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Transanal Delivery of Angiotensin Converting Enzyme Inhibitor Prevents Colonic Fibrosis in a Mouse Colitis Model: Development of a unique mode of treatment

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Abstract

Background—We have previously shown that angiotensin converting enzyme-inhibitor (ACE-I) improved colonic inflammation and apoptosis in a dextran sodium sulfate (DSS)-induced colitis model. This study attempted to determine whether ACE-I could prevent the development of colonic fibrosis.

Methods—Colitis was induced in C57BL/6 mice with 2.5% DSS water for 7-days, followed by 7-days without DSS (fibrosis development). Study groups: Control (Naïve or non-treated), DSS +Placebo (polyethylene glycol (PEG), and DSS+ACE-I (using enalaprilat and PEG which are not absorbed through intact mucosa). Placebo and ACE-I were delivered daily via transanal route. Colonic mucosal fibrosis and inflammation were evaluated based on histological findings and cytokine expression.

Results—Transanal administration of ACE-I/PEG dose-dependently decreased the severity of fibrosis and pro-inflammatory cytokine expression. We next investigated if ACE-I acted on the TGF-β/Smad signaling pathway as a mechanism of this anti-fibrosis action. Results showed a significant down-regulation of TGF-β1 expression; as well, downstream signaling of the Smad family, known to mediate fibrosis, showed a decline in Smad 3 and 4 expression with ACE-I/PEG.

Conclusion—ACE-I/PEG is effective in preventing colonic fibrosis and pro-inflammatory cytokine expression in a DSS colitis model, most likely by down-regulating the TGF-β signaling pathway. ACE-I/PEG may be a potential new option for treating inflammatory bowel disease.

Keywords

angiotensin converting enzyme inhibitor; dextran sodium sulfate; colitis; fibrosis; TGF-β/Smad-signaling

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INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are the two major forms of inflammatory bowel disease (IBD) and are characterized by non-specific chronic inflammation and intestinal tissue damage. Although the etiologies of both diseases are still unknown, recent advances in the understanding of IBD pathogenesis increasingly suggest that chronic intestinal inflammation is caused by an excessive and uncontrolled immune response (1–3). In inflamed intestine, healing of the damaged wall requires reconstruction of the tissue framework and remodeling of extracellular matrix (ECM) components. However, deposition of excessive collagen which is one of the major components of the ECM, may result in fibrosis. Fibrosis, is commonly seen in Crohn's disease and can lead to stenosis and obstruction of the intestinal lumen. Despite recent advances in IBD therapy, the development of intestinal fibrotic strictures remains a challenging complication of patient's with IBD. New effective treatments are needed to improve the clinical outcome of patients suffering from this complication. Identification of key mediators of ECM remodeling and development of strategies to block the action of these mediators is now the focus of many investigators. Recently, investigators have shown that two key mediators of fibrosis development in non-gastrointestinal organ systems are Angiotensin II (Ang II) and transforming growth factor (TGF)-β1(4,5).

TGF-β1 is known to be an important cytokine for the process of wound healing and collagen deposition (3,5–7), and functions to accelerate wound healing and the production of ECM. Furthermore, expression of TGF-β1 has been shown to be increased significantly in the inflamed mucosa of IBD patient (2,7). Prolonged expression of TGF-β1 during chronic inflammation may lead to excessive collagen deposition and eventually fibrosis (4,6). Considerable evidence recently has shown that overproduction of TGF-β1 is associated with a susceptibility to fibrosis in many organs, including kidney, vasculature and liver (4,5,8). In fact, therapies that reduce TGF-β1 levels have led to clinical and histological improvements in experimental models (8,9).

The renin-angiotensin-system (RAS) exerts multiple biological functions including cell growth, inflammation, and fibrosis contributing to the progression of tissue damage (10). Although drugs that block the actions of Ang II are known to have the beneficial effects of reduced inflammation and fibrosis in several organ systems (vasculature, kidney and liver) (4,8,10), the function of ACE in intestine is not well understood. We previously showed that ACE is expressed at particularly high levels in intestinal epithelium, and to be critically important in promoting the development of intestinal epithelial cell apoptosis (11,12). The effect of ACE and Ang II are mediated through a series of cell surface Ang II receptors, and interestingly, these receptors are also found in the intestinal mucosa (13). Recent studies using pharmacologic ACE inhibitors (ACE-I) have shown that ACE-I significantly reduces injury and fibrosis in the vasculature and liver (5,8). Other investigators have demonstrated that fibrogenic response to injury is mediated through Ang II induction of TGF-β1 expression in a number of tissues such as the kidney and lung (4,6). Further, blockade of Ang II by ACE-I, or Ang II receptor antagonists, reduces injury effects and fibrosis; findings that were closely correlated to the reduction of TGF-β1 expression (4–6,8). Taken together, these findings suggested that Ang II is a central mediator in the pathogenesis of fibrosis, and neutralization of Ang II could be a beneficial therapeutic target through a reduction of this fibrogenic cytokine in colitis.

In this study, we hypothesized that transanal administration of an ACE-I would decrease inflammatory cytokine expression and colonic fibrosis in a mouse experimental model of dextran sulfate sodium (DSS)-induced colitis. As systemic administration of an ACE-I has the significant potential to lead to hypotension, this study addressed this problem by developing

a novel compound which delivered the ACE-I to the colonic mucosa with little to no systemic absorption. The present study, first investigated the effect of ACE-I on the development of colonic fibrosis, and then examined potential mechanistic pathways.

MATERIAL and METHODS

Animals

Specific pathogen free male, 8 week old C57BL/6 mice (Taconic Farms Inc, Germantown, NY) were maintained in a 12-h night rhythm at 23°C and a relative humidity of 40–60%. Animals were fed standard rodent chow (LabDiet® 5001Rodent Diet, PMI Nutrition International, LLC, Brentwood, MO) *ad libitum*. All experiments were approved by the University Committee on Use and Care of Animals at the University of Michigan.

Induction of colitis

Colitis was induced by 2.5% (W/V) reagent-grade dextran sulfate sodium (DSS; Molecular weight; 36,000–50,000, ICN Biomedicals, Inc, Aurora, OH) dissolved in drinking water, which was administered *ad libitum*.

ACE-I Inhibition / polyethylene glycol compound

We used the ACE inhibitor, enalaprilat (Molecular weight; 384, Abbot Laboratories, North Chicago, IL) which is a first generation ACE-I, and because it is very poorly absorbed from the gastrointestinal tract is normally given via the parenteral route (14). Enalaprilat was suspended in polyethylene glycol (PEG, Molecular weight, 1500: Sigma, St Louis, MO). Macromolecular PEG exceeding a molecular mass 900 has been reported to have little or no intestinal absorption(15). Therefore this new 5compound, enalaprilat suspended in PEG-1500, is not absorbed through an intact intestinal mucosa.

Experimental design

DSS was administrated through drinking water for 7 days (colitis phase), and then replaced with plain water (without DSS) for an additional 7 days (fibrosis developmental phase). Mice were randomly divided into 3 groups. In the DSS-placebo group, mice were given PEG (total volume 0.25-ml) each day as a control (n=13). Two DSS-ACE-I groups were studied consisting of enalaprilat suspended in PEG at a total volume of 0.25-ml. Enalaprilat dosing was: 14.5μg or 145μg (n=11 in each dose) per day, respectively; and daily dosing was given for the entire 14 days of the study. The study drugs were administered using a blunt needle via the transanal route. Preliminary testing confirmed that this amount of drug evenly coated the entire colon. A final group of mice consisted of naïve mice as an additional control group (n=6) received plain drinking water *ad libitum* and received only PEG (0.25-ml, transanal) without ACE-I.

Assessment of colitis

The body weight of each mouse, stool characteristics, and intestinal bleeding were recorded and scored to obtain a disease activity index (DAI) as described by Murphy et al (16). All animals were evaluated daily. By this index, scores ranging from 0 to 4 were assigned to weight loss, variation in stool consistency, and presence of occult or gross intestinal bleeding. Occult bleeding was tested using a hemoccult-card test (Beckman Coulter Inc, Fullerton, CA). Mice were euthanized using carbon dioxide asphyxiation at the specified number of days, and laparotomy with total cololectomy was immediately performed.

Histologic assessment

A 0.5 cm segment taken from the distal half of the colon was excised and placed into 10% formaldehyde. Formalin-preserved sections of distal colon were preserved in paraffin with

standard technique. Transverse 5 μm sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome.

Colitis Score—All grading of colitis was performed in a masked fashion (investigator blinded to the study group) according to the method as described previously (11). Crypt shortening and distortion, together with inflammatory infiltrative thickening of the lamina propria, were assigned a score 0 (normal) through 4 (complete loss of crypt, ulceration, and severe thickening of lamina propria). The individual colitis score (0–4) from four different area were summed, such that the maximum score for a given section is 16, and the minimum score is 0. At least 2 sections were assessed in this manner for each mouse.

Assessment of colonic fibrosis

Fibrosis Score—Masson's trichrome, which stains for collagen, was used to assess the distal colon, and a qualitative histologic score for fibrosis was performed according to the method described by Theiss et al (17). In this method, the severity of increased collagen deposition is assigned a score of 0 (normal) through 5 (the most severe fibrosis, represent a progressive increased collagen deposition throughout all layers from mucosa to serosa). The final scores are assessed by above methods.

Collagen Density—Trichrome stained sections were also analyzed by quantitative image analysis. Briefly, tissue images were photographed with a Nikon TS-100 microscope. Images were then digitally recorded with an Evolution MP 5.1 CCD camera, and saved in tagged image file (TIF) format. The image white background was removed with Adobe Photoshop CS3 version 10.0.1 (Adobe Systems Incorp.). Color segmentation analysis was then performed with MatLab software (R2007a; copyright 2007, The MathWorks, Inc., Natick, Mass.). Color augmentation of red, green, blue (RGB) multispectral composite images was performed to enhance tissue differentiation. Colors were then converted into CIE (International Commission on Illumination) *Lab* color space (18). Each pixel was categorized by nearest neighbor color classification to identify its likely tissue type. Collagen area was defined as the distinct blue color region and distinguished from muscle, blood and inflammatory cells. Area of collagen and total tissue area were measured by quantification of color-segmented pixels. Collagen area was standardized to tissue section size by dividing collagen area by total tissue area. Standardized collagen area utilizing this method was shown to correlate with tissue collagen as determined by Western immunoblot for type I collagen ($R=0.731$, $p=0.039$).

Mucosal cell isolation

Colonic tissue, not including the cecum, were placed in RPMI cell culture medium on ice, and fecal contents were gently flushed out. Colonic epithelium was isolated for RNA and mucosal protein isolation, as described previously (11). Briefly the colon was opened longitudinally and rinsed with fresh cold RPMI, then the colonic mucosa was mechanically scraped off on a glass slide, and epithelial cells (EC) collected in fresh RPMI with glutamine. These EC were then rapidly pelleted by centrifugation at 330 × g at 4°C for 3-mins. The supernatant was decanted and the EC pellet was then immediately snap-frozen in liquid nitrogen and processed for RNA and protein extraction.

Real time polymerase chain reaction (RT-PCR)

Real time PCR was performed from isolated RNA based on previous standard protocols using the ΔΔCt method (19). All primers for selected gene sequences were designed using proprietary software (Lasergene, DNA star Inc, Madison, WI). Real-time PCR (RT-PCR) was performed using a Smart Cycler (Cepheid, Sunnyvale, CA) with intercalation of SYBR green I used to

determine the amount of DNA using previously published techniques (19). Expression of results were normalized to β-actin expression.

Immunoblot analysis

Techniques are similar those previously described (12). We used mouse anti-TGF-β1 (1;500: Sigma-Aldrich, St Louis, MO), rabbit anti-Smad 3 (1;3,000: Abcam, Cambridge, MA), rabbit anti-phospho-Smad 3 (1;1,000: Cell Signaling Technology Inc, Danvers, MA), mouse anti-β-actin (1:5,000: Sigma-Aldrich), and rabbit anti-Smad 4 (1;500: Santa Cruz biotechnology INC, Santa Cruz, CA), respectively. Membranes were then washed and incubated for 1-hour at room temperature with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:10,000 : Zymed Laboratories, San Francisco, CA) and goat anti-mouse IgG (1:10,000 : Santa Cruz biotechnology INC, Santa Cruz, CA). Results were expressed as the ratio of target density to the density of β-actin expression.

Statistical analysis

Data are reported as mean ± standard deviation (SD). Results were analyzed using t test for comparison of two means, and a one-way analysis of variance (ANOVA) for comparison of multiple groups. A post-hoc Bonferroni test was used to assess statistical difference between groups. The chi square test was used for categorical data (Prism software; GraphPad Software, Inc., San Diego, CA). A value of $P<0.05$ was considered to be statistically significant.

RESULTS

Effect of ACE-I on clinical parameters

After DSS administration, mice developed colitis which was manifested by loose stool, intestinal bleeding, and weight loss. Figure 1A shows the survival rate of both placebo and ACE-I treated mice. The survival rate of placebo mice was 53% (seven out of 13 died). In contrast, the survival rate of ACE-I treated mice was significantly improved (Placebo versus ACE-I at 14.5μg $p=0.040$; Placebo versus ACE-I at 145μg $p=0.030$). The disease activity index (DAI) reflected the severity of colitis. The DAI increased toward the end of 1 week of DSS administration, and then slowly decreased following withdrawal of DSS administration (Figure 1B). However, ACE-I treatment significantly prevented this increase in DAI. Although the highest DAI score in the placebo group was on day 8 (placebo 3.9 ± 0.1), in the ACE-I treated groups the highest score was on day 9 (at 14.5μg, 2.7 ± 0.4 $p< .001$; at 145μg, 1.7 ± 0.3 $p<.0001$, respectively). There was a significant difference between the placebo and ACE-I (at 145μg) treatment groups on day 7; and a significant difference between the placebo and ACE-I groups (both dose) was observed after day 8. After 14 days, the end of the study period, weight loss was severe in the placebo group (21.5 ± 4.0% weight loss); however, this loss was significantly attenuated in the ACE-I group (14.5μg, 10.5 ± 3.5% $p<.0001$; 145μg, 5.9 ± 3.0%: $p<.0001$, respectively).

Effect of ACE-I on histopathology and biochemical measures of fibrosis

To evaluate if transanal treatment with ACE-I was associated with a reduction in the severity of colitis a blinded histological score at day 14 was measured as previously described (11). DSS-placebo mice showed significant increases in colitis scores compared with DSS-ACE-I mice (Table 1). Mice in the DSS-placebo group also showed an obvious increase in transmural collagen deposition, and had a significant increase in histologic scores for fibrosis (Table 1 and Figure 2). However, ACE-I treated mice had lower fibrosis scores compared to the Placebo group, with the majority of collagen deposition confined to the submucosa, as is found in the naïve colon (i.e., normal mice without any manipulation; Figure 2). In DSS-placebo mice the affected colonic wall area consisted of collagen deposition in which fibroblasts and fibrosis

were evident in the submucosa together with regenerative changes in the overlying epithelium. The colon of ACE-I treated mice showed nearly normal mucosal architecture and only minimal collagen tissue and mild fibrosis in the submucosa.

Expression of pro-collagen I (α1) and (α2) mRNA were assayed as a biochemical marker of fibrosis. DSS-induced colitis resulted in significantly increased mRNA expression of pro-collagen I (α1) and (α2) compared to control mice (naïve). However ACE-I administration significantly reduced mRNA expression of both pro-collagens to control levels (Figure 3). To investigate the effect of ACE-I on intact (naïve) intestine, ACE-I (at 145μg/day) was also given to mice without DSS treatment (n=6). ACE-I led to a slight reduction in pro-collagen I (α1) and (α2) mRNA expression; however, these changes were not significant.

Effect of ACE-I on pro-inflammatory cytokine expression

Expression of TNF-α and IL-1β are two pro-inflammatory cytokines that are known to be up-regulated in the DSS colitis model. Further, these cytokines may be responsible for tissue injury and promote subsequent fibrosis formation (1). Therefore, these were quantified as independent biochemical markers of inflammation. Figure 4 shows mRNA expression of TNF-α and IL-1β in naïve and DSS mice treated with either placebo or ACE-I/PEG treatment. DSS-placebo mice showed significantly increased levels of TNF-α and IL-1β mRNA. However, ACE-I significantly reduced the mRNA expression of both cytokines.

Effect of ACE-I on TGF-β1 expression

TGF-β1 expression was investigated with real time PCR and Western immnunoblotting to assess the effect of ACE-I treatment in DSS mice. DSS-induced colitis treated with placebo was associated with a significant increase in TGF-β1 mRNA expression (3.46±0.73) compared to controls (1.96±0.60). Administration of ACE-I to DSS mice resulted in decreased TGF-β1 expression in a dose-dependent manner (ACE-I 14.5μg 2.00±0.72; and 145μg 1.49±0.30, respectively). A similar trend in TGF-β1 protein expression was also noted, with increased expression in the DSS-Placebo group and a significant reduction with ACE-I treatment (Figure 5) in a dose-dependent fashion.

Effect of ACE-I on Smad expression

Because TGF-β1 is known to mediate fibrosis via intracellular signaling of the Smad family, the expression these factors were then examined using real time PCR and Western immnunoblotting. DSS resulted in significant increases in Smad 3 and Smad 4 mRNA expression (Figure 6). Two other members of the Smad family, 2 and 7 showed no significant change in expression. ACE-I treatment at a dose of 145 μg/day significantly reduced the mRNA expression of Smad 3 and Smad 4. Based on this, we concentrated our analysis of Smad protein analysis to Smad 3 and 4. It has been previously reported that Smad 3 phosphorylation (p-Smad) is correlated with an activation of the pro-fibrotic process (7,8,20), therefore p-Smad was also examined (Figure 7). ACE-I markedly diminished DSS-induced activation of Smad signaling by a reduction in p-Smad in dose-dependent manner; whereas un-phosphorylated Smad showed no significant difference between study groups.

DISCUSSION

Our study showed that treatment with ACE-I significantly reduced the histopathologic grade of intestinal fibrosis. Furthermore, our study showed that the secondary formation of colonic fibrosis was associated with increased expression of pro-collagen I (α1), pro-collagen I (α2), and TGF-β1; and these factors may well play an important role in the development of fibrosis (3,17). Our compound, the ACE-I enalaprilat combined with PEG, led to a significant reduction in the expression of these factors, suggesting a potential mechanism for enalaprilat's action.

Finally, treatment with ACE-I led to a down-regulation of two important Smad proteins (p-Smad 3 and Smad 4); suggesting that inhibition of the TGF-β1/Smad signaling pathway is a potential mechanism for the prevention of fibrosis. Several investigators have shown that ACE and ANG II are implicated as promoters of fibrosis in other organ systems (5,6,10). Further, extensive studies in intestinal cells have demonstrated that ACE can stimulate deposition of collagen. ANG II, produced by ACE, is necessary for fibrosis in pulmonary and vascular smooth muscle cells (6,10). Although it has been demonstrated that ACE is highly expressed in the small and large intestine of rodents and humans (13,21), the functional role for this expression is uncertain. To address this, our laboratory investigated the role of ACE in the formation of fibrosis using a DSS-colitis model.

DSS-induced colitis is a well established model of acute and chronic intestinal inflammation driven by a Th1 mediated cytokine immune response that mimics IBD (22). The present study showed additional beneficial effects of ACE-I on DSS-induced intestinal inflammation, as demonstrated by a reduction in histological scoring of colitis, and a decline in the expression of TNF-α and IL-1β. This reduction in the expression of TNF-α and IL-1β with ACE-I treatment persisted even 7 days after stopping DSS administration. These observed effects of ACE-I on colonic inflammation are consistent with our previous findings with systemic administration of ACE-I in both a DSS-induced colitis and short bowel syndrome model (11,12). Although improvement of fibrosis and deposition of collagen may have been partially mediated by a reduction in the severity of mucosal inflammation, our previous data indicate that intestinal mucosal apoptosis and increased inflammatory cytokine actually occur in a very early phase of DSS induced colitis model. Therefore, we deliberately administered ACE-I therapeutically at the onset of chronic inflammation in this study. The findings of the present study may well have relevance to human IBD. In a report by Jaszewski, et al, colonic mucosal expression of Ang I and Ang II were were shown to be significantly increased in patients with active Crohn's disease (23).

Our study showed a strong over-expression of TGF-β1 with DSS treatment, suggesting an essential role of TGF-β1 in the normal repair process of damaged tissue. Other investigators have shown that the production of both TNF-α and TGF-β1 are stimulated by the activation of ACE or ANGII, and inhibited by ACE-I or ANG II receptor antagonists (1,3,5,24). In addition to its role in the regulation of collagen deposition, TGF-β1, along with TNF-α helps to regulate the acute inflammatory response in IBD (7). In out DSS-colitis model, we found that ACE-I significantly down-regulated TGF-β1 and TNF-α expression. Our results suggest that ACE within the colonic mucosa is involved in the up-regulation of these cytokines, and inhibiting ACE activity with the transanal administration of an ACE-I was effective in reducing both fibrosis and the inflammatory process. However, TGF-β1 is known to be controlled by a complex network of signaling cascades, and it is quite likely that other factors in addition to ACE contributed to the levels of TGF-β1 in our study. The effects of ACE inhibition on fibrosis may involve signal pathways, such as NF-κB, JAK and others, which may affect TGF-β1 expression (7). Further work will be necessary to more fully elucidate the mechanisms involved in ACE signaling in the colon.

In order to further understand the mechanism of ACE-I on fibrosis, we examined changes in the expression of several Smad signaling factors. Smad is critical for TGF-β superfamily signaling from the cell surface to nuclear transcription of down-stream factors (7,8). Upon ligation and activation of TGF-β with its receptor, the phosphorylated Smad 2 and 3 form a complex with the common mediator Smad 4. The Smad2/3 –Smad 4 complex can then translocate into the nuclei where they enhance specific TGF-β target genes. Smad 7 acts as an inhibitor and antagonizes TGF-β signaling by the interfering with the ligation of Smad2/3 with the activated receptor complex. Recently, experimental evidence suggests that disruption of the TGF-β/Smad signaling pathway plays a central role in controlling both chronic tissue

inflammation and fibrosis (7,8). We found that DSS treatment up-regulated the expression of Smad 3 and Smad 4, and treatment with ACE-I prevented this increase. It is reported that Ang II-induced vascular and hepatic fibrosis utilizes this TGF-β/Smad signaling pathway (8,20). In this study, activation of Smad 3 but not Smad 2 was found to be a key mediator of fibrosis. It has also been reported that Smad 7 is induced by TGF-β and participates in a negative feedback loop to control TGF-β responses by competitive interaction with the type I receptor (7,8), and that Smad 7 is mediated by both the expression of TNF-α, interferon-γ (7). Although we noted a slight decline in Smad 7 in the DSS group, and an increase in Smad 7 expression with ACE-I, the changes were not significant. It is possible that that Smad 7 expression was not significantly increased due to the prolong duration and inflammatory state of our study. Our study suggests that the down-regulation of Smad 3 and Smad 4 expression and potentially the up-regulation of Smad 7 expression with ACE-I treatment, along with the decline in TGF-β1, contribute to the mechanisms observed with ACE-I prevention of fibrosis formation in the DSS model.

Although ACE-I produced significant clinical and histological improvements, it is noteworthy that fibrosis was not completely prevented by ACE-I treatment. It is possible that higher dosing may lead to further reductions in fibrosis, or that other mechanisms are responsible for fibrosis formation that are not affected by ACE-I treatment. Our current findings are supported by two other studies. One group studied fibrotic changes in a trinitrobenzene sulfonic acid (TNBS)-induced model of colitis, and noted significantly reduced fibrosis formation with the blockade of the renin-angiotensin system using another ACE-I, captopril (3). In another study, captoril and lisinopril reduced tissue collagen content in a TNBS rat model (25). One of the great advantages of our approach in this study was the use of a novel compound of a very high dose of ACE-I in PEG. Importantly, this compound was given via the enteral route (i.e. enema). This unique approach allowed for the administration of a very high dose (10-fold higher than our previous study in which we gave enalaprilat parenterally (11)) of an ACE-I with little to no side-effects.

In conclusion, the present study demonstrated that DSS administration led to a marked increase in fibrotic regenerative changes after the induction of colitis. The DSS-induced fibrosis was associated with an increased expression of TGF-β1 as well as the pro-inflammatory cytokines TNF-α and IL-1β. Transanal administration of an ACE-I/PEG compound prevented most of these changes, and most likely acted by decreasing the expression of these cytokines, and down-regulating the TGF-β/Smad signaling pathway. ACE-I/PEG may be a potential new option for treating inflammatory bowel disease.

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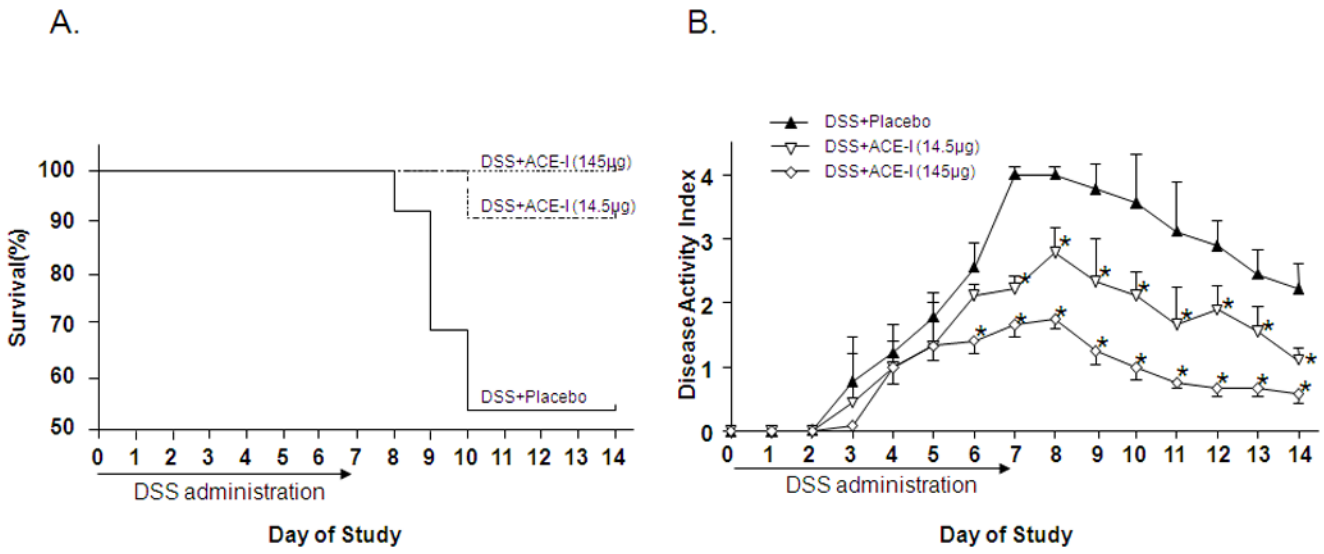

Figure 1.

(A) Survival rates of DSS-placebo and DSS-ACE-I treated mice. A minimum of N=11 mice in each group were given 2.5% DSS, and survival rates were investigated until the last day of the study. (B) Time course of changes in the disease activity index (DAI). All mice received 2.5% DSS in drinking water. DSS was administrated during the first 7 days, followed by 7 days of standard plain drinking water without DSS. All mice were evaluated daily for weight loss, stool consistency, and occult or gross intestinal bleeding. Results are expressed as mean ± SD. (* P <.05 vs DSS+Placebo). In the ACE-I treatment group, the DAI on day 8 was significantly lower compared to mice in the DSS+Placebo group, and this trend continued until the end of the study.

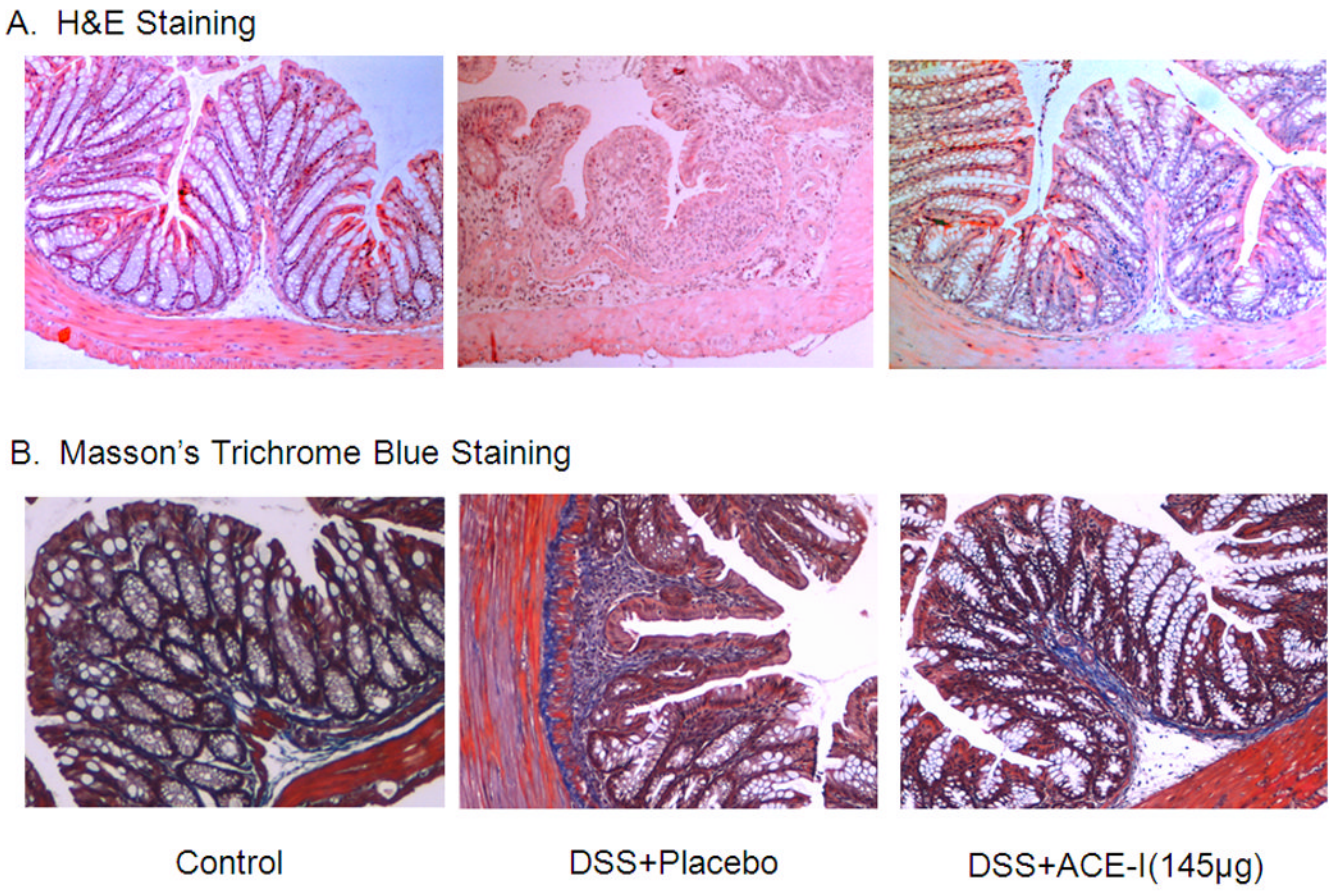

Figure 2.

(A) Representative histologic sections of distal colon are shown after undergoing Hematoxilyn-Eosin staining (x10 magnification). The Control group showed a normal (Naïve) colon without DSS treatment. In the DSS-placebo mice, dense cellular fibrosis was observed in the colonic submucosa with regenerative changes. In ACE-I treated mice, the colon showed normal mucosa architecture and mild fibrosis in the submucosa. (B) Representative histologic sections of distal colon are shown after undergoing Masson's trichrome staining (x10) of the colon. Note the prominent fibrosis in DSS-placebo mice as represented by a dense blue staining. Whereas only mild fibrosis is noted in ACE-I treated mice.

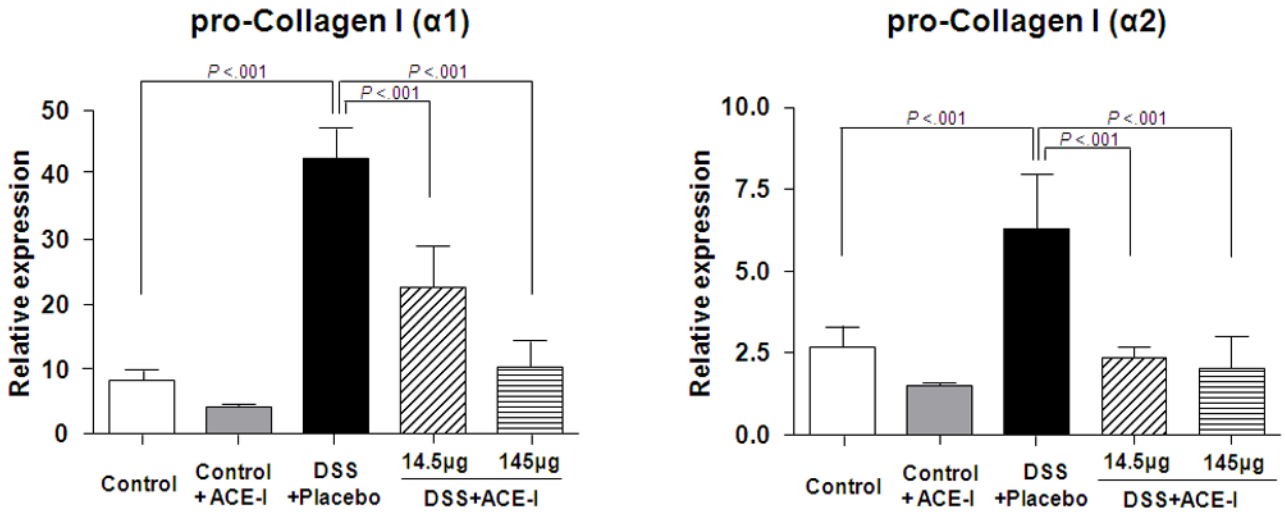

Figure 3.

mRNA expression of pro-collagen I (α1) and (α2) derived from mucosa samples in each group and measured by real time PCR. Result are expressed as $2^{-(-\Delta\Delta Ct)}$ in relation to β-actin gene for PCR. Expression of both pro-collagens were significantly increased in the DSS-placebo group compared to non-treated controls. Administration of ACE-I to DSS-induced colitis significantly reduced the expression of pro-collagens I (α1) and (α2) in a dose-dependent fashion. Note that ACE-I treatment of control mice failed to significantly affect baseline pro-collagen expression. Statistical comparisons are made using ANOVA with a post hoc Bonferroni test.

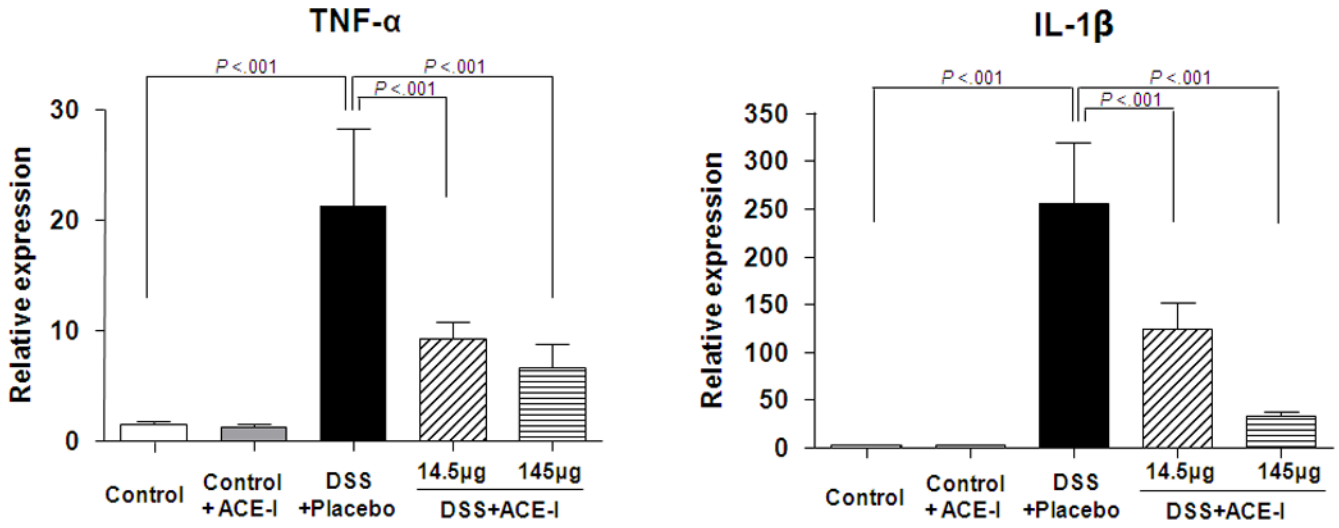

Figure 4.

Expression of mucosal pro-inflammatory cytokines TNF-α and IL-1β as detected by real time PCR. Result are expressed as $2^{-(-\Delta\Delta Ct)}$ in relation to β-actin gene expression. TNF-α and IL-1β mRNA expression were significantly lower in the ACE-I treated groups compared to the DSS-placebo group.

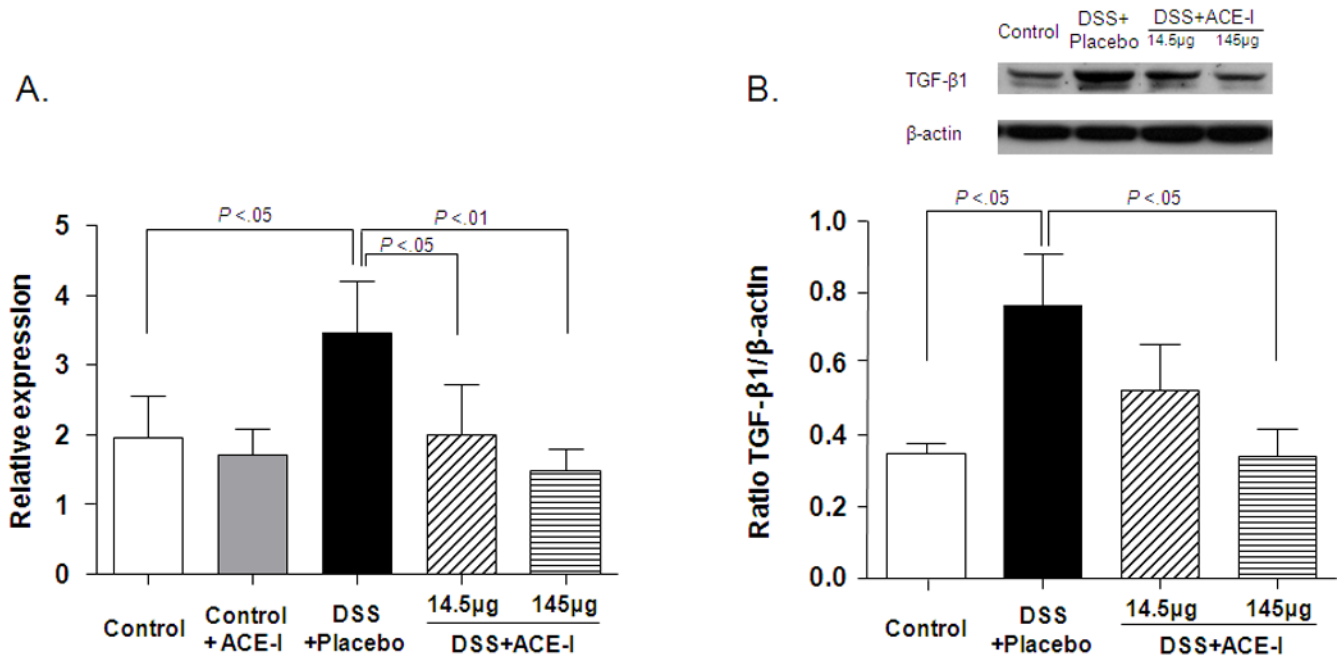

Figure 5.

Expression of mucosal –derived TGF-β1 as detected by (A) real time PCR and (B) Western blot techniques. Result are expressed as $2^{-(-\Delta\Delta Ct)}$ in relation to β-actin gene expression for PCR results; and the ratio of TGF-β1/β-actin for immunoblots. Note that TGF-β1 expression was significantly increased with administration of DSS for both mRNA and protein analysis, and both mRNA and protein expression significantly decreased with ACE-I treatment.

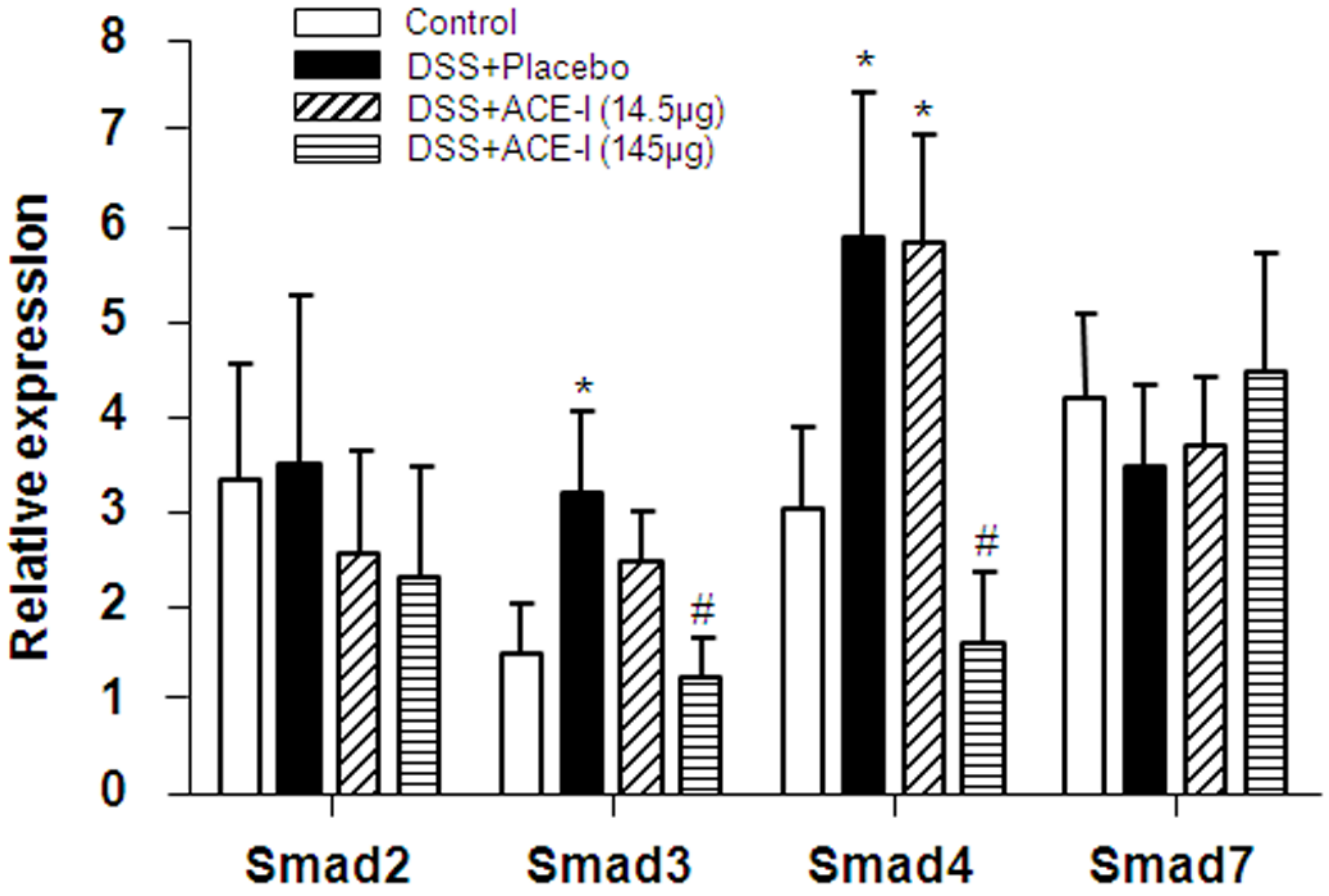

Figure 6.
mRNA expression of Smad signaling genes form colonic mucosal samples as measured by real-time PCR. mRNA expression of Smad 3 and Smad 4 significantly increased in the DSS-placebo group. Administration of high-dose ACE-I to DSS-treated mice returned the expression of these factors to control levels, and expression was not significantly different from control values. There were no significant differences Smad 2 and Smad 7 expression between study groups.

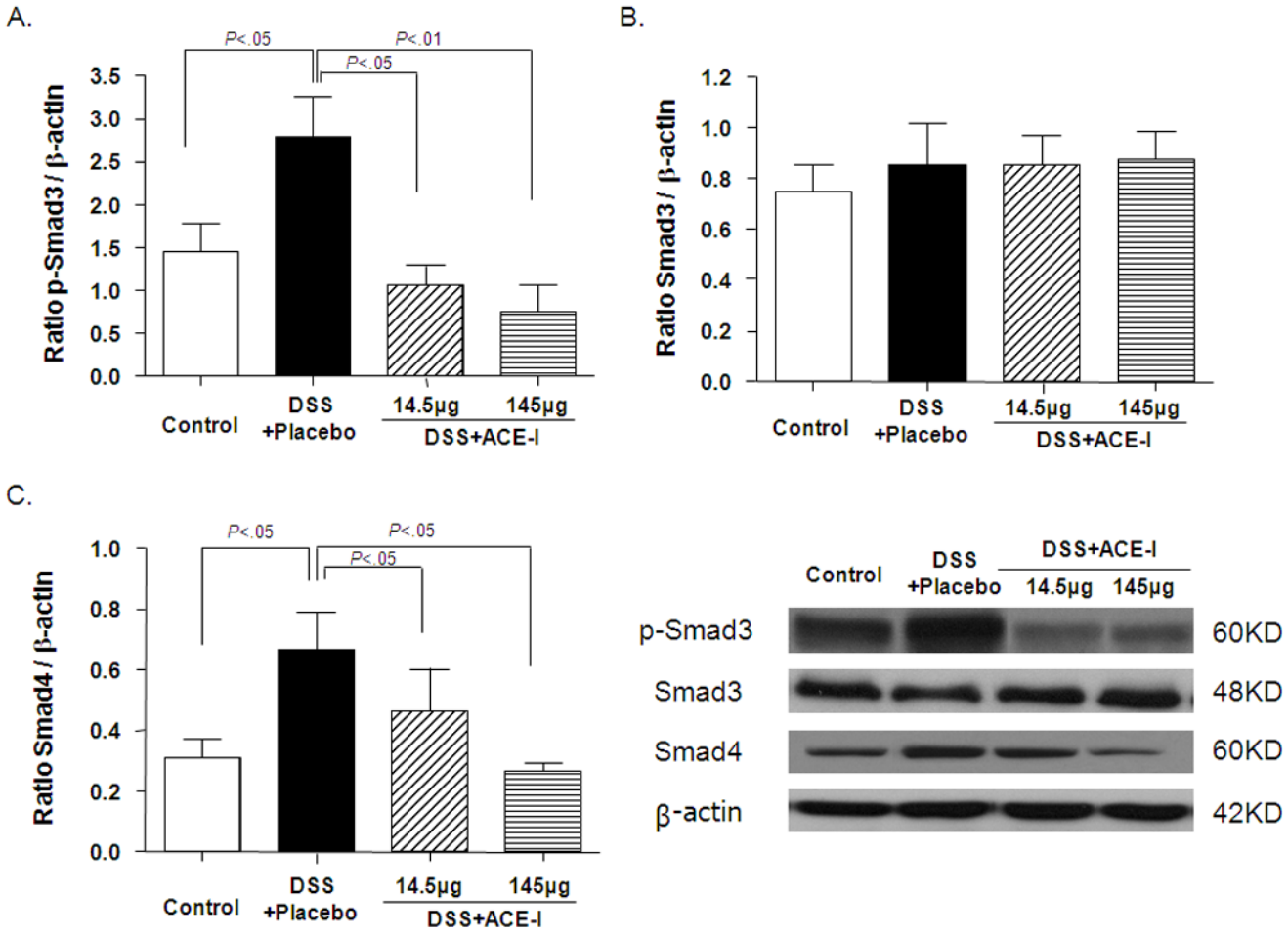

Figure 7.

Expression of phosphorylated Smad 3 (p-Smad 3), Smad 3 and Smad 4 proteins as examined by Western blot methods. Results are reported as the ratio of Smad protein to β-actin protein expression. The expression of p-Smad 3 and Smad 4 were significantly increased in DSS-placebo mice compared with controls. Administration of ACE-I, led to a decline in the expression of these Smad proteins. Interestingly, the increased expression of Smad 3 was confined to the p-Smad (activated protein).

Table 1

Histologic Grading

	Control	DSS+Placebo	DSS+ACE-I	
			14.5 μg/day	145 μg/day
Histological Score	0.2±0.4	4.7±2.1*	0.7±0.9#	0.6±1.1#
Fibrosis Score	0.3±0.5	9.0±1.4*	3.6±1.5#	3.3±1.1#
Collagen Density	0.14±0.02	0.23±0.05*	0.17±0.03#	0.16±0.02#

Histologic evaluation of each study group. Note the histologic score was performed using standard Hematoxylin and Eosin staining. The Fibrosis score was a ranking of the density of fibrosis after Mason's trichrome staining based on an arbitrary range of 0 to 5 for each of 4 quadrants of a cross-section of the distal colonic wall. The collagen density values represent the digitized areas of collagen staining as described in the Methods section. Note that ACE-I treatment resulted in a significant decrease in the amount of fibrosis.

Abbreviations: DSS, dextran sulfate sodium. ACE-I, angiotensin converting enzyme inhibitor.

*P<.05 (ANOVA), Control (Naïve mice, no DSS treatment) versus DSS+Placebo group

#P<.05 (ANOVA), DSS+Placebo versus DSS+ACE-I treated group. Data are mean ± SD.