable in the treatment of a multiplicity of dermatological disorders, especially those characterized by ingress of activated leucocytes into the skin, such as alopecia areata, vitiligo, psoriasis and others.[2] However, there is scarce evidence whether these drugs can be topically applied. One single study was recently conducted by Otuki *et al*.,[3] presenting preclinical evidence that the application of topical simvastatin could be effective in the treatment of skin inflammatory conditions. The efficacy of the other clinically relevant statins, such as pravastatin and atorvastatin, has not yet been investigated.

Ezetimibe belongs to a new class of cholesterol-lowering drugs. It is a potent inhibitor of cholesterol absorption and has been successfully used in combination with statins. Combined ezetimibe + statin therapy provides an improvement in lipid-lowering capacity compared with statins alone.[4,5] Furthermore, previous studies have shown that, in addition

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to their favorable effects on lipid levels, the combination ezetimibe + simvastatin significantly reduced C-reactive protein levels compared with simvastatin monotherapy, possibly consistent with an additional anti-inflammatory effect.[6] In contrast, there is no evidence that such a combination has topical anti-inflammatory properties.

It is well known that permeation of topically applied drugs through the stratum corneum (SC) barrier of the skin is required to achieve therapeutic effects. The ability to cross biological membranes strongly affects the pharmacokinetic behaviour of drugs and their capacity to access the receptor site. An optimal hydrophilic–lipophilic balance is essential for the cutaneous penetration of drugs.[7] Thus, it is reasonable to suppose that drugs with different polarities may pass through the skin to different degrees and that this can affect their pharmacological properties.

Statins, which can be natural fungal (lovastatin, simvastatin, mevastatin, pravastatin) or synthetic (atorvastatin, fluvastatin, rosuvastatin) compounds, possess different polarities. Pravastatin is hydrophilic, while simvastatin and atorvastatin are lipophilic drugs.[8] These drugs may therefore pass through skin to different degrees by passive diffusion. In fact, it has been already shown that pravastatin does not easily cross cellular membranes whereas simvastatin does.[9]

Therefore, the present work had two main purposes: one was to compare the topical anti-inflammatory effects of pravastatin, simvastatin and atorvastatin; the other was to investigate whether ezetimibe, or the simvastatin + ezetimibe combination possess further beneficial topical anti-inflammatory effects in the croton oil model of skin inflammation in mice.

Materials and Methods

Chemicals

Croton oil, dexamethasone, hexadecyltrimethylammonium bromide, phosphate-buffered saline and o-dianisidine dihydrochloride were purchased from Sigma (St Louis, USA). Hydrogen peroxide was purchased from Merck (Darmstadt, Germany). Simvastatin, atorvastatin, pravastatin and ezetimibe were obtained from Galena (Campinas, Brazil). Simvastatin was activated from its inactive lactone proform to its active dihydroxy open acid forms by alkaline hydrolysis, as described previously.[10] All other reagents were of the highest commercially available grade.

Animals

Male Swiss mice, 30–40 g, were used in the experiments. The mice were kept in boxes on a 12-h day–night cycle, with food and water freely available. All experiments were approved by the Animal Ethics Committee of the State University of Maringá.

Ear oedema induction

Ear oedema was induced by croton oil inflammation.[11] Simvastatin, atorvastatin, pravastatin, ezetimibe, ezetimibe + simvastatin combination and dexamethasone (dissolved in 20 µl of 70% acetone) were topically applied simultaneously with croton oil (200 µg/ear, dissolved in 20 µl of 70% acetone) at the inner surface of each ear (treated groups). The inflamed control group (CO + V) received only topical application of croton oil plus vehicle and the non-inflamed control group received only vehicle (V). Ear oedema was evaluated 6 h after the inflammatory stimulus as the increase in ear weight. Equation 1 was used to determine the percentage of oedema inhibition.

$$\% \text{ inhibition} = \frac{[\text{inflamed ear weight (CO + V)} - \text{treated ear weight}]}{[\text{inflamed ear weight (CO + V)} - \text{non-inflamed ear weight}]} \times 100 \quad (1)$$

Myeloperoxidase activity

Myeloperoxidase activity (MPO), indicative of polymorphonuclear cell migration, was assessed 6 h after the inflammatory stimuli. The ear tissue was disrupted using a van Potter homogenizer and appropriate buffer (0.5% hexadecyltrimethylammonium bromide in 50 mM potassium phosphate buffer, pH 6.0), following 30 s of sonication. The mixture was then incubated for 1 h at 60°C (for catalase inactivation) and after that, sonication was repeated. The homogenate was centrifuged and the supernatant was used for enzyme activity measurement. MPO was assayed spectrophotometrically: 10 µl of the supernatant were mixed with 200 µl of a reaction medium containing o-dianisidine dihydrochloride (0.167 mg/ml) and hydrogen peroxide (0.0005%) in phosphate buffer (50 mM, pH 6.0). MPO activity was expressed as increase in optical density at 460 nm.[12] A relation similar to that shown in Equation (1) was used to calculate the percentage of MPO activity inhibition.

Statistical analysis

Results were expressed as means ± standard errors of the mean (SEM) and evaluated by analysis of variance complemented by Tukey's post-hoc testing, considering $P < 0.05$ as indicative of significance.

Results

The comparative effect of simvastatin, atorvastatin and pravastatin on ear oedema in mice can be seen in Figure 1a. Topical application of croton oil induced cutaneous inflammation in the ears of mice, which caused a significant increase in ear weight compared with untreated ears. As a positive control, dexamethasone (0.1 mg/ear) significantly inhibited the ear edema by 89%. Simvastatin had the greatest effect of all statins, reducing ear oedema in a dose-dependent manner. The calculated inhibition was 79% and 55% with 0.3 mg/ear and 0.03 mg/ear, respectively. Atorvastatin also reduced ear oedema in a dose-dependent manner, causing 67% of inhibition with 0.3 mg/ear and 40% with 0.03 mg/ear. Pravastatin exhibited the lowest anti-inflammatory effect, achieving only 40% of inhibition with 0.3 mg/ear or causing no reduction in ear oedema with 0.03 mg/ear.

Another event associated with the topical application of croton oil is a massive tissue polymorphonuclear cell infiltration, reflected by the increased MPO activity (Figure 1b). Treatment with both simvastatin and atorvastatin produced a

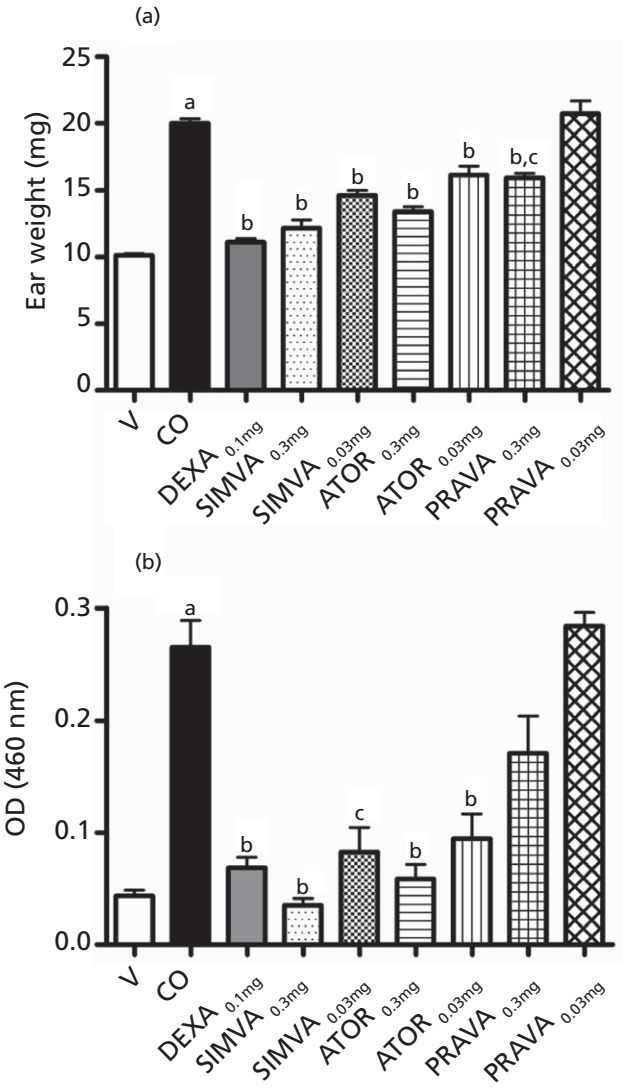

Figure 1 Effect of simvastatin (SIMVA), atorvastatin (ATOR), pravastatin (PRAVA) or dexamethasone (DEXA) topically administered on croton oil-induced mouse ear oedema (a) and myeloperoxidase activity (b). The ear oedema was measured at 6 h after croton oil treatment. MPO activity was measured in the homogenate supernatant from croton oil-treated ears 6 h after croton oil treatment. Each bar represents the mean ± SEM for 8–10 mice. [a]$P < 0.001$ compared with vehicle (V) group; [b]$P < 0.001$ compared with croton oil (CO) group; [c]$P < 0.001$ compared with $SIMVA_{0.3mg}$ group.

remarkable inhibition of MPO activity, completely suppressing polymorphonuclear cell migration at the highest dose (0.3 mg/ear). For this parameter, the calculated inhibition with 0.03 mg/ear was 81% and 77%, respectively, for simvastatin and atorvastatin. Once again, pravastatin was the least efficient cell infiltration blocker, causing only 40% of inhibition at the highest dose and producing no benefit at the lowest dose.

To investigate whether other hypocholesterolaemic drugs exhibit anti-inflammatory properties, experiments with ezetimibe were performed. The effect of this drug alone or combined with simvastatin on ear oedema and MPO activity can be seen in Figures 2 and 3. Treatment with ezetimibe inhibited ear oedema by 64% and 44% with a dose of 0.3 and 0.03 mg/ear, respectively (Figure 2a). MPO activity was also inhibited by 92% at both concentrations (Figure 2b). The treatment with the simvastatin + ezetimibe combination (0.6 mg + 0.15 mg/ear), however, did not cause any additional reduction in ear weight or MPO activity. The inhibitory effect of this combination of drugs was compared with that seen when the ears were treated with the same concentration of simvastatin alone (Figure 3).

Discussion

These findings consistently support the hypothesis that hypocholesterolaemic drugs possess topical anti-inflammatory properties. With statins, the effect is dependent on statin polarity. Pravastatin is relatively hydrophilic with an octanol–water partition coefficient (log P) equal to 2.20, while simvastatin ($\log P = 4.7$) and atorvastatin ($\log P = 5.7$) are lipophilic drugs.[8,13] In this work the order of topical anti-inflammatory activity was pravastatin $<<<$ atorvastatin $\leq$ simvastatin, albeit there was no significant statistical difference between the anti-inflammatory effect of simvastatin and atorvastatin ($P > 0.05$).

These results are in accordance with a previous study showing that the statins elution order in an immobilized artificial membrane system is pravastatin $<<<<$ atorvastatin $<<$ simvastatin, which indicates that pravastatin exhibits the lowest affinity for phospholipid monolayers.[13] In general terms this sequence corresponds to the sequence for increasing anti-inflammatory activity as given above. Pravastatin, which presents greater hydrophilicity, clearly has lower rates of passive diffusion and presents also the lowest topical anti-inflammatory activity. Therefore, the results of this study corroborate the well-established notion that the more a molecule crosses the cellular membrane by passive diffusion the greater the amount reaches its target and can exert its therapeutic effect.

The anti-inflammatory effect of statins has been intensively investigated in the past few years. The most probable molecular mechanism of their action is that by blocking the HMG-CoA reductase activity they reduce the production of mevalonate. The latter is the precursor of isoprenoids, farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP). These compounds in turn serve as lipophilic attachments for various signalling molecules, such as the small GTPases, Ras, Rac and Rho, involved in cell proliferation, cytoskeletal organization and activation of transcription factors.[14] The mechanisms underlying diminished leucocyte migration may be associated with the downregulation of chemokines and cytokines, such as monocyte chemoattractant protein-1 (MCP-1), RANTES, β2 integrin lymphocyte-function-associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1) as well as blockade of the induction of nitric oxide synthase. Statins seem also to interfere with the expression of cytokines in endothelial cells (interleukin (IL)-1β and IL-6) and monocytes (IL-6 and tumour necrosis factor (TNF)-α)[1,3] and might thereby inhibit leucocyte cell migration observed in the croton oil inflammatory process.[14]

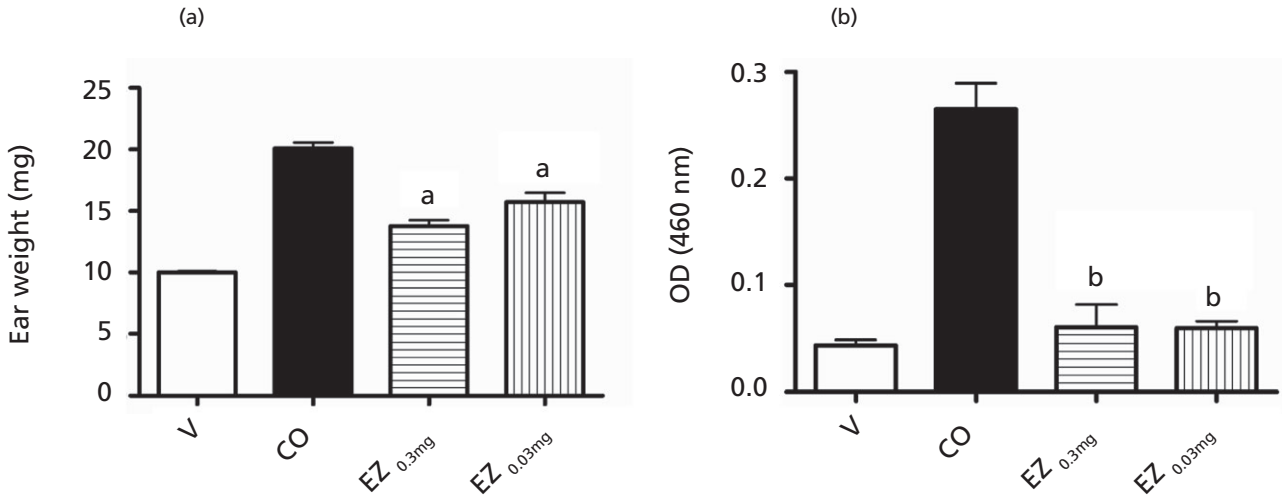

Figure 2 Effect of ezetimibe (EZ) topically administered on croton oil-induced mouse ear oedema (a) and myeloperoxidase (MPO) activity (b). Ear oedema and MPO activity was assayed 6 h after croton oil treatment. Each bar represents the mean ± SEM for 8–10 mice. [a]$P < 0.001$ compared with CO group; [b]$P < 0.01$ compared with CO group.

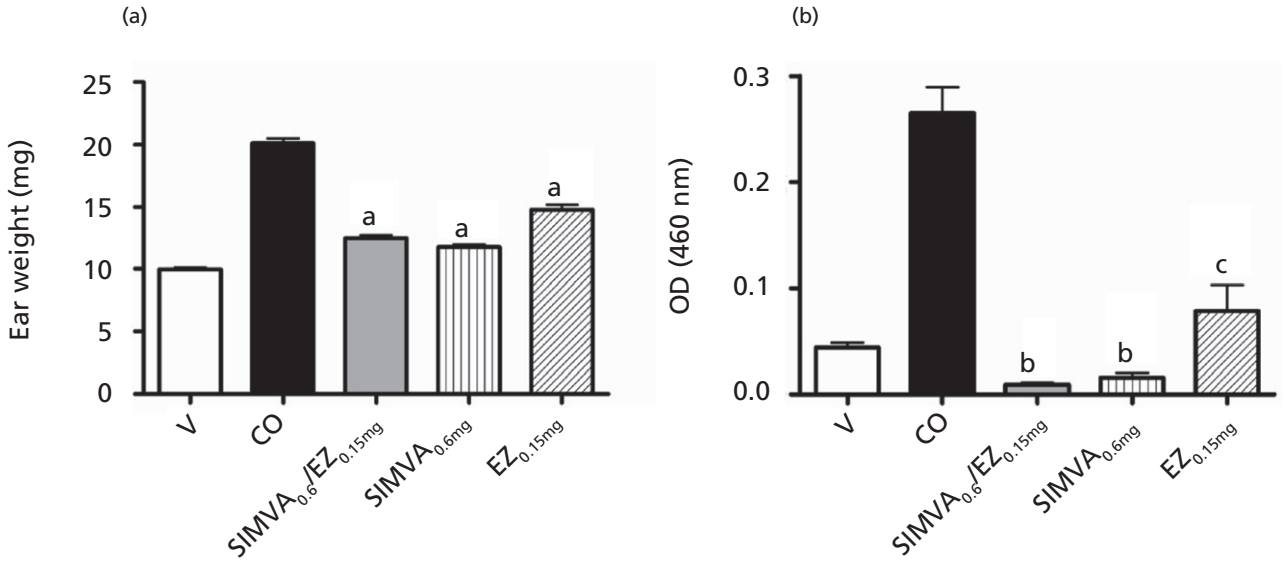

Figure 3 Effect of simvastatin$_{0.6mg}$ + ezetimibe$_{0.15\ mg}$ combination (SIMVA/EZ), simvastatin$_{0.6\ mg}$ and ezetimibe$_{0.15mg}$ on croton oil-induced mouse ear oedema (a) and myeloperoxidase (MPO) activity (b). Ear oedema and MPO activity was assayed 6 h after croton oil treatment. Each bar represents the mean ± SEM for 8–10 mice. [a]$P < 0.001$ compared with CO group; [b]$P < 0.01$ compared with CO group; [c]$P < 0.05$ compared with CO.

It was also demonstrated here that, although ezetimibe alone exhibited a topical anti-inflammatory activity, the combined simvastatin + ezetimibe therapy did not exert any further beneficial effects in the croton oil ear oedema model of topical inflammation in mice. Ezetimibe is hydrophobic (log $P = 4.5$) and should therefore cross the stratum corneum. It is thus quite surprising that ezetimibe presented topical anti-inflammatory properties, since its action is mediated by the selective blockage of the intestinal sterol transporter Niemann–Pick C1-like 1 protein.[4] Probably, other mechanisms are involved in the topical anti-inflammatory effect of ezetimibe observed in the present study, since pleiotropic effects of ezetimibe are rather controversial.[15] Nonetheless, there is substantial evidence that monotherapy with ezetimibe does not significantly improve inflammatory markers such as the C reactive protein (CRP),[16] although the combination with a statin provides a further decrease in the CRP levels compared with statin monotherapy upon oral administration.[5,6,17] Further investigations are needed to elucidate ezetimibe's anti-inflammatory mechanisms.

Conclusions

This study demonstrates that hypocholesterolaemic drugs such as statins and ezetimibe act as topical anti-inflammatories and that their performance is dependent on

the polarity of each specific molecule. Simvastatin and atorvastatin presented higher topical anti-inflammatory activity than pravastatin. Ezetimibe also exhibited anti-inflammatory activity, but when administered in combination with simvastatin, no further benefits were observed when compared with simvastatin alone. Therefore, our study supports the notion that statins can be invaluable in the treatment of dermatological disorders. Polarity of the molecule, however, is a factor that should be considered before recommending use. It also points to new perspectives on the pleiotropic effects of ezetimibe.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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