Lovastatin and mevastatin reduce basal and cytokine-stimulated production of prostaglandins from rat microglial cells in vitro: evidence for a mechanism unrelated to the inhibition of hydroxy-methyl-glutaryl CoA reductase

Giuseppe Tringali, Mauro Vairano, Cinzia Dello Russo, Paolo Preziosi, Pierluigi Navarra*

Institute of Pharmacology, Catholic University Medical School, Largo Francesco Vito 1, 00168 Rome, Italy

Received 12 May 2003; received in revised form 25 September 2003; accepted 30 September 2003

Abstract

Statins were recently shown to possess anti-inflammatory activities, which might be responsible for their favourable effects in cardiovascular or CNS disorders independently from the inhibition of hydroxy-methyl-glutaryl CoA reductase. Here we investigated the effects of the statins lovastatin and mevastatin on prostanoid production in primary cultures of rat cortical microglia and astrocytes. We found that both statins significantly reduce prostaglandin E\(_2\) (PGE\(_2\)) release from microglia, either under basal conditions or after stimulation by interleukin-1\(\beta\). Lovastatin also tends to reduce, although not in a significant manner, basal and interleukin-1\(\beta\)-stimulated PGE\(_2\) release from astrocytes. Precursors and intermediates in cholesterol biosynthesis – mevalonic acid and geranyl and farnesyl pyrophosphate – also reduce PGE\(_2\) production, and potentiate the inhibitory effects of statins, suggesting that the latter might not depend on the inhibition of hydroxy-methyl-glutaryl CoA reductase.

© 2003 Elsevier Ireland Ltd. All rights reserved.

Keywords: Lovastatin; Mevastatin; Hydroxy-methyl-glutaryl CoA reductase; Prostaglandin E\(_2\); Microglia; Astrocyte; Rat

The enzyme 3-hydroxy-methyl-glutaryl-coenzyme A (HMG-CoA) reductase catalyzes the conversion of HMG-CoA into mevalonate. HMG-CoA not only regulates intracellular cholesterol synthesis but also acts as a precursor of many isoprenoid compounds [5]. A class of competitive inhibitors of HMG-CoA reductase, the statins are effective lipid-lowering agents. These drugs have been associated with a host of different biological actions [1,6,8,9,14,18,19,20], which might or might not be associated with their hypocholesterolemic properties. In particular, putative anti-inflammatory properties of statins have been thoroughly investigated, since these may be, at least in part, responsible for the observed reduction of cardiovascular events and the plaque-stabilizing effects [2,8,12].

It is currently agreed that the pathogenesis of different central nervous system (CNS) disorders is of a common immune-inflammatory background [10,13]. Regardless of the aetiology of disease, inflammatory reactions within the CNS are peculiar insofar as the so-called ‘innate’ immune response is by far prevailing with respect to adaptive immunological reactions, i.e. those reactions based on immune-competent lymphocytes [10]. In this regard, a pivotal role is played by the resident macrophages, i.e. microglial cells, and, to a lesser extent, by astrocytes [10].

The present study was addressed to investigate the effects of statins on the production of inflammatory mediators by CNS cells. We used lovastatin – also referred to as mevinolin – and mevastatin, and primary cultures of rat cortical microglia and astrocytes to seek whether these drugs can influence the production of prostaglandins, and whether such influence – if any – is associated with the inhibition of HMG-CoA reductase. Prostaglandin E\(_2\) (PGE\(_2\)) released from microglia or astrocytes, either under basal conditions or after stimulation by the pro-inflammatory cytokine interleukin-1\(\beta\) (IL-1\(\beta\)), was taken as an index of cyclo-oxygenase (COX) activity.

Primary cultures of neonate rat cortical astrocytes and microglia were prepared as previously described in detail [16]. In experiments on astrocytes, culture medium was changed with serum-free Dulbecco’s Modified Eagle’s
Medium (DMEM, Gibco) containing 0.2% bovine serum albumin (BSA; Sigma) and antibiotics 18 h before the experiments, and then again at the beginning of the experiments. The latter change was made with the plain medium (control group) or medium containing test substances (treatments). In experiments on microglia, 24 h after the last change culture medium (DMEM with 10% foetal calf serum and antibiotics) was replaced with fresh medium (control group) or with medium containing test substances (treatments). At the end of the experiments, media were collected under sterile conditions and stored at −35 °C until assays were performed. Lovastatin, mevastatin, mevalonic acid, farnesyl and geranylgeranyl pyrophosphate ammonium salts were purchased from Sigma. The substances were dissolved in absolute ethanol and diluted to working concentrations in incubation medium. IL-1β was obtained from Boeringher (Mannheim, Germany). The cytokine was dissolved in phosphate-buffered saline containing 0.1% BSA and further diluted in incubation medium. None of these substances interfered with PGE2 assay. The latter was performed by radioimmunoassay as previously described in detail [16]. One hundred and 250 µl of incubation media, from microglia and astrocyte experiments, respectively, were assayed. The detection limit of the assay was 2 pg/tube and the EC50 28 pg/tube. Intrainter-assay variability was 5 and 10%, respectively.

Data are expressed as pg of PGE2/ml of incubation medium. All results are means ± 1 standard error of the mean (SEM) of (n) replicates per group. Data were pooled from at least two different experiments, each performed with at least four wells (astrocytes) or six wells (microglia) of a multi-well plate receiving the same treatment. Data were analyzed by one-way ANOVA and post-hoc Newman–Keuls test for multiple comparisons among group means, or Student’s t-test where appropriate, using a Prism™ computer program (GraphPad, San Diego, CA). Differences were considered statistically significant if P < 0.05.

Astrocytes produce and release tiny, albeit detectable, amounts of PGE2; in the experiment shown in Fig. 1A, about 15 pg of PGE2/ml was measured after 24 h of incubation under basal conditions. Previous studies with the protein synthesis inhibitor cycloheximide showed that such basal production is already associated with some degree of activation of the inducible COX isoform, COX-2, most probably caused by culture procedures [17]. Lovastatin did not modify basal PGE2 release at all concentrations tested, and tended to reduce release stimulated by the addition of 1 ng/ml of IL-1β (Fig. 1A), although such reduction did not reach statistical significance.

Under the same experimental conditions (i.e. 24 h of incubation in plain medium), microglial cells produced and released about 20-fold higher levels of PGE2 with respect to astrocytes (Fig. 1B). IL-1β also induced significant increases in PGE2 release. At variance with astrocytes, lovastatin in the range of concentrations 0.1–10 µM completely counteracted the increase in prostanooid release induced by the cytokine; the drug was also able to significantly reduce basal PGE2 production by microglial cells (Fig. 1B). To clarify whether these actions of lovastatin...
are shared with other HMG-CoA inhibitors, we carried out experiments with another compound of this group, mevalonate, showing that the latter also reduced both basal and cytokine-stimulated prostanoid release, with significant reductions at 1 and 10 μM (Fig. 1C).

In the second part of the study we attempted to ascertain whether or not the above effects of statins are related to the inhibition of HMG-CoA reductase. Therefore, experiments were carried out with mevalonic acid, the product of HMG-CoA enzymatic activity, either in the absence or in the presence of lovastatin. If reduced PG production by lovastatin was secondary to HMG-CoA inhibition, we would expect the addition of the metabolite downstream of HMG-CoA blockade to revert the inhibitory effect of lovastatin and restore normal microglial PG production. However, mevalonic acid displayed an intrinsic inhibitory effect on PGE2 production in this paradigm and tended to potentiate the inhibitory effect of lovastatin (Table 1). Two other intermediates in the synthesis of cholesterol that are downstream of mevalonic acid, geranylgeranyl and farnesyl pyrophosphate, displayed a similar profile, since they both inhibited PGE2 production per se, and the latter also potentiated inhibition by lovastatin (Table 1).

We also investigated whether the reductions in PG production observed in this study were caused by non-specific toxic actions of test substances on glial cells; for this purpose, we used the MTS assay, which was previously validated by our group to test microglia viability and metabolic activity [16]. None of the substances used in this study reduced cell viability; instead, a significant increase was observed [data expressed as absorbance at 492 nm, the means ± SEM of ten replicates per group; controls: 0.973 ± 0.008; 10 μM lovastatin: 1.085 ± 0.037 (P < 0.05 vs. controls); 10 μM mevalonic acid: 1.270 ± 0.021 (P < 0.001 vs. controls); 10 μM geranylgeranyl pyrophosphate: 1.177 ± 0.034 (P < 0.001 vs. controls); 10 μM farnesyl pyrophosphate: 1.721 ± 0.043 (P < 0.001 vs. controls)].

To our knowledge, this is the first report showing that statins are able to reduce prostanoid production and release from CNS cells; one such interplay has been described by the group of Habib[4] in human aortic smooth muscle cells. These authors found that the use of statins was associated with increased COX-2 gene expression and prostacyclin production; statins were active under basal conditions and also synergized with IL-1β [4]. On the contrary, Inoue et al. [7] found that these drugs were able to reduce mRNA levels of COX-2 as well as of other pro-inflammatory gene products, such as the interleukins (IL) 1 and 6, on primary cultures of human umbilical vein endothelial cells. Pahan et al. [11] investigated the role of statins on inflammation mechanisms in rat primary astrocytes and microglia, as well as in macrophages. Although the COX pathway was not investigated, these authors found that lovastatin and phenylacetate inhibit the LPS-mediated induction of NOS and of the inflammatory cytokines IL-1β, IL-6 and tumour necrosis factor alpha. These findings, along with the clinical evidence of protective actions exerted by statins in

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PGE2 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>529.25 ± 107.79 (6)</td>
</tr>
<tr>
<td>Mevalonic acid</td>
<td></td>
</tr>
<tr>
<td>0.01 μM</td>
<td>350.64 ± 73.79 (6)</td>
</tr>
<tr>
<td>0.1 μM</td>
<td>321.12 ± 106.12 (6)</td>
</tr>
<tr>
<td>1 μM</td>
<td>186.93 ± 38.57 (6)*</td>
</tr>
<tr>
<td>10 μM</td>
<td>103.20 ± 50.18 (6)*</td>
</tr>
<tr>
<td>Controls</td>
<td>497.22 ± 23.43 (6)</td>
</tr>
<tr>
<td>Lovastatin 1 μM + mevalonic acid 10 μM</td>
<td>296.85 ± 35.39 (6)**</td>
</tr>
<tr>
<td>Lovastatin 10 μM + mevalonic acid 10 μM</td>
<td>232.31 ± 9.75 (6)**</td>
</tr>
<tr>
<td>Mevalonic acid 10 μM</td>
<td>247.37 ± 9.76 (6)**</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>Lovastatin 10 μM</td>
<td>177.54 ± 2.153 (6)**</td>
</tr>
<tr>
<td>10 μM</td>
<td>162.44 ± 8.26 (6)**</td>
</tr>
<tr>
<td>Lovastatin 10 μM + geranylgeranyl PP 10 μM</td>
<td>501.26 ± 35.60 (12)</td>
</tr>
<tr>
<td>Geranylgeranyl PP 10 μM</td>
<td>364.27 ± 21.77 (12)*</td>
</tr>
<tr>
<td>Lovastatin 10 μM + farnesyl PP 10 μM</td>
<td>281.32 ± 34.64 (6)**</td>
</tr>
<tr>
<td>Farnesyl PP 10 μM</td>
<td>275.13 ± 46.41 (6)**</td>
</tr>
</tbody>
</table>

A dose-response curve of mevalonic acid given alone is also shown. The results are expressed as pg PGE2/ml of incubation medium, the means ± SEM of (n) replicates per group. Data analyzed by Student’s t-test; *P < 0.05, **P < 0.01 and ***P < 0.001 vs. controls; t P < 0.05 and ***P < 0.001 vs. lovastatin 10 μM given alone.
inflammatory CNS disorders [3], support the view that statins may have a general anti-inflammatory action in the brain, and part of this effect might be related to the inhibition of prostaglandin production.

What is the mechanism through which statins inhibit PG production in glial cells? Precursors and intermediates in cholesterol biosynthesis through the HMG-CoA reductase pathway all consistently inhibited PGE2 production in our model. This phenomenon might be mediated by increased cholesterol availability; however, it is more likely that prenyl intermediates, namely farnesyl and geranylgeranyl moieties, play a role via the mechanism of protein prenylation. The latter has been proved by time to be critical in the general regulation of cell function, either through the generation of proteins in the active form, or via the docking of proteins to plasma membranes, or else by favouring protein–protein interactions [15]. COX isoforms do not appear to be directly involved in prenylation processes, although one such mechanism was found to indirectly enhance COX activity in endothelial cells [4].

While the downstream effect of activating the HMG-CoA reductase pathway in microglia appears to be the inhibition of PG biosynthesis, it clearly emerges that the effects of statins in the same paradigm are unlikely to be related to their interference in HMG-CoA reductase activity, as effects opposite to those of mevalonic acid and prenyl compounds would be expected in this case. Indeed, lovastatin significantly enhances the inhibitory effects of mevalonic acid, and farnesyl and geranylgeranyl pyrophosphate, suggesting that different underlying mechanisms work in an additive manner to reduce COX activity. Growing evidence is showing that statins exert biological activities through mechanisms that are additional or alternative to HMG-CoA reductase inhibition; Inoue et al. [7] have recently demonstrated that several anti-inflammatory actions in endothelial cells, including a decrease in COX-2 gene expression, are mediated through the modulation of peroxisome proliferator-activated receptors-α, a mechanism which might account for at least a part of the pleiotropic activities of these drugs.

Acknowledgements

This study was supported by Consiglio Nazionale delle Ricerche, targeted project ‘Aging’ and MURST-CNR 5% 2001.

References