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Type I collagen and its daughter peptides for targeting mucosal healing in ulcerative colitis: A new treatment strategy

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ABSTRACT

Ulcerative colitis, particularly the chronic persistent form is characterized by the presence of active inflammation and extensive areas of ulceration in the colonic mucosa. The existing treatment protocol aims at only reducing intestinal inflammation, rather than targeting mucosal ulceration. In this study, type I collagen and its daughter peptides called collagen hydrolysate, highly popular reconstructive materials for tissue engineering applications, are hypothesized as healing matrices to target the recuperation of internal mucosal ulceration. The clinical assessments on day 10 of dextran sodium sulfate induced colitis in mice model revealed that both the collagen (1.56 ± 0.29) and collagen hydrolysate treatments (1.33 ± 0.33) showed a significant reduction in the rectal bleeding compared to the reference mesalamine treatment (2.50 ± 0.33) and untreated negative control (2.40 ± 0.40). VEGF, a potent angiogenic growth factor, over expressed during UC was down-regulated by collagen hydrolysate (1.06 ± 0.25) and collagen (1.76 ± 0.45) to a greater extent than by mesalamine (2.59 ± 0.51) and untreated control (4.17 ± 0.15). The down-regulation of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 also follows the same pattern. Histological observations were in accordance with the clinical indicators. Both collagen and collagen hydrolysate treatments showed significant reduction in mucosal damage score and facilitated faster regeneration of damaged mucosa.

1. Introduction

Ulcerative colitis (UC) is a chronic debilitating disorder characterized by the presence of both active inflammation and ulceration within the colonic mucosa (Odze, 2003). According to the Centers of Disease Control and Prevention, the worldwide incidence or diagnosis of new cases of UC varies considerably between 0.5 and 24.5 per 100,000 people, with the prevalence being high in developed countries.

Severe UC is manifested by the presence of extensive areas of ulceration in the distal colon, which if left untreated leads to complications mandating surgical intervention, including toxic mega colon, significant bleeding and gastrointestinal perforation (Rutgeerts et al., 2007). The most common treatment for UC involves anti-inflammatory agents such as 5-amino salicylic acid (5-ASA) compounds, systemic corticosteroids, and topical corticosteroids, which are aimed exclusively at reducing intestinal inflammation, rather than healing mucosal ulceration (Sands, 2000). Recent clinical evidences suggest that mucosal healing could be a reliable indicator of treatment efficacy and a key prognostic marker of long-term outcome (Froslie et al., 2007; Rutgeerts et al., 2007). Therefore, a strategy that addresses both mucosal inflammation and ulceration is important to achieve an optimal therapeutic outcome.

Currently, mucosal healing is not considered as a primary end point although this is clearly the most important clinical outcome (Rutgeerts et al., 2007). The importance of mucosal healing is now acknowledged in new guidelines, which recommend incorporating complete mucosal healing (along with symptom resolution) into the primary endpoints of remission in all therapeutic studies (D'Haens et al., 2007). Mucosal healing has been rather neglected as a treatment goal in inflammatory bowel disease (IBD) because most treatments are not disease modifying and effective treatment strategies are not available to heal bowel mucosa (Rutgeerts et al., 2007). Numerous clinical studies have revealed that mucosal healing can be achieved by prolonged therapy with 5-ASA drugs (Kamm et al., 2009). However, the need of the hour is faster mucosal regeneration because UC severely decreases quality of life leading to major economic loss caused by the inability to work.

Collagen is the main structural protein of the extracellular matrix (ECM) of the intestinal mucosa (Bornstein and Sage, 1980; Leblond and Inoue, 1989). Patho-physiologically, ulceration in the mucosal and

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sub-mucosal areas of patients with UC is due to excessive degradation of ECM (Wang and Mao, 2007). Regeneration of the ECM is the ultimate solution to ulceration. Recently we have developed a collagen mesalamine in situ rectal gel which was effective for ulcerative colitis, where collagen served as a drug carrier and reconstructive matrix for damaged mucosa (Ramadass et al., 2013). Type-I collagen hydrogels has been widely used for soft tissue engineering applications as they self-assemble into a fibrillar gel in vivo and are biocompatible, biodegradable, and naturally support cell adhesion and growth.

Collagen hydrolysate, the daughter peptides of type I collagen, has begun to emerge as a valuable source material for diverse tissue engineering applications (Ficai et al., 2013; Pei et al., 2013; Ramadass et al., 2014). Being in the form of smaller peptide fragments, collagen hydrolysate would have better bioavailability than collagen to the damaged host tissue. Additionally, collagen hydrolysate would have the advantage of higher solubility than collagen and hence higher therapeutic loading is feasible. In our recent findings, we have highlighted the advantages of collagen hydrolysate as dermal scaffolds for wound healing (Ramadass et al., 2014).

Both collagen and collagen hydrolysate have proven to be good matrices for healing external ulcers (Ruszczak, 2003). It has also been proven in a preclinical study that a combination of mesalamine and collagen promoted mucosal healing better than conventional mesalamine therapy (Ramadass et al., 2013). Therefore, we hypothesize that collagen and collagen hydrolysate could be possible solutions to address the problem of inadequate intestinal mucosa regeneration in existing UC therapy. This study investigates collagen and collagen hydrolysate as potential candidates to promote intestinal mucosal healing in UC.

2. Materials and methods

2.1. Materials

Pepsin treated Type 1 collagen was extracted from rat tail tendon as reported earlier (Tanaka et al., 1988). Type I collagen hydrolysate was prepared through gelatinization followed by enzymatic hydrolysis from rat tail tendon collagen as described elsewhere (Ramadass et al., 2014). Dextran sodium sulfate (DSS, MW: 36,000–50,000) was purchased from MP Biomedicals Pvt. (India) Ltd. PCR primers were purchased from Xcelris genomics, Gujarat, India. Other PCR reagents and chemicals were procured from Bioneer, Republic of Korea.

2.2. Animals

Adult male Balb/C mice were obtained from Laboratory Animal Medicine Unit, TANUVAS, Chennai, India. Animals were housed in groups on soft bedding with food and water available ad libitum, in a temperature controlled environment with a light dark cycle of 12:12 h. All animals were allowed to habituate to the housing facilities for at least 1 week prior to surgery. Guidelines of "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academic Press 1996; NIH publication number #85-23, revised 1996) were strictly followed throughout the study. All experimental procedures were approved by the Institutional Animal Ethical Committee, Central Leather Research Institute, constituted according to the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India (IAEC NO: 07/2013 (a)).

2.3. Induction of colitis in mice

6–8 week old (25–32 g), male Balb/c mice were randomly divided into five groups based on their body weights ($n = 18$/group), (1) normal control (No DSS induction), (2) vehicle control (DSS induction followed by vehicle treatment), (3) mesalamine (positive control - DSS induction followed by mesalamine treatment) and test groups (DSS induction followed by collagen (4), and collagen hydrolysate (5) treatments). DSS induced colitis was performed as described previously with minor modifications (Ramadass et al., 2013). All groups excluding normal control were fed 3.5% DSS dissolved in sterile, drinking water ad libitum from days 1 to 5 followed by treatment (therapeutic model). Collagen (20 mg/kg b.w.), collagen hydrolysate (100 mg/kg b.w.) and mesalamine suspension (20 mg/kg b.w.) were administered intra rectally from days 6 to 15 into the lumen of the colon. Both induction and treatment progress were assessed by (1) clinical score evaluation and (2) histological analysis. The body weight was assessed on alternate days and clinical score parameters such as stool consistency and rectal bleeding were assessed daily. The scoring was performed by two investigators blinded to the treatment protocol and scoring was performed as described previously (Hartmann et al., 2000). For stool consistency, 0 points were given for well formed pellets, 2 points for pasty and semiformed stools, and 4 points for liquid stools. Bleeding scored 0 points for no blood in hemoccult, 2 points for positive hemoccult, and 4 points for gross bleeding. At sampling interval of days 6, 10, and 15, three mice in each group were sacrificed by cervical dislocation anesthetized using isoflurane to evaluate the reversal of colitis. The distal colon was excised for measurement of mRNA expression of inflammatory and growth factor markers and histological evaluation.

2.4. Real time PCR

Total mRNA from colon tissues were extracted and reverse transcribed into cDNA using Gent Bio RT-PCR Kit (Biobase, Germany). Individual mRNA levels were relatively quantified using Real-Time PCR (Agilent Startagene, Germany). Each reaction contains 2 L cDNA sample, 1 L forward primers (10 ng), 1 L reverse primers (10 ng), 10 L SYBR Green (2× green star qPCR master mix, K6251) and 6 L water in a total volume 20 L per sample. The primers are listed in Table 1.

All measurements were performed in triplicate. Real-time RT-PCR data were represented as Ct values, the Ct or threshold value of the target sequence is directly proportional to the absolute concentration when compared with the threshold value for reference genes. The relative expression level of target gene were plotted as fold change compared to control and determined by the $2^{-\Delta\Delta ct}$ method (Livak and Schmittgen, 2001), a relative quantification algorithm. The factor X by which the amount of the changed gene can be calculated with the formula: $X = 2^{-\Delta\Delta ct}$. where $\Delta\Delta ct$ = (Ct of target) control − (Ct, of target × β-actin) sample.

2.5. Histology analysis

Sections of distal colon were fixed in 10% neutral buffered formaldehyde and embedded in paraffin for histological analysis before staining with hematoxylin and eosin. The histological scoring was performed based on the observation of mucosal damage and inflammatory cell infiltrate. For infiltration of inflammatory cells, rare inflammatory cells in the lamina propria were counted as 0; increased numbers of inflammatory cells in the lamina propria as 1; confluence of inflammatory cells, extending into the submucosa as 2; and a score of 3 was given for transmural extension of the infiltrate. For mucosal damage assessment, intact mucosa was scored as 0, discrete lymphoepithelial lesions were taken as 1, surface mucosal erosions was 2 and the score for extensive mucosal damage was given as 3 and evaluated by pathologist as treatment-blinded assessment.

For CD 34 immunohistochemical analysis (Mikalsen et al., 2011), deparaffinised sections were microwaved in Tris/EDTA (pH 9.0), followed by 5 min treatment with 0.03% hydrogen peroxidase. The sections were incubated with anti-CD4 QBEND-10 (Biogenex, Fremont) against CD34 at room temperature for 30 min, then with peroxidase-labelled polymer conjugated to goat antimouse antibody for 30 min, and finally with 3-3′-diaminobenzidine tetrahydrochloride for 10 min. Counterstaining was performed using hematoxylin. Appropriate negative and positive controls were included.

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Table 1
Primer sequences for RT-PCR.

Primer name	Primer sequence	Annealing temperature	Product size
β-actin	Sense-5′-TGGAATCCTGTGGCATCCATGAAA-3′, Antisense - 5′-TAAAACGCAGCTCAGTAACAGTCC-3′	55.2	348 bp
IL-1β	Sense - 5′-GCAACTGTTCCTGAACTCA-3′, Antisense - 5′-CTCGGAGCCTGTAGTGCAG-3′	54.2	384 bp
VEGF	Sense - 5′-GTTACCTCAGCAAGACGTTGT-3′ Antisense - 5′-AGGAAGTGTGATTGGCAAAACT-3′	53.2	190 bp
FGF	Sense - 5′-ACCAAACTATACAGCCGACAAG Antisense- 5′- AGGGATGAGGTTAAACAGAGTGT-3′	54.0	108 bp
TNF-α	Sense - 5′-GGCAGGTCTACTTTGGAGTC-3′, Antisense - 5′-ACATTCGAGGCTCCAGTGAATTCG-3′	53.5	307 bp
IL-6	Sense - 5′-TGGAGTCACAGAAGGAGTGGCTA-3′ Antisense - 5′-TCTGACCACAGTGAGGAATGTCC-3′	53.5	155 bp

2.6. Statistical analysis

All data except histology score are presented as mean ± SEM. Statistical analysis was performed using GraphPad Prism Software (Version 5; Inc.). Each treatment group was analyzed against all other groups using two way analysis of variance (ANOVA) followed by Bonferroni post-test. Histology scoring is presented as median with range. Statistical analysis for histopathological scores was carried out using Mann Whitney test. The level of significance was set at $P < 0.05$.

3. Results and discussion

3.1. Clinical activity index

Mucosal healing is the ultimate goal of UC therapy. This fact is widely acknowledged and is rapidly gaining prominence in clinical practice (Lichtenstein and Rutgeerts, 2010; Rutgeerts et al., 2007). Evaluation of UC earlier had been predominantly dependent on symptomatic observations. While we do accept that this might not be a comprehensive way to evaluate UC, it cannot be denied that these clinical parameters, namely rectal bleeding, stool consistency and weight loss, provide a good indication of overall disease status (Hartmann et al., 2000; Siegmund et al., 2001). The ultimate aim of this study is to establish the efficacy of collagen and collagen hydrolysate in aiding mucosal regeneration.

DSS does not directly cause intestinal inflammation, rather it exerts chemical injury to the intestinal epithelium, resulting in exposure of the lamina propria and submucosal compartment to luminal antigens and enteric bacteria, triggering inflammation. Also, DSS model phenotype lacks the chronic changes observed in humans unless the animals are treated with multiple pulses of DSS (Low et al., 2013). Moreover, cryptitis and crypt abscesses are common histological features of human IBD but rarely reported in DSS-induced colitis (Melgar et al., 2005). Nevertheless, DSS is the most commonly used experimental model that mimics many of the signs and symptoms of human ulcerative colitis, including diarrhea, bloody feces, weight loss, mucosal ulceration, and shortening of the large intestine (Elson et al., 1995; Okayasu et al., 1990).

Mice fed with 3.5% DSS in drinking water developed clinical signs of UC from day 3 and showed severe clinical signs on day 6, as mice produced loose stools or diarrhea, gross rectal bleeding and showed significant loss in the body weight (Fig. 1). The therapeutic efficiency of intrarectally delivered collagen and collagen hydrolysate was compared to that of mesalamine, which served as the positive control, while the vehicle acted as negative control. The three clinical assessment parameters, rectal bleeding, stool consistency and loss in body weight, indicated an improvement in the disease condition with collagen, collagen hydrolysate and mesalamine treatment when compared to the negative control, which is shown in Fig. 1A. On day 10, both collagen ($P < 0.05$) and collagen hydrolysate ($P < 0.01$) treatment gave a very significant reduction in rectal bleeding scores (1.56 ± 0.29 and 1.33 ± 0.33, respectively), compared to the mesalamine treatment (2.50 ± 0.33). The interesting observation here is that the rectal bleeding score for mesalamine treatment was almost same as that of the negative control on day 10. By day 13, both collagen and collagen hydrolysate treatments were successful in completely reversing rectal bleeding, while it still persisted for mesalamine and vehicle groups (Fig. 1B). However, mesalamine fared better when it came to stool consistency, achieving a score of 1.00 ± 0.38 on day 10 compared to collagen (2.00 ± 0.00) and collagen hydrolysate (1.56 ± 0.33) (Fig. 1C). Earlier clinical studies with mesalamine treatment have clearly reported significant reduction in the stool frequency and improved stool consistency (Bafutto et al., 2011; Lichtenstein et al., 2007). It may be noted that the difference in stool consistency scores (on day 10) between mesalamine and collagen hydrolysate is not significant. By day 14, collagen hydrolysate and mesalamine treatment resulted in well formed stool pellets, while the symptom mildly persisted in the case of collagen treatment.

With respect to body weight, collagen hydrolysate treatment restored the body weight of the animals to normal levels by day 15, whereas collagen and mesalamine treatments were able to show a significant improvement over the vehicle control. The clinical activity index, a measure of the overall severity of the disease, showed that collagen hydrolysate gave better overall recovery than collagen and mesalamine (Fig. 1D).

Of the three clinical parameters, rectal bleeding is the one that is an indirect indicator of the extent of mucosal ulceration. Reduced bleeding can be considered as a sign of healing of the mucosa. As mentioned above, both collagen and collagen hydrolysate held a significant advantage over mesalamine in this parameter, which is a strong, even though indirect, evidence that collagen and collagen hydrolysate support mucosal regeneration. This outcome is not surprising considering the fact that collagen and collagen hydrolysate have already been widely used for tissue regeneration applications (Ramadass et al., 2014; Ruszczak, 2003). Collagen is an abundant protein of the extracellular mucosal layer. Ulceration in the mucosal and sub-mucosal areas of patients with UC is due to excessive degradation of ECM (Wang and Mao, 2007). Both collagen and collagen hydrolysate treatments would have served as a regenerative matrix for damaged mucosa and aided in faster healing. However, it is to be recognized that the use of regenerative approach for the treatment of mucosal damage is highly novel and is the first of its kind.

3.2. VEGF levels in colon tissue

Pathological (abnormal) angiogenesis has been implicated to play a pathogenic role in inflammatory bowel diseases and increased expression of vascular endothelial growth factor (VEGF) has been proven to play a central role in this angiogenesis (Tolstanova et al., 2009). The role, however, is not clearly defined. There are a lot of evidences that demonstrate the upregulated serum and tissue levels of VEGF in

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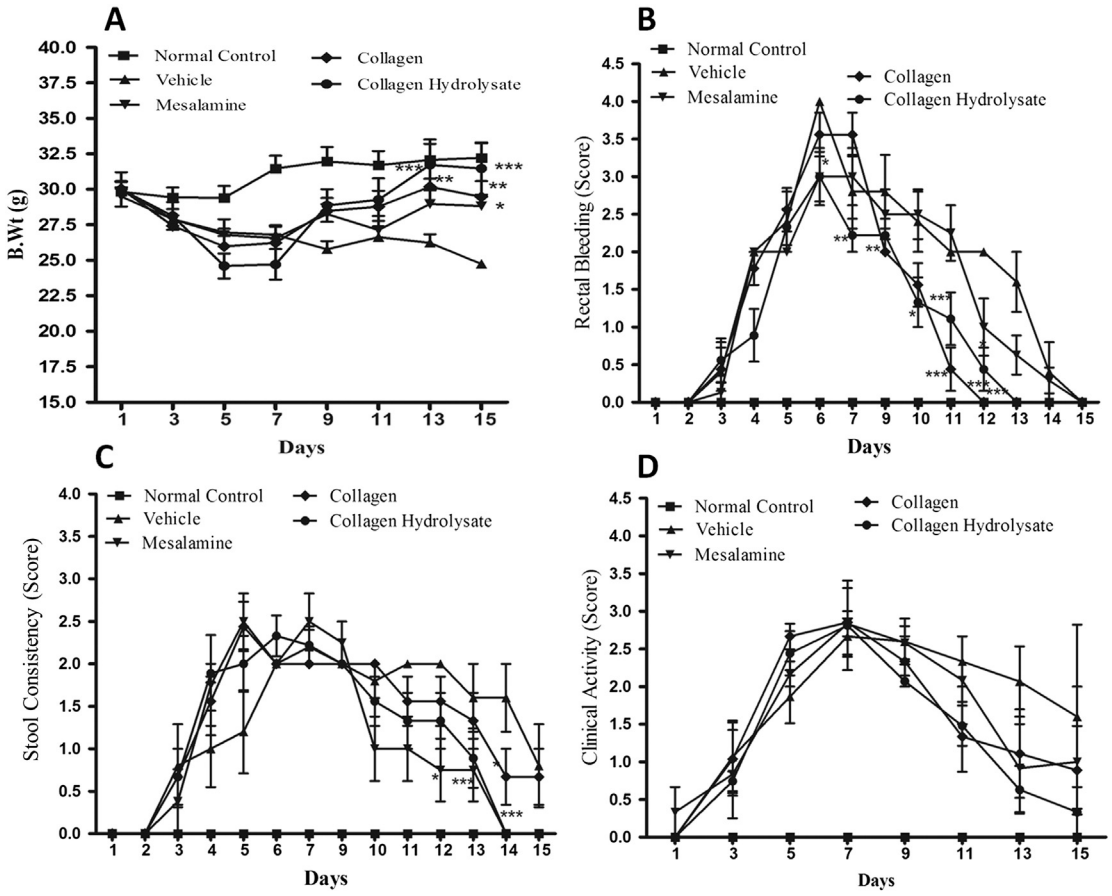

Fig. 1. Effect of collagen and collagen hydrolysate on clinical score assessment. (A) Body weight, (B) rectal bleeding, (C) stool consistency and (D) clinical activity index in DSS induced ulcerative colitis in mice. The mice were subjected to drinking water containing 3.5% DSS for 5 days, followed by the treatment through intra-rectal administration for 10 days. Each column represents the mean ± SEM of 6 mice. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, compared with the vehicle group.

patients with active UC and in animal models of UC (Griga et al., 1998; Griga et al., 1999; Kanazawa et al., 2001; Sandor et al., 2006; Tolstanova et al., 2009). Up-regulated VEGF increases vascular permeability in colonic mucosa; thus, it facilitates the infiltration of inflammatory cells at the site of injury and promotes persistent chronic inflammation (Sartor, 1994). This is what led to the implication of VEGF in UC pathogenesis. However, this relation is a paradox considering the fact that VEGF has been widely proven as one of the most critical factors

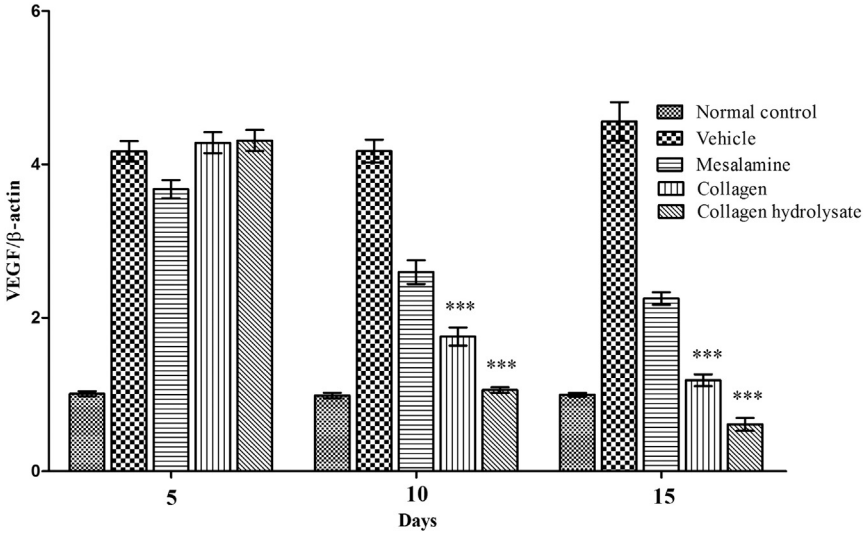

Fig. 2. Effect of collagen and collagen hydrolysate on the VEGF mRNA expression in DSS induced ulcerative colitis in mice. VEGF levels in colonic tissue were determined by real time-PCR. All the treatment groups caused significant increase in VEGF mRNA levels followed by DSS induction by day 5. Collagen hydrolysate treatment showed marked reduction in VEGF expression levels followed by collagen and mesalamine treatments on both days 10 and 15. Each column represents the mean ± SEM of 3 samples. $^{***}P < 0.001$, compared with the positive control mesalamine treatment.

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in healing of tissue injuries and ulcers (Elson, 1996). But, the correlation between elevated VEGF levels and active UC is very widely established (Griga et al., 1998; Griga et al., 1999; Kanazawa et al., 2001; Sandor et al., 2006; Tolstanova et al., 2009).

All groups that were induced by DSS showed significantly increased levels of VEGF, which is an indication of disease severity (Fig. 2). Among the groups that underwent therapy, collagen hydrolysate (1.06 ± 0.25) showed marked reduction in VEGF expression by day 10, followed by

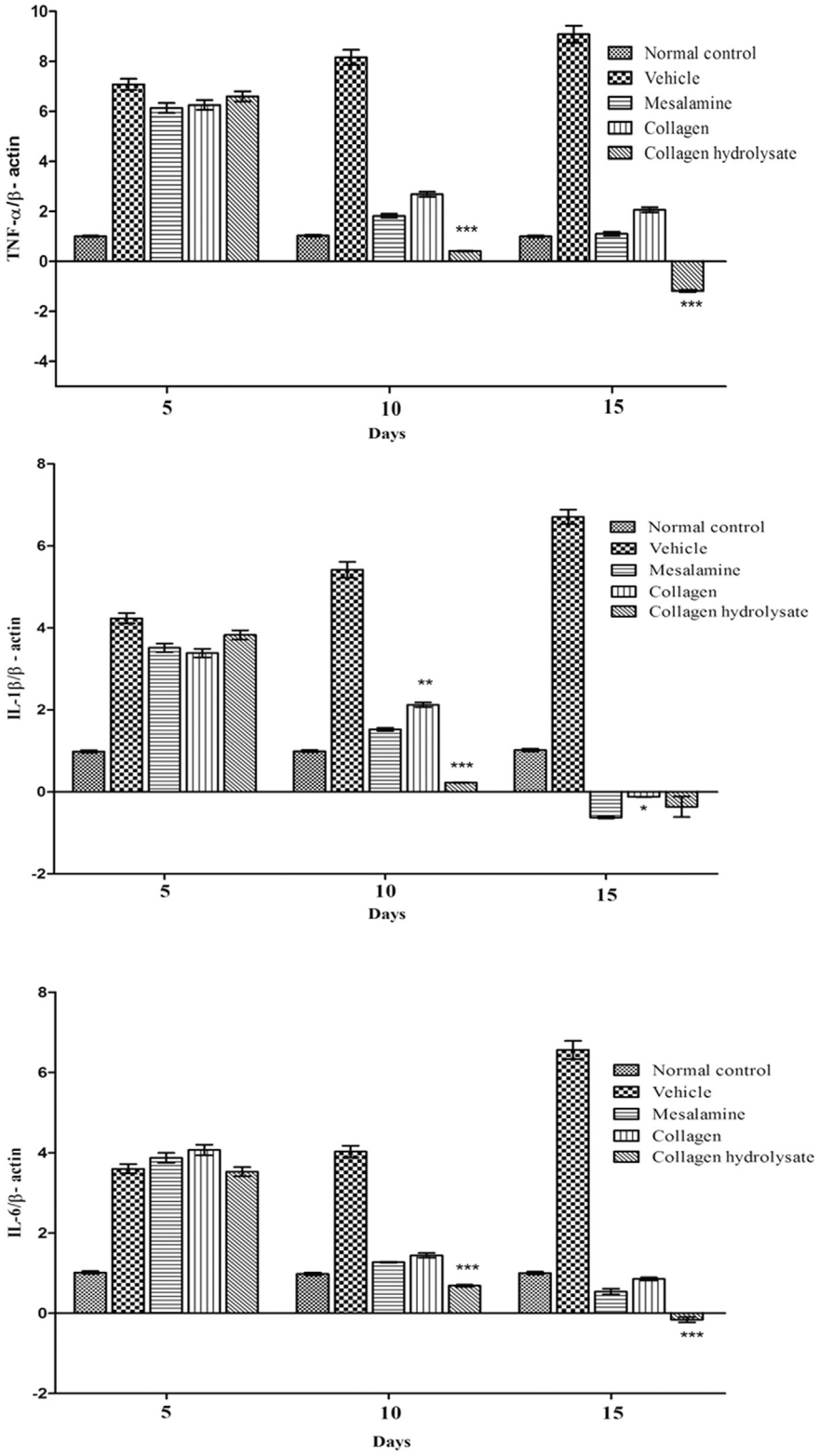

Fig. 3. Effect of collagen and collagen hydrolysate on the mRNA expression levels of pro-inflammatory cytokines. (A) TNF-α, (B) IL-1β and (C) IL-6. Collagen hydrolysate treatment was very effective in decreasing the expression levels of TNF-α, IL-1β, and IL-6 by day 15, followed by mesalamine and collagen treatments. Each column represents the mean ± SEM of 3 samples. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, compared with the positive control mesalamine treatment.

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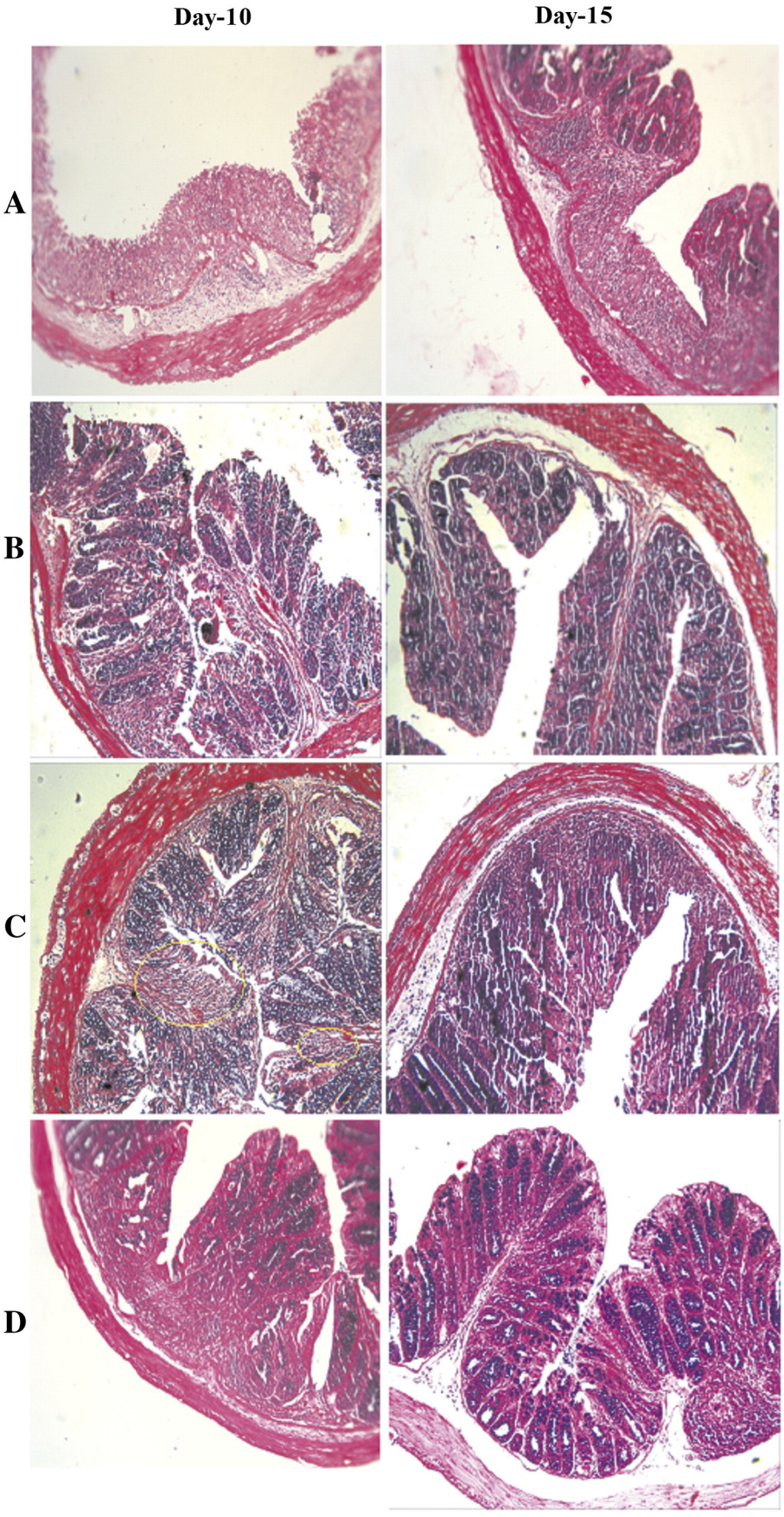

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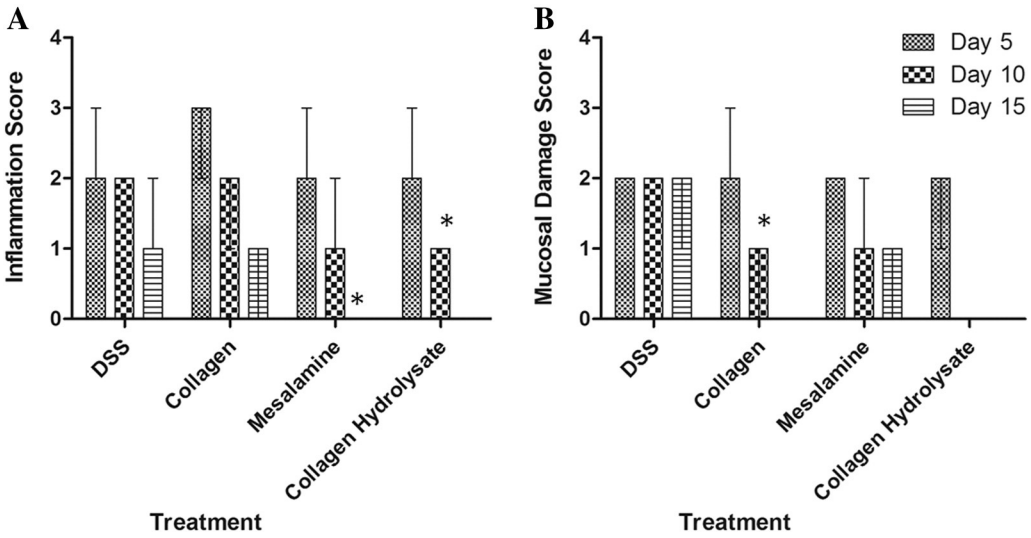

Fig. 5. Histological scoring of distal colonic mucosa for (A) inflammatory infiltrate and (B) mucosal damage observation during treatment at days 6, 10 and 15. Each column represents the median and range of 3 samples. *$P < 0.05$, compared with the vehicle group.

collagen (1.76 ± 0.45) and mesalamine (2.59 ± 0.51). By day 15, VEGF had been downregulated below normal control levels in all therapeutic groups. Here too, collagen hydrolysate showed maximum downregulation followed by mesalamine and collagen. The mechanism of VEGF downregulation by collagen and collagen hydrolysate warrants further investigation.

3.3. RT-PCR of pro-inflammatory cytokines

Cytokines are involved in a variety of biological processes, including cell activation, growth, and differentiation, and they are central to the development of inflammation and immunity (Elson, 1996; Sartor, 1994). In UC, TNF-α, IL-1β and IL-6 are proinflammatory cytokines that have been reported to be up regulated during mucosal damage (Brynskov et al., 1992; Kusugami et al., 1995; Ligumsky et al., 1990; MacDonald et al., 1990). In agreement with previous findings, all DSS-induced groups showed significant upregulation of TNF-α, IL-1β and IL-6 mRNA levels as compared to normal group. It has to be noted that the vehicle treated group did not show any reduction in upregulation of inflammatory markers throughout the course of the treatment. Mesalamine, a first line drug used to treat mild-moderate UC, is known to bring about a significant suppression in the expression on the levels of all three proinflammatory cytokines.

Of the three pro-inflammatory cytokines, the downregulation of TNF-α is expected to have the most positive therapeutic outcomes because of the multiple roles that TNF-α plays in UC. TNF-α disrupts the epithelial barrier, induces apoptosis of the villus epithelial cells, and stimulates the secretion of chemokines from the intestinal epithelial cells (Paunovic et al., 2011). Most importantly, TNF-α, which acts early in the cytokine cascade, has been identified as a potential target for therapeutic intervention (Sands, 1997). In our study, it has been observed that collagen hydrolysate treatment was very effective in downregulating TNF-α expression by day 10, followed by mesalamine and collagen treatment (collagen hydrolysate - 0.41 ± 0.02, mesalamine - 1.81 ± 0.08, collagen - 2.68 ± 0.10). Even further reduction was observed by day 15 (Fig. 3A) (collagen hydrolysate - 1.18 ± 0.04, mesalamine - 1.10 ± 0.08, collagen - 2.06 ± 0.10). TNF-α downregulation gains further importance because of the fact that it is known to upregulate other proinflammatory mediators such as IL-6 and IL-1β, thus amplifying the early sequences of the inflammatory cascade (Rossetti et al., 2004).

Collagen hydrolysate showed significant reduction in the expression levels of both IL-1β (Fig. 3B) and IL-6 (Fig. 3C) followed by the mesalamine and collagen (IL-1β, collagen hydrolysate - 0.23 ± 0.01, mesalamine - 1.53 ± 1.13, collagen - 2.12 ± 0.06 on day 10 and collagen hydrolysate - 0.36 ± 0.05, mesalamine - 0.62 ± 0.02, collagen - 0.12 ± 0.00 on day 15 and IL-6, collagen hydrolysate - 0.69 ± 0.03, mesalamine - 1.27 ± 0.01, collagen - 1.40 ± 0.06 on day 10 and collagen hydrolysate - 0.16 ± 0.06, mesalamine - 0.54 ± 0.07, collagen - 0.85 ± 0.04 on day 15).

Apart from the inflammation cascade, TNF-α also plays a key role in mucosal ulceration by activating NF-κβ-dependent pathways, which trigger the release of matrix metalloproteinases (MMP) (Louis, 2001). MMP 1, an intestinal collagenase, degrades collagen and plays a major role in ECM degradation (Wang and Yan, 2006). It has been reported that the expression of MMP 1 is increased by 230 fold in the colonic mucosa of UC patients compared to that of normal control (Baugh et al., 1999). Elevated MMP levels are common in chronic external ulcers too, and in such cases collagen based wound dressings are thought to act as sacrificial substrates by providing an alternative collagen source for the MMPs to cleave (Boyer, 2013), leaving the endogenous native collagen unharmed to mediate the wound healing for regeneration of normal tissues. Thus, collagen and collagen hydrolysate therapy can effectively counter the major inflammatory pathways involved in UC, and at the same time might also be able to provide protection to the intestinal mucosa from MMP action.

3.4. Histological analysis

Histological examination of distal colon and scoring are given Figs. 4 and 5, respectively. DSS induced animals showed extensive mucosal erosion and crypt damage, with dense inflammatory cell infiltration extending to the sub mucosal layer. The severity of the disease was confirmed by histology score assessment on day 5, with mucosal damage and inflammatory infiltrate scores having median scores of 2 respectively. The negative control showed gradual improvement in the clinical

Fig. 4. Effect of collagen and collagen hydrolysate on the histological manifestation of DSS induced UC. Representative cross sectional images of transverse distal colon of DSS treated mice with PBS vehicle (A), mesalamine positive control (B), collagen (C), and collagen hydrolysate treatments (D). Vehicle treatment (negative control) showed partial mucosal regeneration no significant reduction in the inflammatory infiltration. Mesalamine treatment showed regional destruction of colonic mucosa and both collagen and collagen hydrolysate treatments effectively reversed the mucosal damage by day 10. Magnification of the images is 10-fold.

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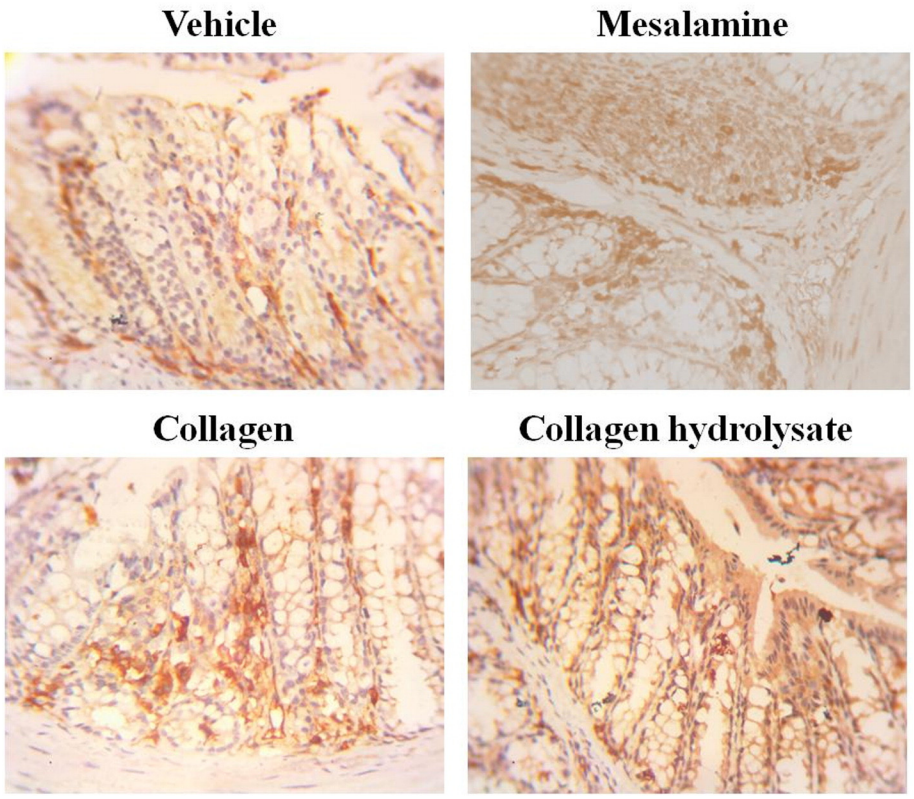

Fig. 6. CD 34 Immunohistochemical analysis. Representative cross sectional images of vehicle control, mesalamine positive control, collagen and collagen hydrolysate treatments (magnification: 400×).

signs (See 3.1) after DSS discontinuation, and histological observation revealed partial mucosal regeneration as expected; however, no reduction in the inflammatory infiltration was observed throughout the duration of the study (Fig. 4A).

Interestingly, on day 10, collagen and collagen hydrolysate treated mice showed no mucosal damage and complete crypt formation, showing that even a short term treatment using collagen and collagen hydrolysate is very effective in regenerating the damaged mucosa (Fig. 4C & D). However, mesalamine treatment could not facilitate mucosal healing by day 10 as evidenced from the median mucosal damage score of 1 (range of 1–2) (Fig. 4B). But, mesalamine treatment showed significant reduction in the inflammatory score. Inflammation was still persistent with collagen treatment on day 10 as indicated by an inflammatory score of 2 (range of 2–3) and collagen hydrolysate showed significant improvement in the reversal of histological signs of colitis (score 1 (range of 0–1) for inflammation and 0 (range of 0–1) for mucosal damage). On day 15, collagen, collagen hydrolysate and mesalamine treatment groups showed no histology signs of ulcerative colitis as evidenced by the absence of inflammation and complete crypt formation. These findings suggest that collagen and collagen degradation peptides might function as chemotactic stimuli for fibroblasts in vivo and attract these cells to initiate repair of damaged tissue (DiCosmo, 2009). The significant reduction in mucosal damage and faster regeneration of damaged mucosa seen with collagen and collagen hydrolysate treatment is consistent with the rectal bleeding scores mentioned earlier (Fig. 1).

Immunohistochemical analysis revealed that CD 34 expression was observed in all tissue specimens in the area of colonic mucosa and the capillaries of lamina propria. Both collagen hydrolysate and collagen treatment showed more expression of CD 34 compared to the mesalamine and vehicle groups (Fig. 6). No significant changes in the expression of CD 34 exist between collagen hydrolysate and the collagen group. All the treatment groups showed higher expression compared to the vehicle control.

4. Conclusion

Ulcerative colitis is characterized by two problems, inflammation and ulceration. While existing therapies effectively address inflammation, they are not successful in repairing the damage that ulceration causes to the intestinal mucosa. The study has proven that collagen and collagen hydrolysate, the popular tissue regeneration matrices are as effective, and in some aspects even more effective, than mesalamine in countering the pathological mechanisms of UC. Both collagen and collagen hydrolysate held a significant advantage over mesalamine in reducing the rectal bleeding, which is an indirect measure of mucosal healing. More importantly, the expression levels of key molecular factors shows that collagen and collagen hydrolysate have a role that is greater than just regeneration. In particular, the results observed with downregulation of VEGF, TNF-α, IL-1β and IL-6 establish that collagen and collagen hydrolysate have played a role at the molecular level. The mechanisms of these actions need to be further investigated. But, the present evidence is good enough to consider both collagen and collagen hydrolysate as potential candidates for comprehensive therapy for UC in the near future.

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