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Simvastatin inhibits NF-κB signaling in intestinal epithelial cells and ameliorates acute murine colitis

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Abstract

Statins, HMG-CoA reductase inhibitors exert pleiotropic anti-inflammatory properties *in vitro* and *in vivo*, and are associated with the risk reduction of colorectal cancer. It remains unknown, however, whether statin is effective for the treatment of inflammatory bowel disease (IBD). Therefore, we investigated anti-inflammatory effects of simvastatin on intestinal epithelial cells (IEC) and on an experimental murine colitis model, and elucidated its molecular mechanisms. Simvastatin (50 μM) significantly inhibited TNF-α-induced IL-8 gene expression in COLO 205 cells. Simvastatin (50 μM) blocked TNF-α-induced NF-κB transcriptional activity, IκB phosphorylation/degradation and DNA binding activity of NF-κB. Administration of simvastatin significantly reduced the severity of dextran sulfate sodium (DSS)-induced murine colitis as assessed by body weight, colon length, DAI, and histology in a dose-dependent manner. These results suggest that simvastatin inhibits proinflammatory gene expression by blocking NF-κB signaling in IEC, and attenuates DSS-induced acute murine colitis. Simvastatin could be a potential agent for the treatment of IBD.

Keywords: Statin; Simvastatin; NF-κB; Intestinal epithelial cells; Murine colitis; Inflammatory bowel disease

1. Introduction

Inflammatory bowel disease (IBD) (ulcerative colitis and Crohn's disease) is a chronic intestinal inflammatory disease characterized by tissue edema, increased gut epithelial permeability, and extensive infiltration of the gut by leukocytes [1,2]. Although great advances have been made in the management of the disease with the introduction of immunomodulators and biologic agents, a curative therapy does not yet exist. Therefore, it is still challenging to develop novel specific therapies for IBD [3].

Abbreviations: NF-κB, Nuclear transcription factor κB; IBD, Inflammatory bowel disease; IEC, Intestinal epithelial cells; TNF-α, Tumor necrosis factor-α; IκB, Inhibitor of κB; DSS, Dextran sulfate sodium; DAI, Disease activity index; IL, Interleukin; TNBS, Trinitrobenzene sulphonic acid; HMG-CoA, 3-hydroxy-3-methyl-glutaryl coenzyme A; KCLB, Korean Cell Line Bank; EMSA, Electrophoretic mobility shift assays; LFA-1, Leukocyte function antigen-1; MAdCAM-1, Mucosal vascular addressin cell adhesion molecule 1; IAP, Inhibitor of apoptosis; c-FLIP, Cellular flice inhibitory protein.

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Recent studies have demonstrated increased production of proinflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and IL-8 in IBD that are known to play a key role in the modulation of intestinal immune system [4–6]. The nuclear transcription factor κB (NF-κB) is a key regulator of the inducible expression of numerous genes involved in immune and inflammatory responses in the gut [7–9]. Sustained activation of NF-κB is thought to be pivotal in the pathophysiology of chronic intestinal inflammation. Increased NF-κB activation has been detected in the intestinal lamina propria of patients with Crohn's disease [10,11], and in a murine TNBS (trinitrobenzene sulphonic acid) colitis model [12]. Activation of this transcription factor then upregulates the expression of numerous proinflammatory genes involved in intestinal inflammation. Growing evidence in the association of NF-κB activation and IBD suggests that the modulation of NF-κB signaling pathway could be the main target for the anti-inflammatory treatment of IBD [8,13].

Statins are a new class of anticholesteremic 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors and have several beneficial effects on the cardiovascular system not strictly related to their effects on cholesterol metabolism [14–20]. During the past several years these beneficial effects of statins have been identified which includes anti-inflammatory and immunomodulatory properties. Despite the increasing number of reports describing anti-inflammatory effects of statins, the molecular mechanisms of these effects remain to be elucidated. In every inflammatory process, the vast majority of cellular events require NF-κB transcriptional activity. Several studies have revealed that anti-inflammatory effects of statins are related to the modulation of NF-κB signaling pathway in vascular endothelial and smooth muscle cells, and monocytes [21–26]. In addition, two studies reported that pravastatin [27] and rosuvastatin [28] reduced disease activity and colonic inflammation in dextran sulfate sodium (DSS)-induced colitis in mice. However, the anti-inflammatory mechanisms of statins on intestinal epithelial cells (IEC) and the experimental colitis still remain unknown.

Therefore, we aimed to investigate anti-inflammatory effects of simvastatin on IEC and on an experimental murine colitis model, and to elucidate its molecular mechanisms.

2. Materials and methods

2.1. Cell culture and treatments

The human colon cancer cell line COLO 205 [KCLB 10222, Korean Cell Line Bank (KCLB), Seoul, Korea] was used between passages 15 and 30. Cells were grown as described previously [29,30]. Simvastatin (MERCK and CO., Inc., Rahway, NJ, USA) was dissolved in ethanol and NaOH followed by neutralization to pH 7.2 for activation as a 10 mM stock solution and stored at −20 °C. Cells were pretreated for 24 h with various concentrations of simvastatin (0–50 μM) or with vehicle, after which COLO 205 cells were stimulated with TNF-α (10 ng/ml; Biosource, Camarillo, CA, USA) for various times.

2.2. Mice

Seven–eight-week-old male C57BL/6 mice were purchased from Orient (Seongnam, Korea). Animals were maintained on a 12-h light/12-h dark cycle under the specific pathogen-free condition. The mice had ad libitum access to a standard diet and water until reaching the desired age (8–9 weeks) and/or weight (18–21 g). All procedures using animals were reviewed and approved by the Institutional Animal Care and Use Committee of Seoul National University Hospital.

2.3. RNA extraction and real-time RT-PCR analysis

Simvastatin-pretreated cells were stimulated with TNF-α for 1 to 4 h. Total cellular RNA was isolated using Trizol (Invitrogen, Carlsbad, CA, USA) and 1 μg of total RNA was reverse-transcribed as described previously [31]. Real-time PCR was performed using an ABI prism 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA) with specific primers for human IL-8. As an endogenous control, β-actin for human IL-8 was used. All primers were designed by Primer Express v2.0 (Applied Biosystems). The primer sequences used in the present study are as follows: IL-8 (S): AAACCACCGG AAGGAACCAT, (AS): CCTTCACACAGAGCTGCAGAAA; β-actin (S): ACGGGGTCACCCACACTGTGCCCATCTA, (AS): CTAGAAGCATTGCGGTGGACGATGGAGGG. PCR was conducted using an SYBR Green PCR Master Mix (Applied Biosystems) according to the manufacturer's instructions. The thermal cycler conditions were as follows; one cycle of 10 min at 95 °C, followed by 40 amplification cycles [denaturation (15 s at 95 °C) and combined annealing/extension (1 min at 60 °C)]. Amplifications were performed in triplicate and the data were normalized versus β-actin for human IL-8.

2.4. Adenoviral transfections and NF-κB luciferase reporter assay

To determine NF-κB luciferase activities, cells were infected for 16 h with Ad5κB-LUC (kindly provided by Dr. Christian Jobin, University of North Carolina, Chapel Hill, NC, USA) at a multiplicity of infection of 50 [32]. Cells were stimulated with TNF-α for 12 h with/without simvastatin. Cell extracts were prepared using cell lysis buffer (Promega, Madison, WI, USA), and luciferase assays were performed using a Microplate Luminometer LB 96V (EG & G Berthold, Bad Wildbad, Germany). Extract protein concentrations were normalized using Bio-Rad protein assay kits (Bio-Rad, Hercules, CA, USA).