The preventive effects of asperuloside administration on dextran sodium sulfate-induced ulcerative colitis in mice

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Abstract

Eucommia ulmoides leaf extract (ELE) is a widely used Chinese medicine; asperuloside is the active substance in ELE and has anti-obesity properties. This study examined the effects of asperuloside administration on dextran sodium sulfate (DSS)-induced ulcerative colitis. We produced a mouse model of ulcerative colitis by administering DSS for 5 days. Asperuloside (160 mg/kg) was also orally administered concomitantly with the DSS for the initial 5 days. DSS-induced ulcerative colitis was ameliorated after administration of asperuloside. Blood levels of interleukin (IL)-6, tumor necrosis factor (TNF)-α, histamine, acetylcholine (ACh), and reactive oxygen species (ROS) increased in DSS-treated mice. In contrast, blood histamine and ACh levels were lower in asperuloside + DSS-treated mice than those in mice administered DSS only; however, blood IL-6, TNF-α, and ROS levels did not change. Although expression of the ACh receptor (AChR) increased in mast cells from DSS-treated mice, the expression decreased after administration of asperuloside. These results suggested that asperuloside administration is effective as a prophylaxis/treatment of DSS-induced ulcerative colitis in mice.

Introduction

Ulcerative colitis is an inflammatory disorder attributable to autoimmune disorders [1] or bacterial infections [2] and is characterized by inflammation of the colon tunica mucosa, with repeating periods of remission and recrudescence. Inflammation begins in the intestinal rectum near a fundament and spreads toward the colon, inducing loose bowels, bloody and mucous-containing stools, thermacogenesis, and weight loss [3,4]. Numerous animal models of ulcerative colitis have been used to elucidate the underlying mechanisms. Dextran sodium sulfate (DSS) can induce inflammation limited to the colon in mice, with symptoms resembling those observed in human ulcerative colitis [5]. Therefore, we used a DSS-induced ulcerative colitis mouse model to search for a corrective strategy to ameliorate symptoms.

Eucommia ulmoides leaf extract (ELE) is a Chinese medicine with various reported effects [6,7]. ELE contains several active substances, including geniposidic acid, asperuloside, and chlorogenic acid. Particularly, asperuloside, iridoid glucosides shows decreased ATP production in the WAT, accelerated fatty acid β-oxidation in the liver, increased use of ketone bodies and glucose in skeletal muscle, and may have increased non-shivering thermogenesis due to UCP1 expression in the BAT [8]. The bile acids secretion by asperuloside promotes these processes, followed by improving accumulation of the visceral fat and the exacerbation of blood fatty acid levels under the high fat diet [9,10]. As such, improvements in lifestyle-related diseases are expected following ingestion of asperuloside.

However, there are no reports regarding the effects of asperuloside on ulcerative colitis. In this study, we examined the effects of asperuloside on a DSS-induced ulcerative colitis mouse model.
= very soft, but formed; 3 = liquid) and fecal bleeding (0 = negative; 1 = faintly blue; 2 = moderately blue; 3 = dark blue; 4 = blood visible; determined via the guaiac paper test); the sum of these two parameters was considered to be the individual’s disease activity score [11]. This study was carried out in strict accordance with the recommendations of the guide for the care and use of laboratory animals of Suzuka University of Medical Science (approval number: 34). All surgeries were performed under pentobarbital anesthesia, and all efforts were made to minimize suffering.

**Results**

**Effects of asperuloside administration in DSS-treated mice**

Diarrhea and fecal bleeding were observed after DSS treatment in mice. The severity of disease was lower in the asperuloside + DSS-treated mice than that in mice treated with DSS only (Figure 1D). DSS-treatment also resulted in a drastic decrease in body weight and colon length. The average colon length in asperuloside + DSS-treated mice was longer than that in mice treated with DSS only (Figure 1A,B,C).

**Effects of asperuloside administration on plasma levels of IL-6, TNF-α histamine, acetylcholine, and ROS in DSS-treated mice**

We measured the plasma levels of IL-6, TNF-α histamine, acetylcholine, and ROS in DSS-treated mice after 5 days of treatment. Plasma IL-6, TNF-α histamine, acetylcholine, and ROS levels were increased after DSS treatment. However, histamine and acetylcholine levels were lower in asperuloside + DSS-treated mice than those in mice treated with DSS only. Plasma IL-6, TNF-α and ROS levels in the asperuloside + DSS-treated mice did not differ from those in mice treated with DSS only (Figure 2).

**Activation of muscarinic acetylcholine receptors (mAChR) during colon disruption**

Mas cell tryptase expression increased in the colons of DSS-treated mice. Furthermore, we observed an increase in mAChR expression, as well as colocalization of these receptors with mast cell tryptases in DSS-treated mice. Expression of these receptors was lower in asperuloside + DSS-treated mice than that in mice treated with DSS only (Figure 3).

**Discussion**

The present study demonstrates that asperuloside, found in ELEs, ameliorates the symptoms of DSS-induced ulcerative colitis. In addition, plasma levels of histamine and acetylcholine, as well as the expression of colon mAChR were reduced after asperuloside administration.

It has been reported that colitis symptoms in the DSS-induced ulcerative colitis mouse model are attributable to TNF-α [14], IL-6 [15], and oxidative stress [16]. In this study, alterations in TNF-α, IL-6, and ROS levels were not observed after asperuloside administration, suggesting they are not directly affected by asperuloside treatment. In contrast, events occurring in mast cells indicate an important role for the onset of colitis [17]; although expression of mast cells following asperuloside treatment did not differ from that in mice treated with DSS only, histamine levels decreased. However, mechanisms underlying the inhibition of histamine secretion from mast cells after asperuloside treatment are unknown. Asperuloside affects the central nervous system [18]; therefore, these results may be attributable to various neurological effects. mAChRs (nicotinic receptors (nAChR) and mAChR) are expressed on the mast cells, and degranulation is induced when a ligand combines with these receptors. It has been suggested that ACh, which is an AChR ligand, is increased in DSS-treated mice, resulting in an increase in the secretion of histamine from mast cells [19,20]. In addition, it has been reported that the mAChR cholinergic signal transmission system is involved in mast cell activation [21]. Asperuloside administration decreases ACh content and the expression of mAChRs in mast cells of mice with DSS-induced ulcerative colitis. These findings suggest that asperuloside suppresses degranulation by inhibiting the expression of ACh/mAChR in mast cells; therefore, we
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Figure 1. The effects of asperuloside administration on dextran sodium sulfate (DSS)-induced ulcerative colitis. Colitis scores (D) and animal body weights (B) are shown. The length of the large intestines (A,C) is shown. Values represent the means ± SD derived from 6 animals. *P<0.05.

Figure 2. An analysis of plasma interleukin (IL)-6, plasma tumor necrosis factor-alpha (TNF-α), plasma histamine, plasma acetylcholine (ACh), and plasma reactive oxygen species (ROS) concentrations after dextran sodium sulfate (DSS)- or asperuloside + DSS-treatment is shown. Values represent the means ± SD derived from 6 animals. * P<0.05.
suggest that asperuloside ameliorates DSS-induced colitis. However, we
do not fully understand the mechanisms by which ACh and mAChR
are decreased, and further examination is necessary.

Conclusion

This study indicates the possibility that asperuloside targets signal
transmission pathways through AChRs on mast cells and could prevent
colitis. Asperuloside is an active substance contained in EUE, which
is used as an auxiliary material for the prophylaxis and treatment of
colitis and is known for its safety and low price. However, information
regarding the effects and mechanisms of asperuloside is still lacking,
and further reports are needed.

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Conflict of interest

There are no conflicts of interest to declare.

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