Ursodeoxycholic acid attenuates experimental autoimmune arthritis by targeting Th17 and inducing pAMPK and transcriptional corepressor SMILE

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ABSTRACT

Background: Ursodeoxycholic acid (UDCA) has been known that UDCA has prominent effects on liver, however, there is little known about its influence on autoimmune disease. Here, the benefit of UDCA on arthritis rheumatoid (RA) in vivo was tested.

Methods: RA mouse were induced using collagen II (CIA, collagen induced arthritis) where the disease severity or UDCA-related signaling pathway such as AMP-activated protein kinase (AMPK) or small heterodimer partner interacting leucine zipper protein (SMILE) was evaluated by westerblot and immunohistochemical staining. Gene expression was measured by realtime-polymerase chain reaction (PCR).

Results: The administration of UDCA effectively alleviated the arthritic score and incidence with decreased cartilage damage and lipid metabolic parameters. UDCA also suppressed the secretion of pro-inflammatory cytokines. It was confirmed that UDCA upregulated the expression of SMILE and transcriptional activity of PPARγ via controlling AMPK or p38 activity.

Conclusions: In the present study, the therapeutic effect of UDCA inducing SMILE through AMPK activation in rheumatoid arthritis mouse as well as other autoimmune disease was proposed.

1. Introduction

Ursodeoxycholic acid (UDCA), also known ursodiol (USAN), is one of the secondary bile acids (BA). UCDA is produced in the liver or intestinal bacteria, and composes approximately 5% of the total BA [1,2]. UDCA was first used for the therapy of gallstone disease in the 1970s, and extended its usage for liver protection or transplantation [3–5]. However, there is minimal reports on UDCA and its effect on autoimmune disease including autoimmune hepatitis. There was an attempt to cure autoimmune hepatitis using UDCA, but no effect was found [6]. However, it suppressed eosinophil mediated inflammation by inhibiting dendritic cell through farnesoid X receptor [7], which showed its influence on T cell function by DC and T cell interaction. UDCA may show therapeutic activity in inflammatory disease because it decreased the expression of TNF-α-induced IL-8 from monocytes in recent [8]. These studies imply that UDCA might has an immunomodulation role in autoimmune disease.

Here, we examined the therapeutic effect of UDCA on rheumatoid arthritis (RA), RA is one of the common autoimmune disease, characterized by progressive joint destruction and functional disability. Accumulating scientific evidence indicates that IL-17-producing T helper (Th17) cells and its secreting cytokine, interleukin 17 (IL-17), play critical roles in RA development [9,10]. IL-17 induces pannus growth, destruction of joints by enhancing osteoclastogenesis and synovial angiogenesis [11–13].

UDCA shows benefits on cholestatic liver disease by anti-apoptotic and anti-fibrotic effects through the PI3 K/Akt/Nrf2 pathway [14], and inhibits liver X receptor α-mediated hepatic lipogenesis by the induction of small heterodimer partner interacting leucine zipper protein (SMILE) [15]. SMILE has been known as a nuclear corepressor of various nuclear receptor such as estrogen receptor, glucocorticoid receptor, hepatocyte nuclear factor 4α, and estrogen receptor-related receptor γ [16–18]. In contrast, SMILE directly binds to peroxisome proliferator-activated receptor (PPAR) γ, and enhances its transcrip-
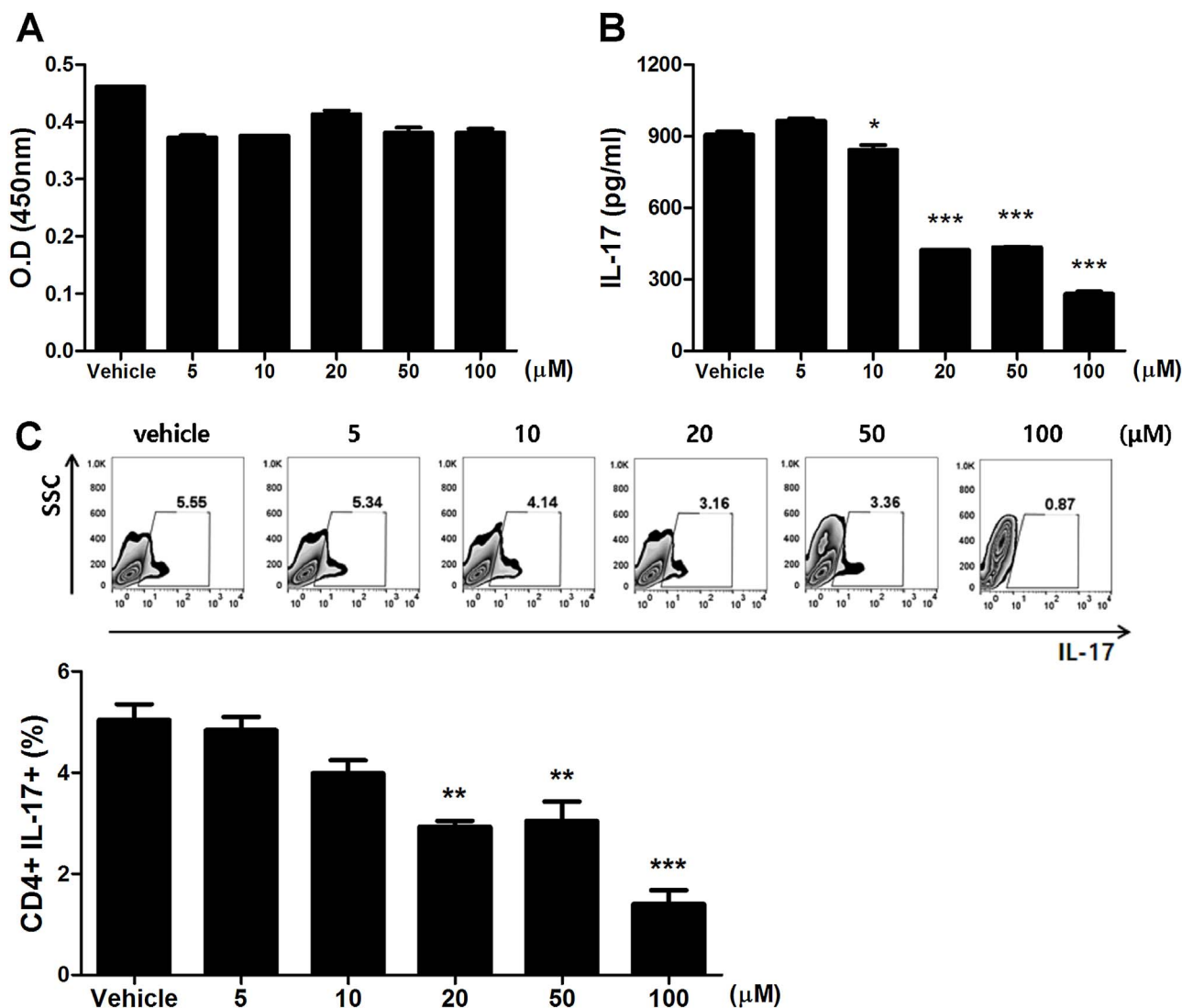
Fig. 1. Inhibitory effect of UDCA on Th17 cells.
Isolated CD4+ T cells of C57BL/6 mice were incubated with UDCA for 2 h in the presence or absence of increasing concentrations 5–100 μmol/L, then cultured with under Th17 polarizing conditions for 3 days. In addition, cells were one more treated UDCA on the second day before the end of the incubation period. (A) The cytotoxicity was measured by CCK-8 on the CD4+ T cells with increasing concentrations of UDCA under null condition for 3 day. (B) The culture medium was collected at the end of the incubation period, and was measured IL-17 by enzyme-linked immunosorbent assay. (C) The cells were stained with anti-CD4 and anti-IL-17. Data represent means ± SD of three independent experiments (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

tional activity [19,20]. PPARs have important roles in T-cell-related autoimmune disease and CD4+ T helper cells including Th1, Th2, Th17, and Treg cells [21]. PPARγ suppressed in Th17 differentiation by inhibition of RORγt in multiple sclerosis [22]. The induction of SMILE is also dependent on adenosine monophosphate-activated kinase (AMPK) [23]. The development of rheumatoid arthritis is related to the disruption of Th17/Treg balance [24]. AMPK inhibits mammalian target of rapamycin (mTOR), which controls T cell differentiation, and suppresses Th17 cell by inhibits mTOR as well as STAT3 [25]. AMPK is known to play a role to regulate Th17/Treg balance and osteoclastogenesis. Activation of AMPK with metformin attenuated RA in collagen-induced arthritis (CIA) mouse model [26].

In the present study, the therapeutic effect of UDCA by inducing SMILE through AMPK activation in rheumatoid arthritis mouse model was investigated. The induction of SMILE by UDCA effectively inhibited the secretion of pro-inflammatory cytokines such IL-17 production.

2. Materials and methods

2.1. Animals

8-week-old male DBA1/J mice and C57BL/6 mice (Orient Bio, Korea) were maintained under specific pathogen-free conditions and fed standard laboratory mouse chow (Ralston Purina, St. Louis, MO) and water ad libitum. All experimental procedures were examined and approved by the Animal Research Ethics Committee of the Catholic University of Korea, which conforms to all National Institutes of Health of the USA guidelines. All surgeries were performed under isoflurane anesthesia and all efforts were made to minimize suffering.

2.2. Induction of arthritis and treatment of UDCA

CIA was induced in DBA1/J mice. Mice were immunized into the base of the tail with 100 μg of chicken CII (Chondrex Inc., Redmond, WA, USA) in complete Freund's adjuvant (Chondrex Inc.). 100 μg of chicken CII in incomplete Freund's adjuvant (Chondrex Inc.) was