別紙様式第４

学　位　論　文　要　旨

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[論文題名]

The HDAC inhibitor, SAHA, prevents colonic inflammation by suppressing pro-inflammatory cytokines and chemokines in DSS-induced colitis

ヒストン脱アセチル化酵素阻害剤である SAHA は DSS 誘導腸炎モデルにおいて炎症性サイトカインとケモカインを抑制することにより大腸の炎症を防止する

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[要　旨]

Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract with two major clinical forms: ulcerative colitis and Crohn's disease. The etiology of IBD is unknown, recent studies have suggested that multiple factors such as genetics, epigenetics, diet, environmental factors, and the innate immune system play an important role in the pathogenesis of IBD. Among these factors, epigenetics is considered an important factor in IBD onset and pathogenesis. Epigenetic alterations such as differential patterns of histone acetylation are found in colitis.

HDAC inhibitors are considered powerful epigenetic regulators and immunomodulators. In the clinical setting, HDAC inhibitors have been introduced for treatment of cancers and inflammatory diseases including rheumatoid arthritis, asthma, and ischemia-reperfusion injury. Among HDAC inhibitors, suberoylanilide hydroxamic acid (SAHA) is a potential therapeutic agent that suppresses inflammation and peritoneal fibrosis, however, the effects of SAHA in IBD pathogenesis is unknown.

Several animal models have been developed to study the pathogenesis of IBD, among all model, dextran sulfate sodium (DSS) is considered the most suitable model to induce colitis. During colitis, local secretion of pro-inflammatory cytokines and

chemokines in colonic mucosa leads to accumulation of migratory macrophages, monocytes, and dendritic cells. Although dendritic cells and macrophages are antigen presenting cells (APCs), they may also play important roles in initiation and progression of inflammation. Therefore, we hypothesized that SAHA treatment may reduce local inflammation and secretion of inflammatory cytokines and chemokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and Ccl2. In this study, we investigated the effects of SAHA in a DSS-induced colitis mouse model.

Materials and Methods

Eight-week-old male C57BL/6 mice were kept in specific pathogen-free conditions with a 12:12 hour light-dark cycle and *ad libitum* access to food and water. The experimental protocol was approved by the animal ethics review committee of Miyazaki University (2012-502-5), and all experiments were performed in accordance with institutional guidelines. The experimental animals were divided into four groups: control, DSS, DSS+SAHA, and SAHA, and each group consisted of 5-10 mice. To induce colitis, 1.5% DSS was dissolved in drinking water, and the DSS and DSS+SAHA mice received DSS for 5 days. On day 5, water with DSS was switched to normal water. SAHA powder was dissolved in 5% dimethylsulfoxide (DMSO)/phosphate-buffered saline (PBS) and administered to the SAHA and DSS+SAHA groups by daily intraperitoneal (IP) injection at a concentration of 25 mg/kg body weight from day 1 to day 26. Control and DSS mice received daily IP injection of 5% DMSO/PBS. Mice were sacrificed by cervical dislocation, and the entire colon was removed. The length and weight of the colon were measured, and then incubated in 4% PFA/ in PBS and snap frozen in optimal cutting temperature compound for immunohistochemistry and fresh tissue samples for qRT-PCR. The histological score was evaluated in a blinded manner by microscopic examination of hematoxylin and eosin (HE) stained tissue.

Results

Our results showed that the severity of colitis is strongly correlated with colon length. The colon length of DSS-treated mice was significantly shorter on day 5 than that of the control, DSS+SAHA, and SAHA-treated groups and this tendency remained

on days 12 and 19. Based on the histopathological evaluation, acute inflammation, including shortening and loss of crypts and infiltration of inflammatory cells in the lamina propria, was observed in DSS-treated mouse colon on day 5. The most severe damage, including crypt abscesses, loss of surface epithelium, and infiltration of inflammatory cells into the lamina propria and submucosa, was found in DSS-treated mouse colon on day 12. Surprisingly, DSS+SAHA-treated mouse colon revealed only mild damage on all days compared to DSS-treated mouse colon.

In DSS+SAHA-treated mouse colon, significantly lower histological changes were found on all sampling days compared to DSS-treated mouse colon. Highest expression levels of IL-6 and TNF-α were found in DSS-treated mouse colon on days 5 and 12, whereas significantly lower expression was found in DSS+SAHA-treated mouse colon. The expression level of Ccl2 was dramatically increased in DSS-treated mouse colon on day 5 and then decreased continually at later time points. Importantly, DSS+SAHA-treated mouse colon showed significantly lower expression of Ccl2 on day 5 compared to DSS-treated mouse colon. To confirm the qRT-PCR results, we performed immunohistochemistry to determine Ccl2 protein expression in colonic mucosa. In DSS-treated mouse colon on day 5, a strong fluorescent signal for Ccl2 was found throughout the colon epithelium, and the signal was especially strong in goblet cells. Although Ccl2 expression remained in DSS-treated mouse colon on days 12 and 19, the staining intensity was decreased significantly, and only a few goblet cells were positive for Ccl2. Consistent with Ccl2 gene expression, DSS+SAHA-treated mouse colon showed a dramatic decrease in the Ccl2 expression on all days compared to DSS-treated mouse colon. SAHA-only and vehicle-only control mouse colons were negative for Ccl2 expression.

DSS-treated mouse colon showed accumulation of CD11b-positive cells in colonic mucosa on day 5. Interestingly, DAPI staining revealed intact crypt morphology on day 5, suggesting that the inflammatory process has just initiated with migrating inflammatory cells. Surprisingly, CD11b-positive cells were significantly increased at inflammatory sites in DSS-treated mouse colon on day 12. Moreover, CD11b-positive cells were localized not only in the mucosa but also in the submucosa and smooth muscle layer, indicating that the most severe inflammation had occurred. In DSS-treated

mouse colon, crypt recovery was observed on day 19 with decreased numbers of CD11b-positive cells. On day 5, DSS+SAHA-treated mice had no obvious damage in the colonic crypt, and only a few CD11b-positive cells were detected. In DSS+SAHA-treated mouse colon on day 12, the number of CD11b-positive cells was dramatically decreased. As expected, SAHA-only treated mouse colon on all days showed only a few positive cells, similar to vehicle only-treated control mouse colon.

Discussion and conclusion

The major finding of this study was that the HDAC inhibitor, SAHA, attenuated inflammatory changes in DSS-induced colitis by suppressing local secretion of pro-inflammatory cytokines and chemokines. Moreover, SAHA suppressed mobilization and accumulation of inflammatory cells such as macrophages, dendritic cells, monocytes, and eosinophils. Although histopathological damage was minor on day 5, the peak of inflammatory gene expression as seen with qRT-PCR was found in DSS-treated mouse colon. In contrast, SAHA treatment dramatically decreased pro-inflammatory gene expression in the DSS-treated colon. These findings demonstrate that SAHA has immunomodulatory effects on the innate immune system, including suppression of local secretion of pro-inflammatory cytokines and chemokines. Moreover, our results demonstrate that both gene and protein expression of Ccl2 were suppressed by SAHA. The severe histopathological damage, as well as the accumulation of APCs, were found in DSS-treated mouse colon on day 12. Surprisingly, fewer migratory cells were seen in SAHA-treated mouse colon on day 12. These results suggest that APCs are negatively regulated by decreased secretion of cytokines and chemokines in colonic mucosa. Therefore, SAHA treatment decreases the mobilization and accumulation of inflammatory cells in colonic mucosa and may have protective effects against inflammation in DSS-induced colitis.

In conclusion, the present study demonstrated that SAHA attenuates inflammatory changes in DSS-induced colitis by suppressing pro-inflammatory cytokines and chemokines as well as accumulation of active inflammatory cells. However, detailed investigations are necessary to reveal the molecular mechanisms of the effects of SAHA in IBD pathogenesis.