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Statins potentiate the IFN-γ-induced upregulation of group IIA phospholipase A_2 in human aortic smooth muscle cells and HepG2 hepatoma cells

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Abstract

The present study shows that the incubation of human aortic smooth muscle cells (HASMC) and HepG2 cells with atorvastatin and mevastatin as HMG-CoA reductase inhibitors potentiated the interferon-γ (INF-γ)-induced group IIA phospholipase A_2 (sPLA$_2$-IIA) expression in a dose- and time-dependent manner. The effect of statins on sPLA$_2$-IIA expression was reduced by mevalonate, farnesyl pyrophosphate and geranylgeranyl pyrophosphate. Inversely, inhibitors of the farnesyl transferase and geranylgeranyl transferase-I mimicked the effects of statins. *Clostridium difficile* toxin B (TcdB), Y-27632 and H-1152, functioning as inhibitors of Rho proteins and Rho-associated kinase, also augmented the sPLA$_2$-IIA expression in combination with IFN-γ. The same effects were observed when inhibitors of mitogen-activated/extracellular response protein kinase kinase (MEK), PD98059 or U0126 were used. Further, the Janus kinase-2 (Jak2)-specific inhibitor, AG-490 and inhibitors of nuclear factor-κB (NFκB) abrogated the sPLA$_2$-IIA elevating effects of statins, TcdB and PD98059 in the presence of IFN-γ. This cytokine alone increased the NFκB p65 and CAAT-enhancer-binding protein-β (C/EBP-β) activity in HASMC nuclear extract, but only C/EBP-β was further augmented when the cells were incubated in addition to IFN-γ with atorvastatin, H-1152, PD98059 or U0126. Moreover, after the incubation of cells with atorvastatin and IFN-γ the stability of sPLA-$_2$IIA mRNA significantly increased in comparison to those after incubation with IFN-γ alone. In conclusion, the obtained data suggest that (i) the expression of sPLA$_2$-IIA is negatively regulated by RhoA/Rho-associated kinase and MEK/ERK signaling pathways and (ii) statins, because of their ability to down-regulate these pathways, can potentiate the IFN-γ-induced sPLA$_2$-II expression at transcriptional and post-transcriptional levels.

Keywords: Statin; HMG-CoA reductase inhibitor; Secretory phospholipase A_2; Inflammation; HASMC; HepG2 cells

Abbreviations: C/EBP-β, CAAT-enhancer-binding protein-β; cPLA$_2$, cytosolic phospholipase A_2; DRB, 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole; ELISA, enzyme linked immunosorbent assay; ERK, extracellular signal-regulated kinase; FCS, fetal calf serum; FPP, farnesyl pyrophosphate; FTI, farnesyl transferase inhibitor; GAPDH, glycerine aldehyde-3-phosphate dehydrogenase; GGPP, geranylgeranyl pyrophosphate; GGTI, geranylgeranyl transferase-I inhibitor; HASMC, human aortic smooth muscle cells; HDL, high-density lipoproteins; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; IFN-γ, interferon-γ; IL-1β, interleukin-1β; IL-6, interleukin-6; Jak, Janus kinase; MEK, mitogen-activated/extracellular response protein kinase kinase; NFκB, nuclear factor-κB; PBS, phosphate-buffered saline; PSI, proteasome inhibitor; RT-PCR, reverse transcription polymerase chain reaction; sPLA$_2$-IIA, secretory phospholipase A_2 of group IIA; STAT, signal transducer and activator of transcription; TcdB, *Clostridium difficile* toxin B; TNF-α, tumor necrosis factor-α

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1. Introduction

Statins represent a group of natural and synthetic drugs that inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol biosynthesis [1]. Numerous in vitro and in vivo studies have provided evidence that statins have beneficial effects in retarding atherosclerosis beyond that of lowering plasma cholesterol levels [2,3]. Indeed, it has been shown that statins may improve the endothelial function by increasing the production of nitric oxide (NO) via the upregulation of endothelial NO synthase expression [4], impair vascular smooth muscle cell proliferation [5,6], and increase the stabilization of atherosclerotic plaques by reducing inflammatory reactions [7,8]. Recently, different gene products such as cyclooxygenase-2 [9], inducible NO synthase [10], E-selectin and vascular cell adhesion molecule (VCAM) [11], expressions of which are induced by proinflammatory cytokines and which play a role in inflammation and atherogenesis, were shown to be modulated by statins.

Another gene product involved in inflammation is represented by secretory group IIA phospholipase A_2 (sPLA$_2$-IIA). In mammals this enzyme is constitutively expressed in a number of cell types such as platelets, neutrophils, macrophages and mast cells as well as in tissues such as spleen, tonsil, intestine and bone marrow [12–14]. Hepatic, endothelial and smooth muscle cells express sPLA$_2$-IIA in vitro upon stimulation by proinflammatory cytokines [15,16]. The serum level of this enzyme is low in healthy humans, but elevated in severe acute inflammatory diseases such as sepsis, septic shock [17,18] and acute pancreatitis [19]. Elevated concentrations of sPLA$_2$-IIA in the serum can also be observed in chronic rheumatoid arthritis [20], multiple injuries [21,22] and in neoplastic diseases [23]. As serum sPLA$_2$-IIA is rapidly increased during diseases involving local or systemic inflammatory reactions, it is regarded as an acute-phase reactant [24,25]. The specific biological functions of sPLA$_2$-IIA are not completely understood. Similar to other phospholipases A_2, sPLA$_2$-IIA may be involved in cell signaling, apoptosis, remodelling of cell membranes, inflammatory response and host defence against bacterial invaders [26–30].

Thus, the activity of statins as potential regulators of gene expression of different cytokines, growth factors and enzymes involved in inflammation together with the strong participation of sPLA$_2$-IIA in this process, prompted us to examine the hypothesis that HMG-CoA reductase inhibitors modulate the expression of sPLA$_2$-IIA in human aortic smooth muscle cells (HASMC) and HepG2 hepatoma cells in the presence of proinflammatory cytokines. The results show that IFN-γ and statins induce the expression of sPLA$_2$-IIA in a strong synergistic manner and that this induction is Janus kinase-2 (Jak2), nuclear factor-kappa B (NFκB) and CAAT-enhancer-binding protein-β (C/EBP-β) dependent.

2. Materials and methods

2.1. Materials

Atorvastatin calcium salt was kindly provided by Pfizer (Ann Arbor, MI, USA). Mevastatin, mevalonic acid lactone, squalene, cholesterol, farnesyl pyrophosphate (FPP), geranylgeranyl pyrophosphate (GGPP), manumycin A, Cys-Val-2-Nal-Met (Cys-Val-2-Naphthyl-3-Alanyl-Met), forskolin, and caffeic acid phenethyl ester (CAPE) were purchased from Sigma-Aldrich (Deisenhofen, Germany). Geranylgeranyl transferase-I inhibitors GGTI-298 and GGTI-286, *Clostridium difficile* toxin B (TcdB), Y-27632, 8-bromo-cAMP, 2′-Amino-3′-methoxyflavone (PD98059), 1,4-Diamino-2,3-dicyano-1,4-bis(2-aminophenylthio)butadiene (U0126), Tyrphostin B42 (AG-490), (s)-(+)-2-Methyl-1-[(4-methyl-5-isoquinolinyl)sulfonyl]-homopiperazine, 2HCl (H-1152), and 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) were from Calbiochem (San Diego, California, USA). Pyrrolidine dithiocarbamate (PTDC) and 20 S Proteasome inhibitor I (PSI) as inhibitors of NFκB were from ICN Biomedicals (Costa Mesa, California, USA). Recombinant human IL-1β, IL-6, TNF-α and IFN-γ were purchased from R&D Systems GmbH (Wiesbaden-Nordenstadt, Germany). Polyclonal rabbit antibodies against secretory group IIA PLA$_2$ were obtained from Cayman Chemical (Ann Arbor, MI, USA). Atorvastatin, mevastatin, mevalonate lactone, squalene, and forskolin were dissolved in ethanol, whereas manumycin A, GGTI-298, GGTI-286, PD98059, U0126, AG-490 and CAPE were dissolved in dimethyl sulfoxide (DMSO). The final concentrations of solvents were 0.3% or less, respectively. Controls using ethanol or DMSO alone were run in all cases. Farnesyl pyrophosphate and geranylgeranyl pyrophosphate were dissolved in methanol and 10 mM aqueous NH_4OH (7:3), and these solvents were also used as controls. Mevastatin and D,L-mevalonic acid lactone were chemically transformed into the active form after dissolving in ethanol by opening the lactone ring in 0.1 N NaOH at 37 °C for 45 min as described [31]. After hydrolysis the pH was adjusted to 7.4 by the addition of 0.1 N HCl. Cholesterol–bovine serum albumin (BSA) complexes were prepared by dissolving 100 mg of cholesterol in 10 ml of absolute ethanol as described [32]. 4 g of BSA was added to the white solution obtained after the addition of 10 ml of double distilled water. Then the pH was adjusted to 7.3, and the solution was centrifuged at 12,000×g and 4 °C for 10 min.

2.2. Cell culture and incubation

HASMC were purchased from Promocell (Heidelberg, Germany) and cultured according to the instructions in smooth muscle cell growth medium-2 containing 5% fetal calf medium, 0.5 ng/ml human epidermal growth factor, 2 ng/ml human basic fibroblast growth factor, 5 μg/ml bovine insulin, 50 μg/ml gentamycin sulfate and 50 ng/ml