

$\Delta 5$ desaturase mRNA levels are increased by simvastatin via SREBP-1 at early stages, not via PPAR α , in THP-1 cells

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Abstract

In addition to inhibiting cholesterol biosynthesis, statins increase the conversion of linoleic acid to its derivatives, in particular to arachidonic acid, both in vivo and in vitro. Desaturases are the rate-limiting enzymes in this metabolic process and statins markedly enhance $\Delta 5$ desaturase activity. To evaluate the $\Delta 5$ desaturase gene expression and the transcription factors involved, THP-1 cells (a monocytic cell line) were incubated with 5 μ M simvastatin for different time periods. The activity of the enzyme, evaluated as product/precursor ratio in the metabolic pathway (starting from [1- 14 C] linoleic acid), increased in treated cells with respect to controls after 24 h, whereas, mRNA levels of the $\Delta 5$ desaturase increased after 12 h of incubation with simvastatin. Fatty acid desaturase genes are regulated by both sterol regulatory element binding proteins (SREBPs) and peroxisome proliferators activated receptors (PPARs). Both PPAR α (WY 14643 and fenofibrate) and PPAR γ (ciglitazone) agonists did not affect linoleic acid conversion and the $\Delta 5$ desaturase activity at any time considered (8–48 h), but they increased the $\Delta 5$ desaturase mRNA levels, after 48 h; only fenofibrate showed a synergistic effect with simvastatin at this time, with a concomitantly increase in PPAR α expression and β -oxidation. Simvastatin alone increased SREBP-1 levels with respect to controls, starting from 8 h of incubation, whereas PPAR α and linoleic acid β -oxidation (a PPAR α mediated process) were not affected after 48 h of incubation. These results taken together suggest that SREBP-1 is involved in the early regulation of $\Delta 5$ desaturase gene by simvastatin, in THP-1 cells.

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1. Introduction

Statins, in addition to reducing cholesterol synthesis by inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, have some pleiotropic effects (Corsini et al., 1993; Hochgraf et al., 1994; Colli et al., 1997). The effects on lipid metabolism involve also the production of the long chain polyunsaturated fatty acids; in fact, statins induce elevation of arachidonic acid levels, as a consequence of increased linoleic acid conversion, in vitro and in vivo (Risé et al., 1997; Hrboticky et al., 1994; Van Doormal et al., 1989; Risé et al., 2001; Nakamura et al., 1998). Linoleic acid (18:2 n-6) is converted to arachidonic acid (20:4 n-6), its most

relevant metabolite, by elongation and desaturation reactions: $\Delta 6$ desaturase catalyzes the step from 18:2 to 18:3 (γ -linolenic acid) and the $\Delta 5$ desaturase the step from 20:3 (eicosatrienoic acid) to 20:4.

Human $\Delta 6$ and $\Delta 5$ desaturase genes have been cloned (Cho et al., 1999a; Cho et al., 1999b): as to the relative abundance of $\Delta 6$ and $\Delta 5$ desaturase mRNA, in different tissues, $\Delta 6$ mRNA levels are higher than those of $\Delta 5$ mRNA, the highest levels being found in the liver (Cho et al., 1999b). In addition, in mice fed diets rich in polyunsaturated fatty acids, the decreased hepatic $\Delta 6$ and $\Delta 5$ desaturase activities reflected a reduction in their mRNA levels (Nakamura et al., 2000). The genes of human $\Delta 6$ and $\Delta 5$ desaturases are located on chromosome 11 where they are positioned in a reverse sequence orientation to each other and, between the start sites of translation for these genes, there is a sequence of about 11 kb. The proximity of the promoters suggests a

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coordinated and similar regulation of desaturase transcription (Leonard et al., 2000).

Fatty acid desaturase genes are regulated by peroxisome proliferator activated receptors (PPARs) and sterol regulatory element binding proteins (SREBPs), two different classes of nuclear receptors (Nakamura and Nara, 2002). Cellular cholesterol and fatty acid homeostasis are regulated by SREBPs; in mammalian cells three isoforms have been identified and characterized, and their distribution and abundance differ in cultured cells and in various animal tissues. Of the three isoforms, SREBP-1a, expressed at very low levels, is responsible for maintaining basal cholesterol levels and fatty acid synthesis; SREBP-1c regulates genes involved in lipogenesis and SREBP-2 is more specific in the control of cholesterol homeostasis. Recently, Matsuzaka et al. (Matsuzaka et al., 2002) reported that $\Delta 6$ and $\Delta 5$ desaturase mRNA levels are elevated in mice overexpressing SREBP-1. The SRE (sterol-regulatory element) sequence, that binds SREBP-1, was identified in human $\Delta 9$ and $\Delta 6$ desaturase promoters (Bené et al., 2001; Nara et al., 2002).

PPARs are transcription factors that bind to specific DNA sequences (PPREs) on the promoter of target genes. All the three isoforms, α , β/δ and γ , are important modulators of lipid metabolism. PPAR α is highly expressed in liver, muscle, heart, kidney and stimulates fatty acid β -oxidation; PPAR γ is expressed mainly in the adipose tissue and promotes lipid storage and cell differentiation; PPAR β/δ is ubiquitous and appears to control adipogenesis (Barbier et al., 2002; Vosper et al. 2002). Peroxisome proliferators, such as fibrates and 4-Chloro-6-(2,3-xylydino)-2-pyrimidinylthioacetic acid (WY-14643), ligands of PPAR α , also induce $\Delta 9$ and $\Delta 6$ desaturase mRNA and it was found that mouse $\Delta 9$ desaturase and human $\Delta 6$ desaturase promoters have a consensus sequence similar to PPRE (Miller and Ntambi, 1996; Tang et al., 2003).

We have previously shown that, in THP-1 cells (a human monocytic cell line), 5 μ M simvastatin significantly increases the conversion of [$1\text{-}^{14}\text{C}$] 18:2 n-6 to its long chain polyunsaturated fatty acid derivatives. The increment of 18:2 n-6 conversion is related to increased desaturase activity, $\Delta 6$ and $\Delta 5$ desaturase activities being enhanced versus controls respectively of about 100% and 380%, in treated cells (Risé et al., 1997). $\Delta 5$ desaturation step is more affected than $\Delta 6$ after statin, oxysterol and cigarette smoke treatments, in different types of cells (Risé et al., 1997; Risé et al., 2004; Marangoni et al., 2004). $\Delta 5$ desaturase is an enzymatic system more specific than that of $\Delta 6$ desaturase in humans and animals. In fact, a restricted range of higher plants synthesize 18:3 n-6 from 18:2 n-6, due to the presence of $\Delta 6$ desaturase, but not arachidonic acid or eicosapentaenoic and docosahexaenoic acids (Tucker, 2003; Napier et al., 1999) that are direct products of $\Delta 5$ desaturase.

Based on these considerations, the purpose of the present study is to investigate in detail, in THP-1 (the cell line used in our previous experiments), the effects of simvastatin on the expression of $\Delta 5$ desaturase and also to study the possible transcription factors involved in the action of simvastatin.

2. Materials and methods

2.1. Chemicals

RPMI 1640 medium, penicillin, streptomycin, glutamine and foetal calf serum were from Sigma-Aldrich (St. Louis, MO, USA). All the solvents used were from E. Merck (Darmstadt, Germany); [$1\text{-}^{14}\text{C}$] 18:2 n-6 (specific activity 55 mCi/mmol) and [$1\text{-}^{14}\text{C}$] acetic acid sodium salt (specific activity 57 mCi/mmol) were from Amersham. All the reagents for the reverse transcription-polymerase chain reaction (RT-PCR) were from Invitrogen, Life Technologies (Carlsbad, CA, USA), whereas the TaqMan probes were from Eurogentec S.A. (Ougrée, Belgium).

All the reagents for protein electrophoresis were purchased from Bio-Rad (Hercules, CA, USA).

The PPAR α agonists, 4-Chloro-6-(2,3-xylydino)-2-pyrimidinylthioacetic acid (WY-14643) and fenofibrate, were a kind gift of Dr. Cristina Banfi whereas the PPAR γ agonist ciglitazone was from Tocris (Cookson Ltd. U.K.).

Simvastatin in lactone form (Merck, Sharp & Dohme Research Laboratories, Woodbridge, NJ) was dissolved in 0.1 M NaOH to obtain the active, open β -hydroxy acid form.

2.2. Experimental plan

THP-1 cells, a human monocytic cell line (Tsuchiya et al., 1980), were grown in RPMI medium with 10% foetal calf serum, 100 μ g/ml penicillin, 100 IU/ml streptomycin and 2 mM glutamine.

At the time of the experiments cells were centrifuged at 200 g for 10 min, the pellet was resuspended in RPMI without foetal calf serum and cell concentration adjusted to 10^6 cells/ml. THP-1 cells were incubated with 5 μ M simvastatin and [$1\text{-}^{14}\text{C}$] 18:2 n-6 (0.1 μ Ci/ml) for different time periods (8, 16, 24 and 48 h) in experiments devoted to evaluate the 18:2 n-6 total conversion and the activity of $\Delta 5$ desaturase (expressed as product/precursor ratio) (Riserus et al., 2005) or with [$1\text{-}^{14}\text{C}$] acetic acid sodium salt (0.5 μ Ci/ml) to assess the cholesterol biosynthesis.

2.3. Lipid extraction

Cell lipid extraction was carried out according to Folch et al. (Folch et al., 1957); the lipid concentration in the extracts, resuspended in a given volume of chloroform/methanol 2:1 v/v, with dibutyl-hydroxy-toluene as an antioxidant, was evaluated by microgravimetry, whereas the radioactivity recovered in the samples was measured with a β -counter and expressed as cpm/ μ l of the extract.

2.4. Linoleic acid metabolism

Total lipids were transmethylated (with $\text{CH}_3\text{OH}/\text{HCl}$ 3N, for 1 h at 90 °C) and the radioactivity associated to the individual fatty acids was determined after separation by HPLC equipped with a radiodetector (Flow Scintillation Analyzer 500TR, Perkin Elmer) as previously reported (Risé et al., 1997).

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