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HMG-CoA reductase inhibitors enhance phagocytosis by upregulating ATP-binding cassette transporter A7

Nobukiyo Tanaka[a,b], Sumiko Abe-Dohmae[a,*], Noriyuki Iwamoto[a,1], Michael L. Fitzgerald[c], Shinji Yokoyama[a,**]

[a] Biochemistry, Nagoya City University Graduate School of Medical Sciences, Nagoya, 467-8601, Japan
[b] Department of Cardiovascular Medicine, Ichinomiya Nishi Hospital, Ichinomiya, 494-0001, Japan
[c] Lipid Metabolism Unit and Partners Center for Computational and Integrative Biology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA

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ABSTRACT

We recently reported that the endogenous ATP-binding cassette transporter (ABC) A7 strongly associates with phagocytosis, being regulated by sterol regulatory element binding protein 2. We therefore examined the effect of statins on phagocytosis in vitro and in vivo through the SREBP–ABCA7. Phagocytosis was found to be enhanced by pravastatin, rosuvastatin and simvastatin and cyclodextrin in J774 macrophages, as cellular cholesterol was reduced and expressions of the cholesterol-related genes were modulated, including an increase of ABCA7 mRNA and decrease of ABCA1 mRNA. Conversely, knockdown of ABCA7 expression by siRNA ablated enhancement of phagocytosis by statins. In vivo, pravastatin enhanced phagocytosis in wild-type mice, but not in ABCA7-knockout mice. We thus concluded that statins enhance phagocytosis through the SREBP–ABCA7 pathway. These findings provide a molecular basis for enhancement of the host-defense system by statins showing that one of their "pleiotropic" effects is in fact achieved through their reaction to a primary target.

1. Introduction

Statins, competitive inhibitors of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, have been marketed as cholesterol lowering drugs for more than 20 years. They lower plasma low-density lipoprotein (LDL) by up-regulation of the LDL receptor gene through the sterol regulatory element binding protein (SREBP) system that senses cellular cholesterol levels [1]. The clinical trials for secondary and primary prevention of coronary heart diseases [2,3], established the benefits of these drugs on mortality and morbidity. While many other studies have also provided evidence for the clinical benefits of lowering LDL by statin treatment to prevent atherosclerotic diseases, a new viewpoint of statin treatment has been introduced by some authors, suggesting that statins have extra beneficial effects beyond lowering LDL [4–7], which are attributed to various types of anti-inflammatory or anti-oxidative effects through regulation of proinflammatory mediators [8,9]. These effects may be interpreted as the consequence of inhibition of the reactions in the mevalonate cascade, such as isoprenylation of the membrane proteins including Ras [10]. Involvement of Rho GTPases has been proposed for enhancement of phagocytosis by statins [11]. The molecular pharmacological grounds for these effects remain quite ambiguous.

Phagocytosis is one of the fundamental functions of animal cells and is an important responsive reaction to infection, injury and apoptosis. Some ATP-binding cassette transporter (ABC) proteins have been reported to affect the phagocytic function of cells [12–17]. ABCA7, a membrane protein and highly homologous to ABCA1 [18] that mediates biogenesis of high-density lipoprotein (HDL) [19–21], plays no significant role in the generation of HDL [16,22,23] and is actively associated with phagocytosis [16,17]. Interestingly, the ABCA7 gene is regulated by sterol regulatory element binding protein 2 [16] in the opposite direction to the liver X receptor-mediated regulation of ABCA1 with respect to cellular cholesterol [24–26]. We recently demonstrated that helical apolipoproteins of HDL enhanced phagocytosis by inhibiting degradation of ABCA7 [27]. These findings shed light on the question of the relationship between sterol homeostasis and the host defense system. Here, we examine the effect of statins on phagocytosis in vitro and in vivo based on the hypothesis that statin treatment enhances the SREBP–ABCA7 pathway.

* Corresponding author. Tel.: +81 52 853 8141; fax: +81 52 841 3480.
** Corresponding author. Present address: Food and Nutritional Sciences, College of Bioscience and Biotechnology, Chubu University, 1200 Matsumoto-cho, Kasugai, 487-8501, Japan. Tel.: +81 568 51 1712x8851; fax: +81 52 841 3480.
E-mail addresses: bc.abedo@med.nagoya-cu.ac.jp (S. Abe-Dohmae), syokoyam@med.nagoya-cu.ac.jp (S. Yokoyama).
[1] Present address: Division of Atherosclerosis, Metabolism and Clinical Nutrition, National Cerebral & Cardiovascular Center Hospital, Suita, Osaka 565-8565, Japan.

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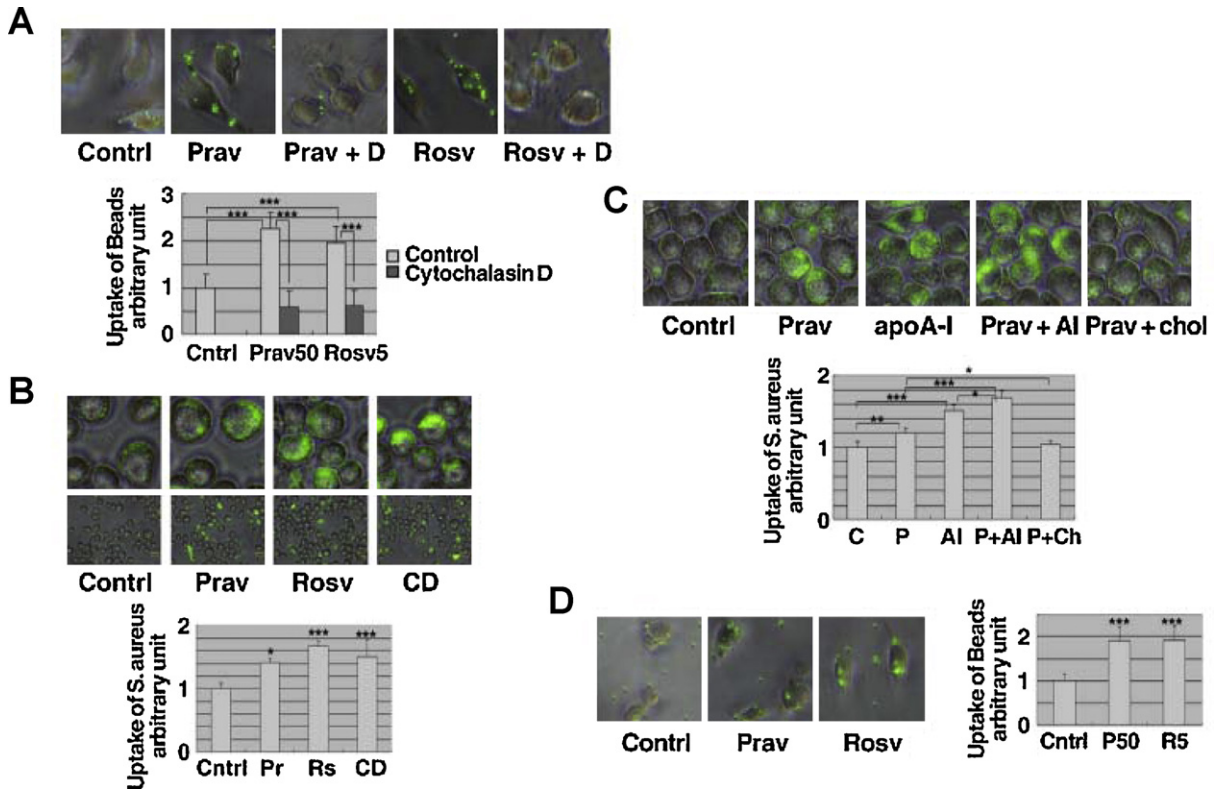

Fig. 1. Pravastatin and rosuvastatin enhance phagocytosis. (A) J774 cells were subcultured as 4×10^3 cells/well for one day. After overnight incubation with and without 50 μM pravastatin (Prav) or 5 μM rosuvastatin (Rosv), quantitative phagocytosis assay for polystyrene beads was performed. The results were expressed as relative phagocytic activity to the blank control (without statins, 0.02% BSA). Cytochalasin D (D) 10 μM was added to inhibit. (B and C) J774 cells were seeded as 5×10^5 cells/well for one day. After overnight incubation with 50 μM pravastatin, 5 μM rosuvastatin, 5 mM cyclodextrin (CD) or 10 μg/mL apoA-I (apoA-I or AI), and 0.3 mM cholesterol (chol), quantitative phagocytosis assay for 3×10^6 (B) or 6×10^6 (C) *S. aureus* was performed. (D) Peritoneal macrophages were collected from wild-type mice. The cells were subcultured in a 96-well tray as 5×10^4 cells/well for one day. After overnight incubation with and without 50 μM pravastatin or 5 μM rosuvastatin, quantitative phagocytosis assay for polystyrene beads was performed. The data in the graphs represent the mean ± SD for 8 samples. Statistical significance is indicated as * for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$ from the control or between the data indicated.

2. Methods

2.1. Reagents and antibodies

Pravastatin and rosuvastatin were provided by Daiichi-Sankyo and AstraZeneca, respectively. Simvastatin was purchased from Sigma (S6196) and activated as previously described [28]. Cytochalasin D was purchased from Merck (250255). (2-Hydroxypropyl)-β-cyclodextrin and anti β-actin antibody were purchased from Sigma (C0926 and A5441, respectively). Monoclonal antibodies against mouse ABCA1 (MABI98-4) and ABCA7 (MABI97-17) were generated at the MAB Institute (Yokohama, Japan) as described previously [16,29]. Apolipoprotein A-I (apoA-I) was isolated from human plasma HDL [30].

2.2. Animals and cells

ABCA7-knockout mice were generated and backcrossed to a C57BL/6 background [22] and bred on the same background [27]. Mouse resident peritoneal macrophages were obtained as described previously [27]. The animal experimental protocols were approved by the institutional animal welfare committee. J774 cells and Jurkat cells were obtained from the Riken Cell Bank and cultured in 10% fetal calf serum (FCS)/Roswell Park Memorial Institute (RPMI) 1640 medium. Cells were maintained at 37 °C in humidified atmosphere of 5% CO_2.

2.3. RNA interference

SiRNAs (Stealth Select RNAi) for ABCA1 and ABCA7 were purchased from Invitrogen. They were transfected by nucleofection (Nucleofector Kit V; AMAXA biosystems). Two different siRNAs were tested and yielded similar results. The data presented represent composite results.

2.4. Lipid assay

J744 cells were subcultured in a 6-well tray in 10% FCS/RPMI1640 medium for 1 day. The cells were washed with phosphate buffered saline (PBS) and incubated overnight in 1 ml/well of RPMI1640 medium in the presence of either 50 μM pravastatin, 5 μM rosuvastatin or 5 mM cyclodextrin. The doses were found by monitoring increase of ABCA7 expression described below. Cellular lipid was determined by colorimetric enzymatic assay [31]. Cellular protein was dissolved in radioimmunoprecipitation assay (RIPA) buffer, and determined with the BCA Protein Assay Kit (Pierce).

2.5. Quantitative analysis of mRNA

Total RNA was isolated by ISOGEN (Wako) and reverse-transcribed by SuperScript III (Invitrogen) with oligo dT primers. Quantitative expression analysis by real-time reverse transcription polymerase chain reaction (PCR) was performed in a StepOnePlus Real-Time PCR system (Applied Biosystems) using SYBR Green technology. The following PCR primers were used for amplification of mouse RNAs: ABCA7, 5′-GCC AGT ATG GAA TCC CTG AA-3′ (for-