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Short communication

Relative potencies of statins in reducing cholesterol synthesis and enhancing linoleic acid metabolism

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Abstract

Simvastatin enhances the conversion of linoleic acid to their long chain polyunsaturated fatty acid derivatives, e.g. arachidonic acid, in addition to typically inhibiting the de novo cholesterol synthesis, in cultured cells. The dose–response relationships for the above effects show that simvastatin, atorvastatin and fluvastatin affect linoleic acid conversion and the Δ5 desaturase step more potently than the synthesis of cholesterol, simvastatin being the most effective in inhibiting sterol synthesis, whereas atorvastatin in stimulating the conversion of linoleic acid.

Keywords: Statins; Linoleic acid; Δ5 desaturase

1. Introduction

Statins, a widely used and very effective pharmacological treatment for the control of hypercholesterolemia through inhibition of the hydroxy-methyl-glutaryl coenzyme A (HMGCoA) reductase, have also been shown to affect the formation of long chain polyunsaturated fatty acids from precursors in vitro (Hrboticky et al., 1994; Risé et al., 1997) and to enhance the levels of plasma long chain polyunsaturated fatty acids in patients (Doormal van et al., 1989; Agheli and Jacotot, 1991; Risé et al., 2001). In clinical interventions the most widely used statins are simvastatin, fluvastatin and atorvastatin, among the lipophilic compounds, and the hydrophilic pravastatin.

Although the potencies of these compounds in inhibiting cholesterol synthesis have been extensively evaluated, their relative and comparative potencies in enhancing the conversion of the 18 carbon polyunsaturated fatty acids (e.g. linoleic acid) to the corresponding long chain polyunsaturated fatty acids (i.e. arachidonic acid) have not so far been investigated.

Aim of our study was therefore to evaluate the dose–response curve of some relevant statins on the inhibition of cholesterol biosynthesis from [1-^{14}C]acetate, the enhancement of arachidonic acid biosynthesis from [1-^{14}C]linoleic acid and of the desaturation steps involved in the process, in cultured cells.

2. Materials and methods

2.1. Chemicals

RPMI 1640 medium, penicillin/streptomycin and all standard compounds were from Sigma (St. Louis, MO, USA), fetal bovine serum was from GIBCO, Life Technologies (UK). All the solvents used were from E. Merck (Darmstadt, Germany); [1-^{14}C] linoleic acid (specific activity 55 mCi/mmol), [1-^{14}C] acetic acid sodium salt (specific activity 57 mCi/mmol) and [6-^{3}H] thymidine (specific activity 27 Ci/mmol) were from Amersham.

2.2. Experimental design

The human leukemic monocytic (THP-1) cells were centrifuged at 200 × *g* for 10 min and the pellet obtained was resuspended in RPMI medium without fetal bovine serum; the concentration of the cells was adjusted to 10^6/ml. Cells were then incubated with different concentrations of simvastatin (0.1, 0.5, 1.0, 2.0 μM), atorvastatin (0.05, 0.5, 1.0, 2.0 μM) and fluvastatin (0.1, 0.5, 1.0, 2.0 μM) for 24 h

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and, at this time, the appropriate labelled substrate ([1-^{14}C] linoleic acid 0.1 μCi/10^6 cells or [1-^{14}C] acetic acid 1 μCi/10^6 cells) was added for additional 24 h. At the end of incubation, cells were centrifuged, resuspended in a given volume of PBS, 50 μl counted with a cell counter, and the total lipids extracted (Folch et al., 1957). Aliquots of total lipid extracts were counted in a β-counter in order to determine the radioactivity recovered in the samples.

2.3. Analysis of fatty acids by HPLC

Long chain polyunsaturated fatty acid synthesis from the precursor [1-^{14}C] linoleic acid was evaluated as radioactivity associated with different fatty acid methyl esters and assessed by HPLC coupled with an on-line radiodetector, as previously described (Risé et al., 1997).

2.4. Separation of lipid classes

The lipid classes (phospholipids, cholesterol esters, diglycerides, triglycerides, free fatty acids) were separated by a monodimensional thin layer chromatography (TLC, hexane/diethyl eter/acetic acid 70/30/1.5 by vol). In order to separate also free cholesterol and monoglycerides another solvent system was used (chloroform/methanol 98:2 by vol). Lipids were detected on plates by exposure to iodine vapors; spots were scraped off and the radioactivity was detected, after addition of 1 ml methanol/water (1:1 by vol) and 10 ml of scintillation fluid, in a β-counter.

3. Results

As expected, in THP-1 cells, all the three statins inhibited cholesterol synthesis from acetate, in a dose-dependent manner (Fig. 1) and simvastatin was more active (IC_{50} 66 nM) than atorvastatin (IC_{50} 154 nM) and fluvastatin (IC_{50} 198 nM).

In contrast, the total conversion of labelled linoleic acid (Fig. 2A) was increased by statins from a value of about

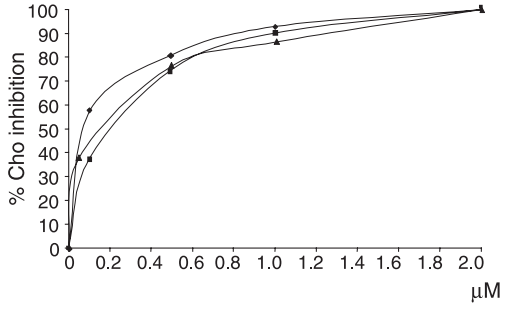

Fig. 1. Dose–response relationships for the effects of statins in inhibiting cholesterol synthesis. The cholesterol synthesis, in THP-1 cells, was evaluated by the incorporation of [^{14}C] acetate in lipids. Values are the mean of five experiments. —▲— Atorvastatin; —◆— simvastatin; —■— fluvastatin; Cho, cholesterol.

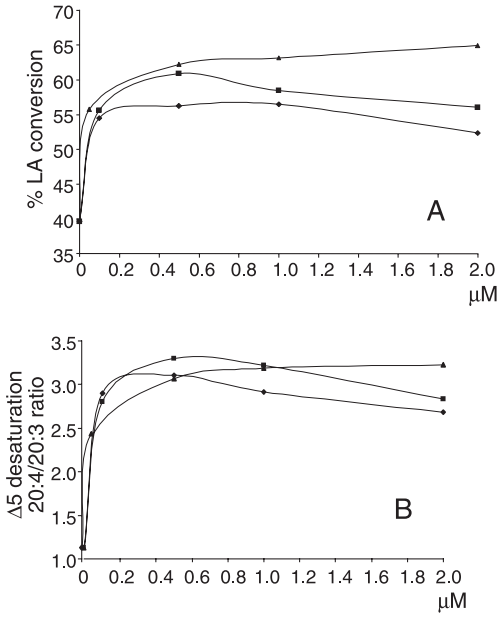

Fig. 2. (A) Dose–response relationships for the effects of statins on total linoleic acid conversion. (B) Dose–response relationships for the effects of statins on the Δ5 desaturation step, in the conversion of LA. Δ5 desaturation was expressed as product (20:4$n-6$)/precursor (20:3$n-6$) ratio. Values are the mean of five experiments. —▲— Atorvastatin; —◆— simvastatin; —■— fluvastatin; LA, linoleic acid.

40% in control cells to a value of about 55% for all statins, at the lowest drug concentration, up to about 58–65% with some difference among treatments at the highest concentrations. The linoleic acid conversion was continuously raised with increasing concentrations of atorvastatin, whereas with fluvastatin the conversion reached a maximum at 0.5 μM followed by a small reduction at higher concentrations, and with simvastatin the conversion reached a plateau at 0.5 μM. The EC_{50} values for the total linoleic acid conversion were: 10 nM for atorvastatin, 24 nM for simvastatin and 39 nM for fluvastatin.

The conversion of linoleic acid to long chain polyunsaturated fatty acids, such as arachidonic acid (20:4$n-6$) is catalyzed by elongases and desaturases, and an index used to assess the activity of these enzymes is the measurement of the product/precursor ratios after chromatographic separation of the fatty acids. Desaturases are the rate limiting enzymes of the whole process: the Δ6 desaturase converts linoleic acid to 18:3$n-6$, while the Δ5 desaturase converts 20:3$n-6$ to 20:4$n-6$, arachidonic acid. In our experiments Δ5 desaturase activity was potently affected by statins, more than the Δ6 desaturase, reflecting the trend observed for the linoleic acid total conversion (Fig. 2B). The EC_{50} for the Δ5 desaturase in the case of atorvastatin, simvastatin and fluvastatin were respectively 15, 29 and 44 nM.

We have also tested pravastatin, the hydrophilic compound, but the effects on linoleic acid conversion were obtained at concentrations higher (5–50 μM) than those used for the other statins.

Finally, the evaluation of cell proliferation by the incorporation of [6-^{3}H] thymidine (1 μCi/10^6 cells) in nucleic acids indicated that the concentrations of statins used in our experiments, were not cytotoxic, since the incorporation of thymidine in treated cells was not significantly different from control cells (the mean differences in the incorporation between controls and treated cells were 2.82 ± 1.56%).

4. Discussion

The most important and relevant effect of statins is the inhibition of cholesterol synthesis; in addition they exert a variety of other effects, i.e. pleiotropic effects, such as a reduced cell proliferation and differentiation, a decrease of platelet aggregation, etc. We, as well as other investigators, have found that statins enhance linoleic acid conversion to long chain polyunsaturated fatty acids; in THP-1 cells we have reported that simvastatin was able to inhibit the cholesterol synthesis and to increase the linoleic acid total conversion, in a dose-dependent (between 0.5 and 5 μM simvastatin) manner (Risé et al., 1997). In the present paper we report the relative potencies of different statins in reducing cholesterol synthesis (simvastatin>atorvastatin>-fluvastatin) and enhancing linoleic acid metabolism (atorvastatin>simvastatin>fluvastatin). In conclusion, our data show that the in vitro effects of statins on linoleic acid conversion are obtained at significantly lower concentrations ($EC_{50\ linoleic\ acid}/IC_{50\ Cholesterol}$ ratios 0.1 for atorvastatin, 0.2 for fluvastatin and 0.44 for simvastatin, and somewhat similar values for the $EC_{50\Delta5\ desaturase}/IC_{50\ Cholesterol}$ ratios) than those for the effects on cholesterol synthesis, suggesting that the activation of polyunsaturated fatty acid metabolism is a major effect of statins.

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