ORIGINAL ARTICLE

Substance-P Ameliorates Dextran Sodium Sulfate-Induced Intestinal Damage by Preserving Tissue Barrier Function

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Received: 8 February 2017 / Revised: 30 August 2017 / Accepted: 11 September 2017 / Published online: 13 October 2017
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Abstract Intestinal inflammation alters immune responses in the mucosa and destroys colon architecture, leading to serious diseases such as inflammatory bowel disease. Thus, the modulation of intestinal integrity and immune responses in IBD can be the critical factor to be considered to reduce the severity of damages. Substance-P (SP), endogenous peptide to be involved in cell proliferation, migration and immune modulation, can exert the therapeutic effect on diverse diseases including cornea damage, rheumatoid arthritis and diabetic complications. SP was found to elevate expression of junctional molecule. Considering the function of SP reported previously, it was inferred that SP is capable of exert the beneficial effect of SP on intestinal diseases by controlling intestinal structure as well as immune responses. In this study, we explored the therapeutic effect of SP on dextran sodium sulfate-induced intestine damage by injecting SP. The effects of SP were evaluated by analyzing crypt structures, proliferating cell pool and infiltration of immune cells. DSS treatment provoked abnormal immune response and disruption of intestine epithelial barrier. However, co-treatment of SP obviously blocked the development of intestinal damages by declining inflammatory responses and sustaining intestinal structure more intact. The treatment of SP to chronic damages also promoted intestinal regeneration by preserving the integrity of colon tissue. Moreover, DSS-induced reduction of epithelial junctional molecule was obviously inhibited by SP treatment *in vitro*. Taken together, our data indicate that SP can reduce intestinal damages, possibly by modulating barrier structure and immune response. Our results propose SP as candidate therapeutics in intestinal damages.

Keywords Substance-P · Tight junction · Intestine · Inflammatory bowel disease

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Abbreviations

BMSCs	Bone marrow stromal cells
DSS	Dextran sodium sulfate
FBS	Fetal bovine serum
IBD	Inflammatory bowel disease
IL-6	Interleukin-6
IL-10	Interleukin-10
IL-17	Interleukin-17
LP	Lamina propria
MEM	Minimum essential medium
MSCs	Mesenchymal stem cells
NK-1R	Neurokinin-1 receptor
PBS	Phosphate-buffered saline
PCNA	Proliferating cell nuclear antigen
SD	Standard deviation

SP	Substance-P
TJ	Tight junction
TNF- α	Tumor necrosis factor-alpha
UC	Ulcerative colitis
ZO-1	Zonula occludens-1

1 Introduction

The ulcerative colitis (UC), one of main type of inflammatory bowel disease (IBD), is the relapsing disease, relating to the structural damage of bowel wall and chronic intestinal inflammation [1–4]. Most of IBD patients have a more difficulties to usual social life because of symptom such as persistent diarrhea, frequently bloody stools and abdominal pain. Although the precise pathogenesis of IBD still remained unclear, it has been suggested that the impairment of intestine barrier in response to overactive immune reaction permits the infiltration of gut lumen of bacteria or noxious antigen to host immune system, leading to development of UC [4, 5]. Thus, it is undeniable that the maintenance of intestine barrier is very important for an effective therapeutic strategy of UC.

Under the inflammatory conditions of intestine such as UC, the several types of cytokines from immune cells provoke the dysfunction of epithelial cell junction and lead to imbalance between cell proliferation and apoptosis [6, 7]. It was reported that the amount of pro-inflammatory cytokine including tumor necrosis factor-alpha (TNF- α) [8, 9], interleukin-6 (IL-6) [10, 11] and interleukin-17 (IL-17) [10] was significantly elevated in tissue and serum of UC patients, compared to those of healthy control. In particular, the high level of TNF- α , the crucial factor for pathogenesis of UC, exaggerates barrier disruption by reducing the tight junction (TJ) molecular proteins such as occludin, claudin and zonula occludens-1 (ZO-1) [12, 13] and promotes the activation of other immune cells, resulting in mucosal inflammation and homeostatic disruption [14, 15]. Thus, anti-TNF- α is commonly applied to UC patients in clinic [15, 16]. As the other therapy, visilizumab induces apoptosis of lamina propria (LP) CD4+ T cell to suppress highly activated immune responses [17]. However, in spite of diverse therapeutics, the use of conventional drugs has been controlled because of its undesirable effects including infections such as tuberculosis. Thus, the safe and effective treatment for UC patients is required.

Substance P (SP), consisting of 11-amino acid neuropeptide, is broadly expressed in immune cell as well as neuronal cells and modulates cellular proliferation and tissue repair [18, 19]. Colonocyte and immune effector cells in the intestine also express SP [20, 21]. Recent evidence revealed that SP is able to promote epithelial cell

proliferation and provides anti-apoptotic effect on injury site of large intestine through binding NK-1R, leading to the accelerated intestinal healing [18, 22]. SP is also found to mobilize stem cells from bone marrow to injury site and inhibit inflammation by upregulating of IL-10 by M2 type of macrophages and Treg and down-regulating in TNF- α /IL-17, which efficacy was proved in diverse diseases including RA and radiation-induced damages [19, 23–25]. Notably, SP was able to increase the expression of junctional protein such as ZO-1 and E-cadherin in epithelial cells [26, 27].

Based on the functions of SP, SP was expected to be capable of preventing intestinal damage by increasing cell proliferation or TJ expression as well as the suppression of inflammation. In order to examine the protective effect of SP on acute intestinal damages *in vivo*, intestine was impaired by DSS administration and SP was daily treated intravenously. Also, to check the recovery effect of SP on chronic intestinal damages, DSS was treated by cyclic administration for long term and then, SP was injected to vein. The effect of SP was evaluated by analyzing total length of intestine, crypt length, intestine structure, collagen deposition, infiltration of F4/80+ macrophage in mucosal injury site, PCNA (+) cell per crypt and the expression of junctional molecule. To explore the direct effect of SP on intestine epithelial cells, Caco-2 was treated by 1.5% DSS and SP was added. The expression level of junctional molecule was determined *in vitro*.

2 Materials and methods

2.1 Materials

Substance P acetate salt hydrate was purchased from Sigma Aldrich (St Louis, MO, USA). Minimum essential medium (MEM) and fetal bovine serum (FBS) were supplied by Gibco (Grand Island, NY). Penicillin/streptomycin, 0.25% trypsin–EDTA solution, and phosphate buffered saline (PBS) were purchased from Welgene (Daegu, Korea).

2.2 Cell culture

Caco-2, human intestinal epithelial cell line, was obtained from the Korean cell line bank and maintained in MEM supplemented with 10% FBS and 1% penicillin and streptomycin. Caco-2 was cultured in a humidified 37 °C, 5% CO₂ incubator and culture medium was changed every 2 days to prevent nutrient deficiency. During all experiment, Caco-2 cells between passages 31 to 40 were used. Serum starvation for 24 h was carried out before treatment of DSS and SP.

2.3 Protein lysis and western blot

Cells were washed with phosphate-buffered saline (PBS) and lysed in 1× lysis buffer (Cell Signaling Technology, USA) for 10 min. The whole protein of cells was centrifuged at 12,000 rpm for 10 min. The protein concentration of the supernatant was measured by the BCA reagents (Thermo, Waltham, MA, USA). Equal amount of proteins was resolved by SDS-PAGE and transferred onto nitrocellulose membrane. The membranes were masked using 5% skim milk in TBS-T for 1 h at room temperature (RT) and incubated sequentially with primary antibody to ZO-1 (1:200) and beta actin (1:5000) (Abcam, Cambridge, MA, USA) and alpha tubulin (1:5000) (Sigma-Aldrich, St Louis, MO, USA). Then, membranes were treated for 1 h with HRP-conjugated secondary antibody (1:5000). Antibody binding was detected using ECL reagents and chemiluminescence (GE healthcare).

2.4 Animal

6 week-old C57BL/6N male mice were purchased from Daehan bio link (Seoul, Korea) and maintained under a 12-h light/12-h dark illumination cycle in an animal holding room. All animals were provided with standard chow and water ad libitum. All animal studies were approved by the Ethical Committees for Experimental Animals at Kyung Hee University (Approval number KHMC-IACUC-14-010).

2.5 Induction of intestinal damages

All C57BL/6N mice (weight 20 g) were randomized into three groups: (1) Normal group, (2) DSS-induced colitis group with saline and (3) DSS-induced colitis group with SP. Dextran sulfate sodium salt reagent (DSS; MP biomedical, Solon-Ohio, Burlingame CA) was dissolved in autoclaved water to a concentration of 1.5% w/v. Mice were provided in drinking water including 1.5% DSS (w/v) and were sacrificed at 4 or 7 days post DSS treatment. During the experiment periods, DSS solution was changed every two day to maintain active DSS. For the induction of chronic colitis, mice were exposed to 1.5% DSS in drinking water for 5 days, followed by drinking water for 5 days. This cycle was repeated three times.

2.6 Administration of SP

In acute DSS model, mice were injected with SP in dose of 5 nmole/kg once a day for 7 days. Saline (JW Pharmaceutical, Seoul, Korea) was used as a vehicle. In case of chronic model, SP was treated twice a week from 7 days post third administration of DSS.

2.7 Tissue preparation

Ketamine (100 mg/kg, Yuhan Co. Seoul, Korea) and Rumpun (1.2 mg/kg, Bayer Healthcare, Gyeonggi-do, Korea) were administrated to all mice to anesthetize during the experiment. Colons were detached and washed with cold phosphate-buffered saline to remove fecal contents. After measuring its linear length, distal half of colon was dissected for histological analysis. Tissue was fixed in 3.7% (v/v) formaldehyde solution for 24 h. The sample was embedded in paraffin using a TP1020 tissue processor (Leica Biosystems, Wetzlar, Germany).

2.8 Hematoxylin and eosin staining

Paraffin-sectioned samples (4 µm-thickness) were deparaffinized by using xylene and rehydrated in a graded ethanol series (100, 90, 80, 70%). After washing in tap water, nuclei were stained with hematoxylin (Sigma-Aldrich, St Louis, MO, USA) for 2 min and washed during 5 min in tap water. To stain the cytoplasm, eosin Y (Sigma-Aldrich, St Louis, MO, USA) was applied for 10 s and washed in tap water. Samples were dehydrated in serial ethanol from 70 to 100% and then, were mounted in synthetic mountantTM (Thermo, Waltham, MA, USA).

2.9 Immunohistochemical staining

Immunohistochemical staining was performed according to the vectastain ABC kit protocol (Vector Laboratories, Burlingame, CA). Tissue sample was deparaffinized in xylene. The slide was washed in PBS for 5 min and boiled in 0.01 M sodium citrate (pH 6.0) for 10 min for the antigen retrieval. The sample was incubated for 10 min with 0.5% H₂O₂ in methanol to block endogenous H₂O₂ and then, permeabilized with 0.3% Triton-X100 for 10 min. After washing in PBS, the sample was blocked using 1% normal horse serum for 1 h at RT and incubated with a primary antibody against PCNA (1:5000), F4/80 (1:100) (Abcam, Cambridge, MA, USA), ZO-1 (1:100) (Invitrogen, San Francisco, CA) and E-cadherin (1:1000) (Cell Signaling Technology, USA). After washing in PBS, a biotin-conjugated secondary antibody was treated for 1 h at RT. After washing in PBS, substrate solution was added for 1 h at RT. After washing in PBS, positive antibody binding was visualized using Nova-Red (Vector Laboratories, Burlingame, CA, USA) and counterstained for nuclei with fast red (Vector Laboratories, Burlingame, CA, USA).

2.10 Statistics

All the data are presented as mean $\pm$ standard deviation of the mean (SD) from 3 independent experiments. Measurements were made with five adjacent fields on the temporal slides. Probability values of p of < 0.05 were interpreted to denote statistical significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3 Result

3.1 SP treatment prevents DSS-induced intestinal damages

To examine the effects of SP on DSS-induced colitis, mice were fed with 1.5% DSS (w/v) water for 7 days. SP (5 nmol/kg) was injected intravenously once a day during DSS administration. All mice were sacrificed at day 7 post DSS treatment (Fig. 1A). Colon length is the important indicator to represent intestinal damage [28, 29]. Gross view for colon tissue showed that DSS treatment caused the reduction of colon length, which was inhibited by SP treatment (Fig. 1B, C). In order to evaluate the severity of inflammation and crypt structure in intestine tissue, H&E staining were performed [30, 31]. As shown in Fig. 1D, colon from DSS-treated mice showed the pathological changes including remarkable crypt damage, goblet cell decrease and infiltration of inflammatory cells. However, the colon of SP-treated mice had more intact crypt and mucosal structure, compared with that of saline-treated mice. The quantitative analysis of crypt length revealed that crypt length of SP-treated group was remarkably longer than that of saline-treated group (Fig. 1E).

During the inflammation, excessive cytokines disturbs hemostasis of immune cells and induces accumulation of collagen and ECM, leading to fibrosis [32–34]. Melgar et al. [35] reported that intestinal fibrosis was discovered in acute or chronic colitis. To identify the effects of SP intestinal fibrosis in DSS-induced intestinal damages, Masson's trichrome staining was performed. The collagen deposit in the mucosa and submucosa increased in saline-treated group but decreased in SP-treated group (Fig. 1F).

Taken together, these results suggest that SP treatment can reduce colon damage resulting from DSS treatment.

3.2 SP protects intestine structure against DSS-induced damage by modulating cell proliferation and junctional protein

For intestinal regeneration, cell repopulation happens in crypt and then, damaged cells are substituted with proliferating healthy cell. SP is known to promote cell

proliferation in various types of cell or tissue. Next, we examined whether SP affected proliferating cell pool in damaged tissue or not. As shown Fig. 2A, PCNA (+) cells in colonic tissue was obviously increased in SP-treated group, compared to that of saline-treated mice. Quantification of PCNA (+) cell per crypt confirmed that SP treatment enhanced cell repopulation within damaged intestine (Fig. 2B).

E-cadherin and ZO-1 is essential for the maintenance of tissue integrity [36, 37]. Down-expression of E-cadherin and ZO-1 is suggested to be one of the pathological features observed in IBD [36]. SP was reported to be able to upregulate the expression of E cadherin in epithelial cells. Treatment of SP on DSS-induced damages blocked the destruction of intestine tissue (Fig. 1). Next, we have checked the suppressive effect of SP on the loss of E-cadherin and ZO-1 due to DSS-induced colitis by immunohistochemical staining. As shown Fig. 2C, D, DSS treatment markedly reduced E-cadherin and ZO-1 expression in intestinal epithelial layer, whereas SP treatment blocked loss of E-cadherin and ZO-1 expression, sustaining integrity of intestine.

These results imply that SP can positively affect DSS-induced damages by promoting cell proliferation and inhibiting loss of junctional molecule in damaged intestine tissue.

3.3 SP suppresses the inflammation response in DSS-induced colitis

In order to investigate the effects of SP on immune response, macrophage infiltration at lesion site was determined by immunohistochemical staining. DSS treatment increased the number of macrophage infiltrated into lesion site of mucosa, comparing with normal mice. In contrast, SP-treated mice rarely showed macrophage infiltrated into mucosa (Fig. 3).

This result means that SP treatment decreased inflammation at damaged site, which might be likely to be caused by SP-induced maintenance of intestinal junction.

3.4 SP exert inhibitory effect on intestinal damages from early time post DSS treatment

This study proved that SP is able to inhibit development of DSS-induced colitis animal models by suppressing immune response and modulating intestinal cell proliferation and junctional protein. In order to check when the beneficial effect of SP on intestinal damages was initiated, the analysis for intestine was performed at day 4 post DSS treatment (Fig. 4A). The effect of SP was assessed based on the crypt structure and the rate of cell proliferation within crypt.

Fig. 1 The effect of SP in DSS-induced intestine damages. Mice received the drinking water with 1.5% DSS for 7 days and SP was administrated to tail vein during the intake of DSS. **A** Experimental schedule for colitis induction and SP treatment. **B** Gross view of intestine tissue. **C** Quantification of colon length (cm). **D** Histological analysis was performed by H&E staining. **E** Crypt length was

measured as a distance from the bottom to apical crypt using Image J program. **F** Collagen deposition was carried out using Masson's trichrome staining. Results are shown as mean values $\pm$ SD. $n = 10/\text{group}$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Scale bar: 50 μm

DSS treatment for 4 days did not affect colon length (Data not shown) but histological analysis showed that compared to the normal group, DSS-treated group showed moderate crypt architectural distortion and decreased length of crypt (Fig. 4B, C). In contrast, SP-treated group had crypt structure similar with normal tissue. PCNA (+) proliferating cell pool in crypt was also decreased by DSS

treatment, which was increased by SP treatment (Fig. 4D, E).

These results indicate that SP can inhibit DSS-induced damage from early time, which leads to the protection of intestine against DSS-induced damages.

Fig. 2 The effect of SP on cell repopulation and integrity in DSS-damaged intestine. **A** PCNA (+) staining to detect the proliferating cells within crypt. **B** Quantitative analysis in PCNA (+) cells per crypt was carried out. **C–D** Immunohistochemical staining for

E-cadherin and ZO-1. Results are shown as mean values $\pm$ SD. $n = 10/\text{group}$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Scale bar: 50 μm

3.5 SP treatment recovers chronic intestinal damage by regulating ZO-1 protein

Our experimental results revealed that SP can protect intestine tissues from DSS-induced colitis by preserving cellular junction and blocking inflammatory cell infiltration. It was attempted check whether SP can restore chronically-damaged intestine tissue or not. After three cycles of DSS intake and recovery phase, mice were injected by SP intravenously twice a week for 1 week (Fig. 5A). Consistent to the effect of SP on acute damages, saline-treated mice exhibited several fecal blood and stool consistency, whereas SP-treated mice did not have fecal blood and showed milder stool consistency (data not shown). Colon length indicates that intestinal injury was severely occurred in saline-treated mice. However, SP treatment promoted recovery of colon length, similar to normal group (Fig. 5B, C). Histological analysis obviously revealed that the reduction of crypt length and crypt collapse was observed in saline-treated mice. On the contrary,

SP-treated mice maintained crypt architecture intact (Fig. 5D, E).

Disruption of epithelial barrier is caused by the loss of junctional molecules, leading to increased infiltration of inflammatory cell into lamina propria and furthermore, exacerbated inflammation in colon. In order to examine if SP-mediated intestine repair is accompanied by preservation of barrier molecule in chronic state, the expression of ZO-1 was detected by immunohistochemical staining. Compared to that of normal group, saline-treated group had the reduced level of ZO-1 remarkably, which was reversed by SP treatment (Fig. 5F).

These results demonstrate that SP treatment is able to facilitate recovery of the chronic intestinal damage, restoring impaired function of barrier.

Fig. 3 The effect of SP on macrophage infiltration at wounded site. Immunohistochemical staining for f4/80 to check infiltrated macrophage into the injured tissue. Scale bar: 50 μ m

Fig. 4 The effect of SP on DSS-induced intestinal damage at early time post damages. DSS-induced colitis was triggered by exposure to 1.5% DSS. During DSS administration, SP was injected intravenously once a day in regular time and after 4 days, all mice was sacrificed. **A** Experimental schedule for colitis induction and SP treatment. **B** H&E staining of damaged tissue from saline- and SP-treated group

colonic section. **C** Crypt length was measured as a distance from the bottom to apical crypt using Image J program. **D** PCNA staining was carried out to check the effect of SP on proliferation cell pool. **E** Quantitative analysis in PCNA (+) cells per crypt was performed. Results are shown as mean values $\pm$ SD. n = 14/group, *p < 0.05, **p < 0.01, ***p < 0.001. Scale bar: 50 μ m

Fig. 5 The effect of SP on DSS-induced chronic damage of intestines. The chronic colitis was induced by administering DSS repeatedly and then, SP was injected to tail vein of mice for two consecutive days. **A** Experimental schedule for colitis induction and SP treatment. **B** Gross view of intestine tissue from each group. **C** Colon length was quantified (cm) **D** H&E staining of damaged tissue from saline- and SP-treated group colonic section. **E** Crypt length was measured as a distance from the bottom to apical crypt using Image J program. **F** Immunohistochemical staining for ZO-1 expression. Results are shown as mean values $\pm$ SD. $n = 10/\text{group}$, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Scale bar: 100 μm

3.6 SP blocks loss of cellular barrier molecule due to DSS treatment through NK-1 receptor

Tight junction protein is important to maintain epithelial integrity. DSS treatment decreases level of tight junction molecules such as ZO-1 and occludin in colonic tissue.¹² This study provided that systemically-injected SP inhibited destruction of tight junction of intestine tissue (Fig. 1). To examine the direct effect of SP on tight junctional molecule, SP was added to caco-2, human intestinal epithelial cell line in the presence of DSS *in vitro* and then, ZO-1 expression was determined by western blot (Fig. 6A).

The exposures to DSS for 24 h reduced ZO-1 level in caco-2 (Fig. 6B, C), compared to non-treated one, which became evident at 48 post DSS treatment (Fig. 6D, E). However, co-treatment of SP could sustain the level of ZO-1, similar with non-treated one. SP regulates the important cellular function including cell proliferation, anti-apoptosis and immune suppressive response by binding NK-1R. To confirm that the inhibitory effect of SP on loss of tight junction molecule is mediated via NK-1R binding, CP 96345 (CP, NK-1R antagonist) was pretreated before SP treatment and then, ZO-1 level was checked. As predicted, CP 96345 treatment obviously abolished the effect of SP.

In result, SP, itself, could augment junctional molecule in intestinal epithelial cell under severe damages and, the direct effect of SP is occurred through NK-1R signaling.

4 Discussion

The UC, a type of IBD, was a medically incurable disease. In the present, the most prescribed drugs to patients with UC were TNF- α blocker such as infliximab, certolizumab and golimumab. Action of these drugs is focused on alleviating symptom related to inflammation rather than treating colitis [15–17]. Accordingly, a new therapeutic strategy based on understanding of the pathophysiological feature is widely investigated.

DSS-induced colitis murine model has a similarity with human colitis [38]. DSS exacerbates disintegration of

epithelial barrier integrity and accelerated penetration of luminal antigen, thereby resulting in overwhelming immune response. DSS treatment significantly reduces TJ molecules implicated in intestine barrier function [12, 13, 36]. DSS-induced leakage of the TJ barrier had been proposed to be pathogenesis factor in UC. Therefore, the maintenance of junctional structure is anticipated to be the initial stage to prevent progression of disease as UC and also, can be indicator to evaluate the efficacy of drug for intestinal diseases.

In this study, histological analysis of intestine from DSS-induced colitis mice revealed that a junctional molecule that has crucial role in maintenance of epithelial integrity was significantly decreased. The destruction of intestine structure caused severed inflammation, accompanied by fibrosis, as predicted. Also, injured tissue has little proliferating cell pool that is required for intestinal regeneration. That is, healing process may be delayed or not happened.

On the other hand, SP treatment mitigated the clinical symptom such as weight loss and blood in stools due to DSS-induced intestinal damages. Histological analysis revealed that SP-treated mice showed preservation of crypt architecture, suppression of the infiltrated inflammatory cells, the reduced collagen deposit and the accelerated cell proliferation in the crypt region, leading to the intact epithelial structure. Notably, because SP was treated prior to damage development, it was inferred that SP can prevent TJ loss by providing compact junctional environment. This was confirmed at day 4 post DSS treatment. At this time, DSS-induced severe damages were not observed but SP-treated group showed the active proliferating cell pool in crypt. Thus, elevated repopulation of cells by SP might cause maintenance of intact TJ, which could inhibit infiltration of inflammatory cells to decline intestinal damages.

ZO-1 plays a key role in the formation and regulation of TJ [39, 40]. Our data demonstrated that DSS-induced epithelial damages evoked the reduction of ZO-1 expression in intestine tissue. Consistent with *in vivo* experiment, DSS treatment declined ZO-1 level in epithelial cells *in vitro*, which was totally suppressed by SP. That is, SP could directly modulate tissue integrity by halting deficiency of junctional molecules due to DSS.

In previously, treatment of SP to damaged intestine reduced inflammatory responses, leading to the accelerated repair in the acute damages [41]. Thus, SP was likely to be able to exert the therapeutic effect on chronic damages. Unlike previous study, we have evaluated the effect of SP on chronic inflammatory damages by injecting SP to mice with DSS for 31 days at this time. As expected, SP treatment enhanced tissue regeneration in chronic damages by facilitating cell repopulation and sustaining junctional structure in crypt.

Fig. 6 The effect of SP on ZO-1 expression in DSS-damaged intestinal epithelial cell *in vitro*. Caco-2 was pretreated with NK-1R antagonist (CP96345) for 30 min, followed by 1.5% DSS (w/v) and SP (100 nM) exposure for 24 or 48 h. 24 h later, SP treatment was repeated. **A** The experimental scheme to CP96345 and SP treatment. **B–C** At 24 h post DSS treatment, ZO-1 expression level was determined by western blots. ZO-1 expression in Caco-2 was

quantified relatively. **D–E** At 48 h post DSS treatment, ZO-1 expression level was determined by western blots. ZO-1 expression in Caco-2 was quantified relatively. Results were shown as mean values $\pm$ SD. All experiments were performed in triplicate. The data was expressed as the means of three independent experiments. CP: CP 96345, NK-1R antagonist

In conclusion, our study strongly supports that the DSS-induced damages can be inhibited or treated through SP-mediated TJ protection and immune control and thus, SP is expected to be able to be utilized as the candidate for the therapy of TJ-damaged disease such as UC and Crohn's disease

Acknowledgements This study was supported by a Korean Health Technology R&D Project grant from the Ministry of Health and Welfare, Republic of Korea (HI13C1479).

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to report.

Ethical statement All animal studies were approved by the Ethical Committees for Experimental Animals at Kyung Hee University (Approval number KHMC-IACUC-14-010).

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