



Mesenchymal Stem Cells for the Treatment of Acetic Acid-Induced Ulcerative Colitis in Rats

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Abstract

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BACKGROUND: Ulcerative colitis (UC) is an autoimmune inflammatory bowel disease, characterized by chronic and relapsing inflammation of the intestinal mucosa. Clinical treatments fail to reduce inflammation and induce side effects in nearly 30% of patients. Mesenchymal stem cells (MSCs) are immunomodulatory agents that can encourage tissue repair and regeneration.

AIM: The aim of the study was to investigate the ability of MSCs to differentiate into enterocytes under the mediation of activin A, fibroblastic growth factor 2, and epidermal growth factors and to study the effect of administering MSCs to rats with acetic acid (AA)-induced UC.

METHODS: MSCs isolated from the umbilical cord were induced to differentiate into enterocytes. The induced cells were morphologically evaluated by flow cytometry and immunocytochemistry. Forty rats were divided into four groups: Control, AA-induced UC, differentiated, and undifferentiated MSC treated groups. The acute UC in rats was induced by 3% AA transrectal administration. Body weight changes, disease activity index (DAI), and histopathological and immunohistochemical CD105 and CD34 staining were recorded. IL-17, IL-10, and TGF- β levels were measured as well.

RESULTS: In both differentiated and undifferentiated MSCs, induced MSCs improved the DAI score and significantly recovered the pathological changes. The favorable effect of MSCs was significantly linked to CD105 overexpression and CD34 low expression. IL-10 and TGF- β levels increased while IL-17 levels decreased.

CONCLUSION: Both differentiated and undifferentiated MSCs showed anti-inflammatory and immunomodulatory effects in our study. Based on our results, MSCs could become potentially useful for regenerative medicine and the clinical treatment of UC.

Introduction

Ulcerative colitis (UC) is a type of chronic inflammatory disorder with typical symptoms of abdominal pain and bloody diarrhea [1].

UC is characterized by recurring episodes of inflammation limited to the mucosal layer of the colon. It commonly involves the rectum and may extend proximally and continuously to involve other parts of the colon [2]. The pathophysiology of UC involves defects in the colon's epithelial barrier, immune response, leukocyte recruitment, and micro-flora [3].

Despite the numerous therapeutic options available, these treatments may result in side effects and/or resistance and/or remission [4]. Treatment resistance may necessitate a total colectomy, which can have a significant impact on quality of life [5].

Mesenchymal stem cells (MSCs) have anti-inflammatory effects due to their hypo-immunogenic, immune-regulatory properties, homing to the injury site,

cytokine release and growth factor expression, altering host immune responses through immune-modulatory protein secretion, and secreting anti-apoptotic factors [6].

Due to expansion capacity and wide activity range, MSCs might have therapeutic potential against UC [7].

Therefore, this study aimed to investigate the ability of MSCs to differentiate into enterocytes and to study the effect of differentiated and undifferentiated MSCs in an acetic acid (AA)-induced UC rat model.

Materials and Methods

MSC isolation

MSCs were isolated from umbilical cord samples obtained from healthy full-term pregnant women during their cesarean section deliveries. Cords were collected in sterile 0.9% NaCl solution, harvested

by explant method, and incubated for 14 days until fibroblast-like cell outgrowth in an incubator with saturated humidity containing 5% CO₂ at 37°C.

MSC identification by flow cytometry

On day 14, harvested MSCs were identified by flow-cytometric analysis (Beckman Coulter cytoflex flow cytometer, USA) of phycoerythrin (PE)-conjugated anti-CD34, PE anti-CD44, and fluorescein isothiocyanate (FITC) anti-CD73 expression according to manufacturer's instructions (Biosciences, 2350 Qume Dr, San Jose, USA).

Osteogenic, chondrogenic, and adipogenic differentiation potential of MSCs

MSCs' differentiation potential into osteogenic, chondrogenic, and adipogenic cells was evaluated using a human mesenchymal stem cell functional identification kit (R and D system, catalog number SC006). Second passage stem cells were cultured in a special culture medium for 21 days and then stained with Alizarin Red-S, Oil red-O, and Alcian Blue to confirm differentiation.

To induce osteogenic differentiation, MSCs were cultured in supplemented media with dexamethasone (100 nM), L-ascorbate-2-phosphate (0.2 mM), and β-glycerophosphate (10 mM) for 3 weeks. For chondrogenic differentiation, insulin-transferrin-selenium-ethanolamine (1%), ascorbate-2-phosphate (50 μg/mL), L-proline (40 μg/mL), sodium pyruvate (100 μg/mL), dexamethasone (100 nM), and TGF-β3 (10 ng/mL) were added to the medium. For adipogenic differentiation, MSCs were cultured in complete media supplemented with insulin (10 μg/m), indomethacin (100 μM), dexamethasone (1 μM), and isobutylmethylxanthine (0.5 mM).

MSCs' differentiation into enterocytes

MSCs were harvested by trypsinization in Petri dishes. Definitive endodermal (DE) differentiation was induced by adding 100 ng/mL activin A to the complete nutrient medium for 3 days; then, DE cells were subsequently induced into intestinal stem cells (ISC)-like cells by adding 250 ng/mL FGF2 to the complete media for 4 days. ISC-like cells were identified by evaluating post-induction morphological changes under an inverted microscope and by immunocytochemical analysis using goat anti-human/mouse/Rat/Musashi-1 antigen affinity-purified polyclonal antibody and northern light™ 557-conjugated Anti-Goat IgG secondary antibody according to manufacturer's instructions (R and D system) (McKinley Place NE Minneapolis, MN55413, USA). Thereafter, 20 ng/mL epidermal growth factor (EGF) was added for 19 days to stimulate the differentiation of ISC-like cells into enterocytes. The media were changed every

2 days. Growth factors were supplied by Elabscience Biotechnology Inc., USA. Cells were incubated in an incubator with saturated humidity containing 5% CO₂ at 37°C. Control cell dishes for comparison of morphological changes were maintained without the addition of growth factors [8]. Enterocytes were evaluated by immunocytochemical analysis of vascular endothelial cadherins (R and D system). Conjugated with goat anti-mouse IgG2b PE conjugate (Santa Cruz Biotechnology, Finnell Street Dallas, Texas, USA) [9].

Animals

Male Wistar albino rats (150–200 g) were purchased and cared for at the animal house at Menoufia University's Faculty of Medicine, Egypt. The rats were housed in plastic ventilated cages with a 12-h light-dark cycle, at 22°C (room temperature), 55% humidity, and food and water *ad libitum*.

The Animal Care and Use Committee of Menoufia University's Faculty of Medicine in Egypt approved all procedures involving the use of rats (IRB No. 19719CPATH15).

UC induction

To induce UC, 2 mL of 3% AA was administered transrectally through a polyurethane tube for enteral nutrition (2 mm in diameter) inserted into the rectum to a depth of 4.5 cm. Rats were placed in the Recumbent position during and for 1 min after rectal installation to prevent solution leakage [10].

Experimental design

Forty rats were blindly assigned to four experimental groups at random (n = 10 per group). G Power software was used to calculate the sample size [11].

Group I (Control group) got 2 mL of saline transrectally; Group II (AA group) got 2 mL of AA transrectally; Group III (Differentiated stem cells treated group) received AA as group II + 1 mL of differentiated stem cells intraperitoneally (i.p.) (1 mL of the cell solution diluted in PBS at 1 × 10⁶ cells/mL) [12], [13]; and Group IV (Undifferentiated stem cells treated group) received AA as Group II + 1 mL of undifferentiated stem cells (i.p.) [12], [13]. Intraperitoneal ketamine (90 mg/kg) and xylazine (15 mg/kg) were used to sedate the rats in the UC subgroups.

After 12 h of fasting, rats received either AA (Sigma-Aldrich, St Louis, MO, USA) or saline transrectally. 72 h after rectal installation, rats in Groups III and IV received either differentiated or undifferentiated stem cells intraperitoneally [12]. Body weights of rats were taken at the day 0 and before scarification. Rats were sacrificed 72 h later.

Distal colonic segments were removed, and then the inner surface was cut open to reveal it, thoroughly washed with normal saline, and macroscopically examined.

For histopathological and immunohistochemical (IHC) analysis, half of the colon was preserved in 10% formalin.

Disease activity evaluation

The disease activity index (DAI) evaluates weight loss, fecal consistency, and rectal bleeding using a scoring system, was used to quantify disease activity. Body weight loss (0, none; 1, 1–5%; 2, 6–10%; 3, 11–20%; and 4, >20%), diarrhea (0, normal; 1, normal; 2, loose stool; 3, loose stool; and 4, diarrhea), and rectal bleeding (0, normal; 1, normal; 2, slight bleeding; 3, slight bleeding; and 4, gross bleeding) were recorded daily. DAI values were calculated using the average of those parameters' values [14].

Biochemical analysis (Cytokine's assay)

The second half of the colon was homogenized in 5–10 mL cold buffer (i.e., 50 mM potassium phosphate, pH 7.5) per gram tissue then centrifuged at 4000 rpm for 15 min. Then, the supernatant was collected and frozen at -20°C until determination. IL-10, IL-17, and TGF- β levels were evaluated with an enzyme-linked sorbent assay kit according to the manufacturer's instructions (Sunlong Biotech Co., Ltd., China).

Pathological analysis

Histopathological assessment

Formalin-fixed paraffin-embedded colon tissues were processed for histological evaluation by hematoxylin and eosin staining [15]. Histopathological scoring of colonic tissues was blindly assessed by the Geboes score on a scale of 0: Absent, 1: Mild, 2: Moderate, and 3: Severe. The parameters to be assessed were as follows chronic inflammation, neutrophils in lamina propria and epithelium, and erosions or ulcerations [16]. In addition, the frequency of apoptosis was assessed on a scale of 1–3 (mild, moderate, and severe) [17].

IHC analysis of colon tissue

A streptavidin-biotin-amplified system was used. After tissue deparaffinization and rehydration and antigen retrieval using a high pH EDTA solution, samples were cooled at room temperature. The sections were incubated overnight at 4°C with the primary antibodies, CD105 polyclonal antibody diluted at 1:10 (Cat. # PA512511) and CD34 polyclonal antibody diluted at 1:1000 (Cat. # PA5-85917), obtained from

ThermoFisher Scientific, Milano, USA. We used an ultravision detection system using anti-polyvalent HRP/DAB, Neomarker; staining was visualized using a DAB chromogen substrate and Mayer's hematoxylin as counterstain. Mouse heart and liver carcinoma tissue were included as positive controls for CD105 and CD34, respectively. Negative control by omitting the primary antibody was included in each run. Each antibody was assessed using a histoscore (H score) as follows: Strong intensity (3) \times percentage + moderate intensity (2) \times percentage + mild intensity (1) \times percentage + negative staining (0) \times percentage. The final score ranged from 0–300 [18].

Statistical analysis

The D'Argostino and Pearson omnibus normality tests were used to assess normal distributions; data were analyzed using an analysis of variance (ANOVA) followed by a *post hoc* Bonferroni test. Kruskal–Wallis's test was used if data were not normally distributed. Post Hoc Test (Dunn's for multiple comparisons test). Chi-square and Monte Carlo tests were used for qualitative data. The p-value for comparing between the studied groups is considered significant if $p < 0.05$.

Results

Morphological identification of MSCs

The attached cells were observed at day 7. At day 14, multi-polar fibroblastoid cells had reached 60–70% confluence (Figure 1).

MSC identification by flow cytometry

MSCs showed positive CD44 expression ($X \pm$ standard deviation (SD), SD; 70.24 ± 5.85), positive CD73 expression (78.16 ± 6.28), and negative CD34 expression (0.93 ± 0.50).

MSC differentiation into enterocytes

As the intestine is an endoderm-derived organ, human MSCs were initially differentiated into endodermal cells using a high activin A concentration, followed by treatment with FGF2 to differentiate into ISC-like cells, which appeared as enlarged spiky shaped cells with a round central nucleus. Immunofluorescence staining revealed Musashi-1 positive staining of ISC-like cells. These cells were stimulated with EGF to differentiate into enterocytes, which showed dome-like structures assumed to contain liquids and cells. Immunocytochemical analysis showed that the induced enterocytes expressed E-cadherin.

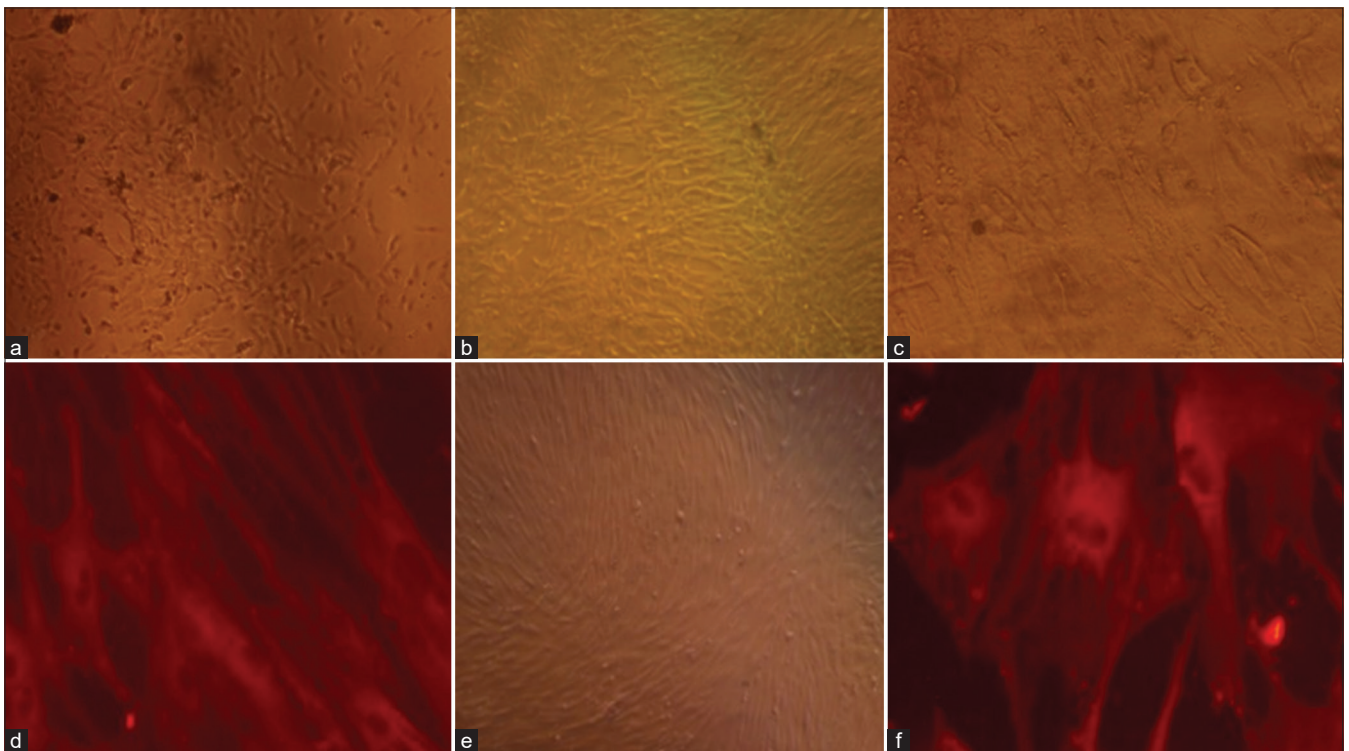


Figure 1: Isolation and differentiation of MSCs; (a): Inverted microscope image $\times 100$ of the adherent fibroblast-like cells grew as spindle shaped cells at day 10; (b): Inverted microscope image $\times 100$ of the adherent MSCs reached 60–70% confluence at about 14 days; (c): Inverted microscope image $\times 200$ of MSCs differentiated into the endoderm using a high concentration of activin A; (d): Microscopic image $\times 200$ of ISC were stimulated by FGF and stained positive for musashi-1 AB; (e): Inverted microscope image $\times 100$ of Musashi-1 positive cells were stimulated with EGF to differentiated into enterocytes; (f): Microscopic image $\times 200$ of immunocytochemistry staining of enterocytes with VE-cadherin

Effect of MSCs on UC clinical symptoms in rats

Rats were monitored at the beginning of the experiment and on the day of sacrifice for weight gain/loss. Except for the control group, all groups lost weight after UC induction. AA rats lost significantly more body weight than the control group (-12.50 ± 1.43 g, 22.40 ± 1.26 g respectively; $p < 0.001$). Compared to the AA group, weight loss improved in the differentiated and undifferentiated MSC treated (-10.10 ± 1.66 g, -9.80 ± 1.93 g; $p = 0.009$ and $p = 0.003$, respectively). There was no significant difference between the differentiated and undifferentiated MSC treated regarding weight loss ($p = 0.975$) (Figure 2).

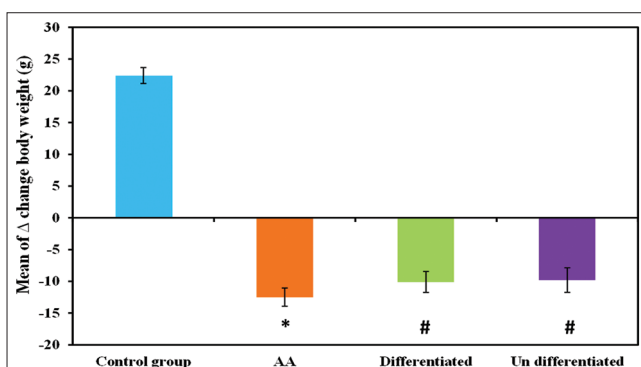


Figure 2: Body weight changes in all study groups. Data are expressed as mean \pm SD ($n = 10$). ANOVA was used for group comparisons. * $p < 0.001$ versus control group, # $p < 0.001$ versus AA

DAI evaluation

Disease severity was evaluated by the DAI scores, which were calculated using three criteria: loss of weight, fecal consistency, and rectal bleeding. The DAI scores of AA rats were significantly higher than those in the control group (3.11 ± 0.39 , 0.00 ± 0.00 ; $p < 0.001$). On the other hand, DAI scores in differentiated and undifferentiated MSC treated rats, were significantly lower than in the AA group (2.15 ± 0.42 , 2.14 ± 0.39 ; $p < 0.001$). There was no significant difference between the differentiated and undifferentiated MSC treated regarding DAI scores ($p = 1.000$) (Figure 3).

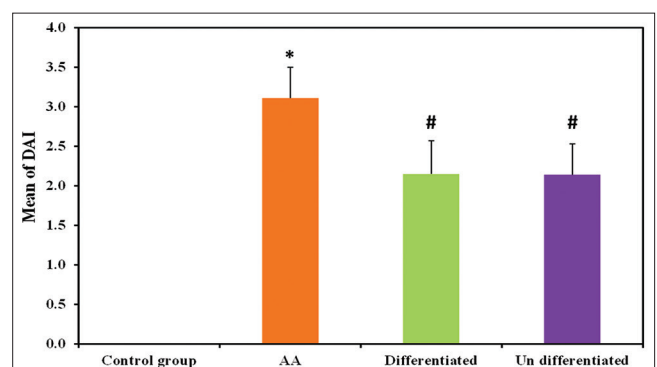


Figure 3: DAI scores of rat colons in all study groups. Data are expressed as mean \pm SD ($n = 10$). ANOVA was used for group comparisons. * $p < 0.001$ vs. control group, # $p < 0.001$ vs. AA

Cytokine's assay

IL-10, IL-17, and TGF-β were statistically significant between groups ($p < 0.001$). IL-10 was significantly increased in AA group then gradually decreased after treatment with no significant difference between rats treated with differentiated and undifferentiated MSCs ($p = 0.722$). While, IL-17 and TGF-β were higher in AA group than control, then after treatment cytokines were significantly increased. Regarding IL-17 there was significant difference between groups treated with differentiated and undifferentiated MSCs (p value 0.003). However, there was no significant difference between groups treated with differentiated and undifferentiated MSCs regarding TGF-β (p value 0.103) (Table 1).

Histopathological analysis

Table 2 shows the histopathological changes in the studied groups. The control group showed preserved colonic mucosal architecture with evenly spaced and regularly arranged crypts. The crypts were lined by goblet cells with preserved crypt architecture. There was no abnormal increase of chronic inflammatory cells, eosinophils, or neutrophils, and few apoptotic bodies were observed at the tip of the villi. However, the UC group showed severe erosions and ulcerations. The lamina propria was distended by mixed inflammatory cellular infiltration with the lymphoid aggregate formation and moderate to marked

activity. An increase in apoptotic activity was evident. These histopathological changes were significantly reduced in both differentiated and undifferentiated MSC treated groups. Both treated groups showed preserved architecture and cellular spacing with mild chronic inflammatory cellular infiltration. Neutrophilic and apoptotic activities were almost absent. There was no significant difference between differentiated and undifferentiated MSC treated groups regarding histopathological changes (Figure 4).

IHC results

Table 3 shows CD105 and CD34 expression in the studied groups. There was no significant difference in CD105 expression between the AA-induced UC and control groups. CD105 expression was significantly increased in both the differentiated and undifferentiated MSC treated groups compared to the AA-induced UC group ($p = 0.036$ and $p = 0.016$, respectively). In addition, there was no significant difference between both treated groups regarding CD105 expression ($p = 0.75$). CD34 expression was significantly increased in the AA-induced UC group compared to the control group ($p = 0.001$). However, CD34 expression was not significantly affected after MSC treatment ($p = 0.78$ and $p = 0.65$) (Figure 5).

Table 1: Comparison between the different studied groups according to IL-17, IL-10, and TGF-β

	Control Group (n = 10)	AA (n = 10)	Differentiated (n = 10)	Undifferentiated (n = 10)	p
IL 17 (pg/ml)					
Min-Max	10.0–20.0	30.0–50.0	25.0–28.0	25.0–30.0	<0.001*
Mean ± SD	14.80 ± 3.61	39.90 ± 6.89	25.80 ± 1.03	27.70 ± 2.0	
Sig bet Groups		$p_1 < 0.001^*$, $p_2 < 0.001^*$, $p_3 < 0.001^*$, $p_4 = 0.722$			
IL 10 (pg/ml)					
Min-Max	31.0–40.0	32.0–52.0	60.0–80.0	66.0–90.0	<0.001*
Mean ± SD	34.90 ± 3.38	41.90 ± 7.22	68.60 ± 5.97	80.0 ± 8.74	
Sig bet Groups		$p_1 = 0.103$, $p_2 < 0.001^*$, $p_3 < 0.001^*$, $p_4 = 0.003^*$			
TGF B1 (pg/ml)					
Min-Max	10.0–45.0	137.0–180.0	170.0–190.0	170.0–230.0	<0.001*
Mean ± SD	31.0 ± 11.99	159.80 ± 16.77	181.40 ± 6.08	196.80 ± 19.70	
Sig bet Groups		$p_1 < 0.001^*$, $p_2 = 0.011^*$, $p_3 < 0.001^*$, $p_4 = 0.103$			

SD: Standard deviation, ANOVA test with *post hoc* Test (Tukey), p_1 : p value for comparing between the studied groups, p_2 : p value for comparing between Control group and AA, p_3 : p value for comparing between AA and differentiated, p_4 : p value for comparing between AA and undifferentiated, p_5 : p value for comparing between differentiated and undifferentiated, *: Statistically significant at $P \leq 0.05$, AA: induction of colitis in rats by acetic acid.

Table 2: Comparison between the different studied groups according to the different histopathological parameters

	Control (n = 10)		AA (n = 10)		Differentiated (n = 10)		Undifferentiated (n = 10)		χ^2	MC p
	No.	%	No.	%	No.	%	No.	%		
Chronic inflammatory infiltrate										
No	10	100.0	0	0.0	0	0.0	0	0.0	50.167*	<0.001*
Mild	0	0.0	0	0.0	8	80.0	7	70.0		
Moderate	0	0.0	6	60.0	2	20.0	3	30.0		
Marked	0	0.0	4	40.0	0	0.0	0	0.0		
Neutrophils in lamina propria/epithelium										
Absent	10	100.0	0	0.0	8	80.0	0	0.0	49.341*	<0.001*
Mild	0	0.0	0	0.0	2	20.0	8	80.0		
Moderate	0	0.0	6	60.0	0	0.0	2	20.0		
Marked	0	0.0	4	40.0	0	0.0	0	0.0		
Erosions/ulceration										
Absent	10	100.0	0	0.0	8	80.0	0	0.0	40.024*	<0.001*
Erosions	0	0.0	6	60.0	2	20.0	10	100.0		
Ulcerations	0	0.0	4	40.0	0	0.0	0	0.0		
Apoptosis										
Minimal	10	100.0	0	0.0	0	0.0	0	0.0	64.390*	<0.001*
Mild	0	0.0	0	0.0	10	100.0	3	30.0		
Moderate	0	0.0	0	0.0	0	0.0	5	50.0		
Marked	0	0.0	10	100.0	0	0.0	2	20.0		

AA: induction of colitis in rats by acetic acid. χ^2 : Chi-square test, MC: Monte Carlo, p: p value, *: Statistically significant at $p \leq 0.05$.

Table 3: Comparison between the different studied groups according to CD105 and CD34 IHC expression

	Control Group (n = 10)	AA (n = 10)	Differentiated (n = 10)	Undifferentiated (n = 10)	p
CD105					
Min–Max.	0.0–140.0	0.0–200.0	0.0–240.0	0.0–300.0	0.028*
Median ± IQR	36.0 ± 42.74	47.60 ± 80.19	114.0 ± 84.62	144.50 ± 116.01	
Sig bet Groups	$p_1 = 0.780, p_2 = 0.036^*, p_3 = 0.016^*, p_4 = 0.750$				
CD34					
Min –Max.	1.0–5.0	1.0–90.0	1.0–80.0	1.0–120.0	0.004*
Median ± IQR	3.60 ± 1.65	38.10 ± 31.77	33.10 ± 28.95	38.60 ± 44.32	
Sig. bet. Groups	$p_1 = 0.001^*, p_2 = 0.780, p_3 = 0.658, p_4 = 0.870$				

IQR: Inter quartile range, Kruskal Wallis test, *: Statistically significant at $P \leq 0.05$, p_1 : p value for comparing between Control group and AA, p_2 : p value for comparing between AA and differentiated, p_3 : p value for comparing between AA and undifferentiated, p_4 : p value for comparing between differentiated and undifferentiated.

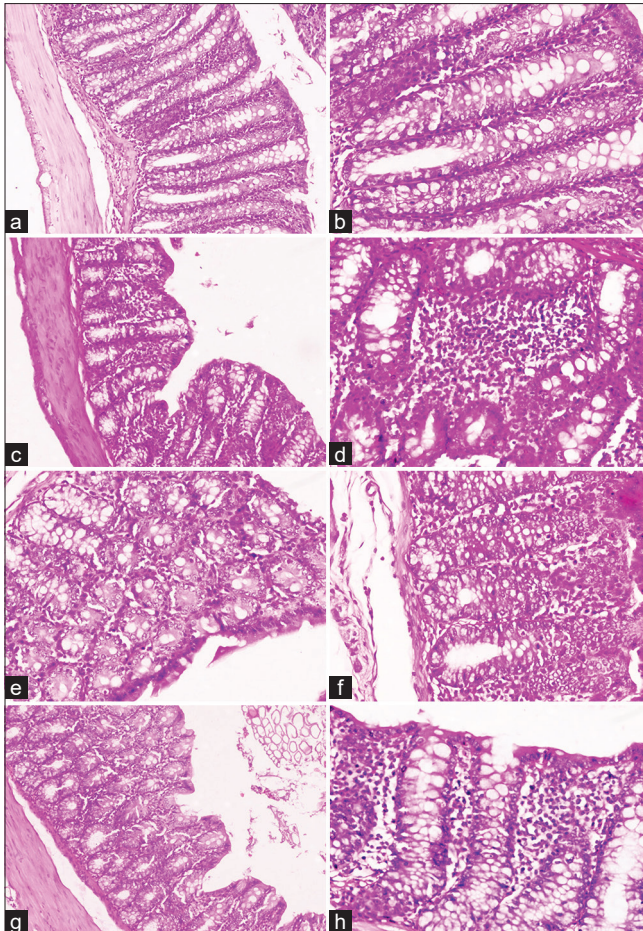


Figure 4: Histopathological changes of the colonic mucosa in the studied groups. a) Preserved architecture with intact surface epithelium in the control group (H and E 40×). b) Minimal inflammatory activity in control group (H and E 200×). c) An untreated UC case shows distorted crypt architecture and erosion (H and E 40×). d) An untreated UC case shows moderate inflammatory infiltrates (H and E 200×). e) An UC case treated with differentiated MSCs shows almost intact surface epithelium (H and E 100×). f) An UC case treated with differentiated MSCs shows almost preserved colonic crypts with minimal inflammatory infiltrate (H and E 200×). g) An UC case treated with undifferentiated MSCs shows mild surface erosion (H and E 40×). h) An UC case treated with undifferentiated MSCs shows mild inflammatory infiltrate (H and E 200×)

Association between CD105 and CD34 expression and histopathological findings

CD105 overexpression was significantly associated with mild chronic inflammation ($p = 0.048$). In addition, CD105 was associated with mild activity, mild erosions, and moderate apoptosis. On the other hand, CD34 expression was significantly associated

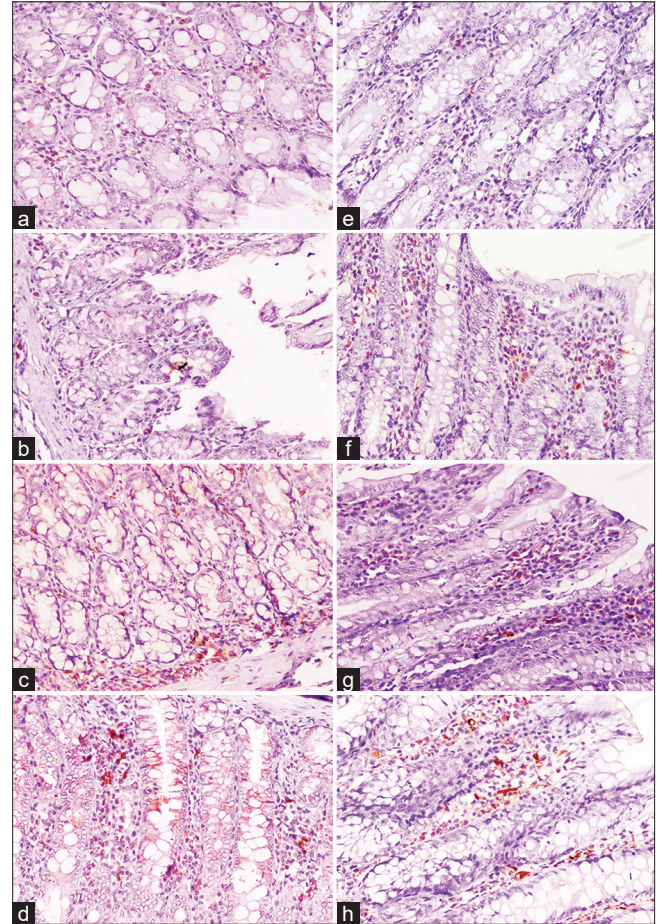


Figure 5: The IHC expression of CD105 and CD34 in the studied groups. a) A normal colonic mucosa shows focal CD105 expression (IHC 200×). b) A colonic mucosa of untreated UC shows minimal CD105 expression (IHC 200×). c) A colonic mucosa of UC treated with differentiated MSC shows strong CD105 (IHC 200×). d) A colonic mucosa of UC treated with undifferentiated MSC shows strong CD105 (IHC 200×). e) A normal colonic mucosa shows absent CD34 expression (IHC 200×). f) A colonic mucosa of untreated UC shows moderate CD34 expression (IHC 200×). g) A colonic mucosa of UC treated with differentiated MSC shows strong CD34 expression (IHC 200×). h) A colonic mucosa of UC treated with undifferentiated MSC shows strong CD34 expression (IHC 200×)

with chronic inflammation, and increased apoptotic activity (Table 4).

Correlation between CD105 and CD34 expression

There was no significant correlation between CD105 and CD34 expression in any of the studied groups ($r = 0.245, p = 0.128$).

Table 4: The relation of CD105 and CD34 IHC expression with several histopathological characteristics

	CD105 Median ± IQR	p- value	CD34 Median ± IQR	p- value
Chronic inflammatory infiltrate		p = 0.048*		0.022*
Absent	25.0 (36.0 ± 42.74)		4.0 (3.60 ± 1.65)	
Mild	150.0 (134.67 ± 92.42)		25.0 (41.33 ± 38.66)	
Moderate	5.0 (68.27 ± 106.71)		20.0 (29.82 ± 29.74)	
Marked	45.0 (72.50 ± 95.0)		25.0 (37.50 ± 35.94)	
Neutrophils in lamina propria/epithelium		0.175		0.111
Absent	50.0 (73.89 ± 77.70)		5.0 (15.67 ± 21.44)	
Mild	150.0 (143.50 ± 115.90)		17.5 (34.60 ± 37.42)	
Moderate	3.0 (45.75 ± 77.17)		40.0 (44.50 ± 42.57)	
Marked	45.0 (72.50 ± 95.0)		25.0 (37.50 ± 35.94)	
Erosions/ulceration		0.753		0.135
Absent	40.0 (69.44 ± 74.56)		5.0 (18.67 ± 25.99)	
Erosions	55.0 (104.50 ± 110.69)		22.5 (36.0 ± 37.77)	
Ulcerations	45.0 (72.50 ± 95.0)		25.0 (37.50 ± 35.94)	
Apoptosis		0.162		0.003*
Minimal	25.0 (36.0 ± 42.74)		4.0 (3.60 ± 1.65)	
Mild	90.0 (102.69 ± 83.43)		20.0 (35.15 ± 37.49)	
Moderate	150.0 (162.0 ± 136.82)		40.0 (45.0 ± 42.72)	
Marked	3.0 (76.33 ± 99.18)		22.5 (34.67 ± 30.01)	

Kruskal-Wallis test, p: p value for comparing between the studied groups, *: Statistically significant at p ≤ 0.05

Discussion

UC is caused by immune dysregulation in the mucosa, an immune inequity among regulatory and effector T cells, and an inflammatory cascade triggered by cytokine production, infection, gene mutations, and external conditions such as diet, lifestyle, and socioeconomic development, which leads to epithelial barrier damage [19]. Both innate and adaptive immunity are involved in disease etiology. The condition is associated with apoptosis and inflammatory responses, as well as tight junction epithelial barrier failure and apoptosis [20].

For UC treatment, MSCs can be easily isolated from a variety of tissues, including bone marrow [21], adipose tissue [22], umbilical cord [23], endometrium [24], placenta [25], intestine [26], amnion [27], amniotic fluid [28], and tonsils [29]. Intraperitoneal, intravenous, subcutaneous, endoscopic, systemic, or anal injection are all possible routes of delivery, which can affect the success of cell transplantation in UC [30].

MSCs help colonic epithelial cells and mucous barriers survive and regenerate by producing exosomes, growth factors, cytokines, and metabolites [31].

In this study, umbilical cord was used as a source of MSCs. Attached cells were observed at day 7; then, multi-polar fibroblastoid cells appeared gradually until reaching 60%–70% confluence on day 14. Flow cytometric analysis showed positive expression of CD44, CD73 and negative expression of CD34 in MSCs. These results agree with Borzou *et al.* [32] and Al Naem *et al.* [33] who documented that MSCs were a group of non-hematopoietic, self-renewing, plastic-adherent, and fibroblast-like stromal cells, that can trans-differentiate into ectodermal and endodermal cells, expressing cell surface markers such as CD44, CD73, CD90, and CD105, and lacking hematopoietic markers such as CD34 and CD45.

In addition, Yu *et al.* [34] and Qiu *et al.* [35] used activin A to treat MSCs for 5 days and found

significantly upregulated DE-specific gene expression on day 3. However, Li *et al.* [36] confirmed that 7-day activin A treated differentiated cells from human induced pluripotent stem cells resulted in higher endoderm-specific gene expression levels at day 7.

Li *et al.* [36] confirmed that FGF2 signaling is important for further DE differentiation to ISC-like cells as it regulates the epithelial-to-mesenchymal transition. Moreover, when cultured with EGFs, Musashi-1 positive cells could differentiate into intestinal absorptive cells.

Differentiated ISC-like cells were confirmed by their morphology similar to that reported by Iwao *et al.* [8]. In addition, cells were Musashi-1 positive. These results agreed with Ye *et al.* [37] who confirmed that Lgr5 and Musashi-1 are two ISC markers, whose expression increased in a time-dependent manner. Maximal expression was observed after 250 ng/mL FGF2 treatment for 4 days. Musashi-1 positive cells were stimulated with EGF to differentiate into enterocytes. Induced enterocytes expressed E-cadherin by immunocytochemical analysis. These results were approved by Ogaki *et al.* [9] and Yu *et al.* [34] who used EGF to complete the differentiation of MSCs into enterocytes; immunocytochemical analysis showed that the induced enterocytes expressed Muc2, CK-18, and E-cadherin.

The current study found that AA-induced colitis resulted in weight loss as evidenced by increased DAI scores. This is in agreement with El-Akabawy and El-Sherif [10] who reported a significantly lower body weight in UC rats when compared to the control group and attributed this change to progressive reduction in food intake and severe diarrhea.

Previous studies have demonstrated that stem cells ameliorate UC symptoms in patients through decreased DAI scores [12], [13], [38]. This agrees with the current study. In the present study, we found that both differentiated and undifferentiated MSCs had similar ameliorating effects on clinical UC signs and stimulating intestinal healing.

MSC therapy decreased the expression level of the pro-inflammatory cytokines TGF- β and IL-17, while increasing that of IL-10. These results are in agreement with Kim *et al.* [39] who documented decreased IL-17 levels in mononuclear cells from the mesenteric lymph nodes and the spleen and elevated TGF- β and IL-10 levels after MSC-CM injection. In addition, the current study corroborates Lee *et al.*'s results [29] of weight gain, DAI reduction, and decreased expression of IL-1, IL-6, and IL-17 after MSC treatment.

The significant improvement of the pathological changes in the treatment groups in our study supported the possible therapeutic role of MSCs in UC management. This is in agreement with previous studies which postulated the potentially safe and effective role of MSC in UC treatment [1]. In addition, the favorable effect of MSCs was significantly linked to CD105

overexpression and CD34 low expression confirming the non-hematopoietic cell nature [3]. On the other hand, CD34 expression was significantly associated with more adverse pathological features. CD34 is overexpressed in AA-induced UC and is essential for eosinophils recruitment and induced inflammatory immune response [2]. Targeting CD34 combined with MSC could be advantageous for the management of UC.

Finally, MSCs were successfully isolated from umbilical cord and were differentiated under the effect of activin A, FGF2 and EGF into enterocytes. Intraperitoneal injection of MSCs (differentiated and undifferentiated) in AA induced UC rats decreased DAI scores and improve UC symptoms.

Conclusion

Taken together, our findings indicate that MSCs had beneficial effects in AA-induced UC due to its anti-inflammatory and immunomodulatory properties. Accordingly, MSCs could be a future useful approach for the treatment of chronic colitis.

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