The potential of a single injection of hUCMSCs in relevant testis injury and related severity of DSS induced acute and chronic colitis

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Research Article

Keywords: inflammatory bowel disease, mesenchymal stem cells, infertility, spermatogenesis, testicle

Posted Date: March 4th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-270686/v1

Abstract

Background A single injection of human umbilical mesenchymal stem cells (hUCMSCs) can improve dextran sulfate sodium (DSS)–induced acute colitis and decrease progression of acute to chronic colitis. We report the therapeutic efficacy of hUCMSCs in acute and chronic colitis-related testicular injury.

Methods Male C57BL/6JNarl mice were divided into control, phosphate-buffered saline 1 (PBS1), PBS2, hUCMSCs 1 (SC1), and SC2 treated groups. Colitis were induced in the mice using 3% DSS. The mice in the SC1 and SC2 groups underwent a single injection of hUCMSCs.

Results The ratio of spermatogenesis impairment of mice in the stem cell groups was lower than that in the PBS groups on days 8 and 25 (P=.029, .043). The overall testicular function impairment and severity of colitis in the PBS2 group were lower than those in the PBS1 group on days 8 and 25 (P=.031, .016). The overall testicular function impairment and severity of colitis in the SC2 group were lower than those in the SC1 group on days 8 and 25 (P=.031, .016).

Conclusions Disease activity of acute colitis is a good predictor of severity and prognosis of testicular injury in mice with colitis. hUCMSCs can reduce the ratio of testicular function impairment in acute and chronic colitis.

Background

Ulcerative colitis (UC) is one kind of inflammatory bowel diseases (IBD). Anatomically, it involves the rectum, parts of the colon or the entire colon.[1] The literature revealed that UC is associated with a variety of extra-intestinal manifestations such as joint, skin, ocular, and oral manifestations, osteoporosis, hepatobiliary disease, amyloidosis, and testicular injury.[2-5] IBD predominantly affects young people of reproductive age, and the age of onset of IBD peaks between 15 and 25 years.[6,7] In Japan, the prevalence of ulcerative colitis in infertile men was 490 per 100,000 men.[8] IBD treatment, active disease, and psychological factors adversely affect male reproductive and sexual function.[9] The outcome of medical therapy varies in the improvement of major symptoms of the disease, and extra-intestinal manifestations, and in the prevention of complications.[10] Finally, patients who become refractory to medical therapy require surgical intervention, and new therapies for the uncontrollable inflammatory activity are needed.[11-13]

Men who were in remission or had mild disease activity had erectile dysfunction rates is similar with those of healthy control subjects. As such, severe IBD activity led to had higher erectile dysfunction rates in men.[14] Generally, maintaining remission was considered as essential in most men with IBD,[15] and inflammation had a negative effect on male fertility.[16] In a recent study, DSS-induced UC was found to be associated with increased inflammation, oxidative stress, cellular damage, and DNA damage in the testes of mice.[5] Moreover, UC also led to a decrease in sperm count and 3β-hydroxysteroid dehydrogenase (3β-HSD) expression.[5] Medications for improving UC-related impairment of testicular function impairment were few, and only 6-gingerol, a phenolic compound isolated from *Zingiber officinale* exerted protective effect against UC-induced testicular damage via mechanisms involving its antioxidant and anti-inflammatory properties.[17]

Mesenchymal stem cells (MSCs) are a pleiotropic population of cells that can differentiate to osteoblasts, adipocytes, and chondrocytes *in vitro*.[18] MSCs promote tissue regeneration in damaged tissues and regulate both innate and adaptive immune responses.[10,19,20] Therefore, MSCs have the potential for the treatment of immune-mediated diseases, including IBD. Human umbilical cord mesenchymal stem cells (hUCMSCs) are isolated from the Wharton's jelly of the human umbilical cord.[21] hUCMSCs have advantages including low immunogenicity, noninvasive harvest procedure, easy expansion *in vitro*, ethical access, and greater anti-inflammatory effect over bone marrow MSCs (BMMSCs).[22,23] Moreover, the properties of anti-inflammation and immunomodulation of hUCMSCs make them as potential candidates for cell-based therapies.[21]

BMMSCs, hUCMSCs, and human umbilical cord blood-derived MSCs have been proven to decrease the severity of colitis in mice.[24-26] In the literature and our previous study of colitis in mice, hUCMSCs have been shown to colonize the inflamed colon and survive where they effectively suppressed the inflammatory and immune responses.[26-28] The medical literature indicates that BALB/c mice can develop an acute form of colitis when exposed to DSS and recover spontaneously about 4 weeks after DSS removal. However, the C57BL/6 mouse strain can develop acute colitis, which can later progress to chronic inflammation.[29] As in our previous study, a single injection of hUCMSCs can improve DSS–induced acute colitis and decrease the progression of acute colitis to chronic colitis in

C57BL/6 mice.[28] In this study, we investigated the correlation between colitis and testicular function and the therapeutic efficacy of hUCMSCs in acute and chronic colitis-related testicular injury.

Materials And Methods

Ethics statement. This work was approved by the institutional review board of Taoyuan General Hospital, Ministry of Health and Welfare, Taoyuan, Taiwan (IRB Number: TYGH104043).

All women provided written informed consent. The research was conducted in accordance with Helsinki Declaration.

All animal experiments were performed in accordance with relevant guidelines and regulations of Animal Ethics Committee of Chung Yuan Christian University (IACUC Approval Number: 105012) accredited for laboratory animal care by Ministry of Health and Welfare of Taiwan, Republic of China.

Culture of hUCMSCs. The hUCMSCs obtained from the Wharton's jelly of human umbilical cords were cultured in a flask with Minimum Essential Medium α (Thermo Fisher Scientific, Waltham, MA) containing 5% UltraGRO (Helios Bioscience, AventaCell BioMedical Corporation, Atlanta GA) and 1% penicillin-streptomycin. TrypLE (Thermo Fisher Scientific) was used to harvest the cells. Oil red O stain and von Kossa stain were used to verify that the cells preserved their differentiation capacity after long-term maintenance in cell culture.[23,30]

The grouping of"vehicle impaired"and"vehicle normal" testis function. The mice were initially divided into three groups: the control group (32 mice), phosphate-buffered saline (PBS) group (64 mice), and the hUCMSCs (SC) group (64 mice). At the end of the experiment, the PBS and hUCMSCs groups were regrouped to the phosphate-buffered saline 1 (PBS1), phosphate-buffered saline 2 (PBS2), hUCMSCs 1 (SC1) and hUCMSCs 2 (SC2) groups according to the lowest epididymal sperm count of control group(18.0 x 10^6/ml on the day 8, 18.6 x 10^6/ml on the day 25) of control group. In a recent study[5], DSS-induced UC led to a decrease in sperm count and 3β-HSD expression. To regroup the PBS and hUCMSCs groups with the lowest epididymal sperm count in the control group was to differentiate the severity of the disease activity. We defined the lowest to highest value of epididymal sperm count in the control group as the normal range of epididymal sperm count in mice with normal testicular function. The epididymal sperm count in the PBS1 and SC1 groups was lower than the lowest epididymal sperm count of the control group. Hence, the PBS1 and SC1 groups represent the group of testicular dysfunction (vehicle impaired) in the PBS and hUCMSCs groups, respectively. The epididymal sperm count in the PBS2 and SC2 groups was higher than the lowest epididymal sperm count of the control group. Hence, the PBS2 and SC2 groups represent the group of normal testicular function (vehicle normal) in the PBS and hUCMSCs groups, respectively.

DSS-induced colitis model and hUCMSC injection. Male C57BL/6Ncrl mice (age, 6–8 weeks) were purchased from BioLASCO Taiwan Company, Ltd. (Taipei, Taiwan). All mice were housed in a temperature and humidity-controlled room and were allowed free access to an in vivo imaging diet (Caliper Life Sciences, PerkinElmer, Waltham, MA).

Care of laboratory animals was in full compliance with standards articulated in the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines; and protocols were approved by the Institutional Animal Care and Use Committee of Chung Yuan Christian University (IACUC Approval Number: 106019).

DSS-induced colitis model. The mice in the PBS and hUCMSCs groups were given 3% DSS (45 kDa; TdB Consultancy, AB, Uppsala, Sweden) ad libitum in their drinking water for first 6 days and plain water for the remainder of the study. Fresh DSS solution was prepared daily. The stages of colitis were defined as follows: day 8 as early colitis (characterized by a considerable neutrophil influx into the colon), and day 25 as chronic colitis (significant numbers of T and B cells).[31] In the control group, the animals received only fresh water.

The daily disease activity index. Body weight, stool consistency, posture, and fur texture were recorded daily to determine the daily disease activity index (DAI). The DAI scoring was assessed blindly (maximum score, 10), as described previously.[32] DAI scoring combined the scoring from weight loss (0–4), stool consistency (0–4), and posture and fur texture (0–2). Briefly, a percentage weight loss score was assessed as 0 = no loss, 1 = 1–3% loss, 2 = 3–6% loss, 3 = 6–9% loss, and 4 = greater than 9% loss in body mass. Stool consistency was evaluated by scoring as follows: score of 0 = no change, 1 = mild change, 2 = loose stool, 3 = loose stool and rectal bleeding, 4 = diarrhea and rectal bleeding. Fur and posture were assessed by applying the following scores: 0 = no change, 1 = mild hunched posture, 2 = hunched posture and reduced movement. The mice in the SC group were via intraperitoneal injected with 2.0 ×

10^6/200 µL passage 3 to passage 5 hUCMSCs on day 1 after receiving DSS. The mice in the PBS groups received PBS 200 µL injected on day 1 after receiving DSS.

Histology. The mice in each group were anesthetized and sacrificed on days 8 and 25. The colons, testes, and epididymides were removed, and the distal 3 cm of the colon were fixed in 10% formalin (Leica: 3800600, Leica Microsystems (SEA) Pte Ltd., Singapore). The testes were fixed in Bouin's solution (Bouin's fluid, Medical Chemical Corporation, Torrance, CA, USA). The distal 3 cm of the colon and testes were also weighted. The colon and testes were stained with hematoxylin (Leica: 3801570, Leica Microsystems (SEA) Pte Ltd., Singapore) and eosin (Leica: 3801570, Leica Microsystems (SEA) Pte Ltd., Singapore) according to standard histological procedures.

Histological quantification was performed in the colon sections by assessing a histological score as previously described.[33] The inflammatory score comprises the inflammatory cell infiltration score and the intestinal architecture score, with maximum values of 3 points each. In the evaluation of inflammatory cell infiltration, no inflammatory cell infiltration received a score of 0, mild mucosal infiltration received a score of 1, mucosal and submucosal inflammatory cell infiltration received a score of 2, and inflammatory cell transmural infiltration received a score of 3. In the evaluation of the intestinal architecture, an intact epithelium received a score of 0, focal epithelial erosion received a score of 1, widespread epithelial erosion received a score of 2, and extensive ulcerations received a score of 3. The inflammatory score was defined as the sum of the scores for inflammatory cell infiltration and intestinal architecture.

Histological quantification was performed in the testicular sections by allotting a Johnsen score following the criteria of scoring as previously described.[34] A Johnsen score of 10 indicates full spermatogenesis, 9 – disorganized tubular epithelium, 8 – few late spermatids, 7 – no late spermatids, 6 – few early spermatids, 5 – no spermatids, 4 – few spermatocytes, 3 – spermatogonia only, 2 – no germ cells, Sertoli cells only and 1 – no seminiferous epithelial cells. Forty seminiferous tubules from each animal were randomly examined, and a Johnsen score was given based on the types of the cells damaged in the seminiferous tubules. The Johnsen score was calculated by dividing the sum of all scores by the total number of seminiferous tubules examined.

Epididymal sperm count. The epididymides were removed after sacrificing the mice and were placed in a microtube containing 1 mL of Hank's Balanced Salt Solution (SH30268.02, General Electric Company., New York, United States) at room temperature. The epididymides were minced into small pieces and were incubated for 30 minutes at 37°C in 5% CO_2 incubator to allow the sperms to swim out. The sperm suspension thus obtained was centrifuged at 1000 rpm for 5 minutes. After centrifugation, 1 mL of the supernatant was taken, and the epididymal sperm count was determined using Neubauer's hemocytometer. The sperm count was expressed as number of sperms per mL of supernatant.[34,35]

Measurement of plasma testosterone level. The plasma level of testosterone (Testosterone ELISA Kit, Crystal Chem, Elk Grove Village, IL, USA) was evaluated using a commercially available kit according to the manufacturer's instructions.[36] Blood was collected from the retro-orbital sinus of the mice before their sacrifice and was placed into microcentrifuge tubes containing 20 µL of EDTA (200 mM). Then, the mixture was centrifuged at 7000 rpm for 7 minutes at 4°C to collect the plasma for testing with the kit. The plasma testosterone levels were expressed as ng/ml.[5]

Immunohistochemistry for the detection of 3β-HSD. Paraffin-embedded testes tissues from the mice in each group were cut into 5-µm-thick sections, placed on poly-L-lysine coated slides, deparaffinized in a 70°C oven for 20 minutes, cleared in xylene for 20 minutes, and rehydrated with graded alcohols. Tissue sections were incubated at 120°C in Epitope Retrieval Solution pH 6 (Leica: RE7113-CE, Leica Microsystems (SEA) Pte Ltd., Singapore) for 15 minutes in an electronic pressure cooker (Panasonic SR-P37, Panasonic Corporation, Osaka, Japan) for antigen retrieval.[5] The immunohistochemistry protocol was performed according to the manufacturer's instructions. Tissue sections were incubated with anti-3β-HSD (rabbit polyclonal IgG) primary antibodies (ab65156, Abcam plc, Cambridge, UK) at 4°C overnight in a humidified chamber. The sections were then incubated with the indicated secondary antibody (KPL, 5220 – 0458, SeraCare Life Sciences Inc., Milford MA, USA) for 30 minutes at room temperature. Horseradish peroxidase activity was detected using 3, 3'diaminobenzidine (DAB), which served as a chromogen (DAB; BioTnA Biotech, Kaohsiung, Taiwan), for 30 seconds. All slides were washed three times in PBS for 5 minutes each in between each step. The sections were finally counterstained with hematoxylin, dehydrated with alcohol, cleared in xylene, and mounted with dibutylphthalate polystyrene xylene.

Statistical analysis. The experimental results are expressed as the mean ± standard error of the mean (SEM). Data analyses (the Mann-Whitney U test and the Chi square test) were performed with SPSS version 21 (IBM Corp., Armonk, NY, USA). Nonparametric analyses between two groups were performed using the Mann-Whitney U test. The comparison of spermatogenesis impairment ratio

between PBS groups and stem cell groups was analyzed using the Chi square test. The comparison of parameters related to IBD severity and testicular function between PBS and stem cell groups was analyzed using the sign and binomial test (https://www.graphpad.com/quickcalcs/binomial1/). $P < .05$ was considered statistically significant.

Results

Ratio of spermatogenesis impairment. Figure 2 shows the comparison of spermatogenesis impairment ratios between the PBS and SC groups. The numbers of mice in the PBS1, PBS2, SC1, and SC2 groups on day 8 were 16, 12, 9, and 22, respectively. The overall ratios of spermatogenesis impairment in the PBS and SC groups on day 8 were 57.1% and 29.0%, respectively. The numbers of mice in the PBS1, PBS2, SC1, and SC2 groups on day 25 day were 14, 8, 11, and 20, respectively. The overall ratios of spermatogenesis impairment in the PBS and SC groups on day 25 were 63.6% and 35.5%, respectively. The ratios of spermatogenesis impairment in the SC groups were statistically lower than in the corresponding PBS groups on days 8 and 25 (*P*=.029, .043 respectively). The single-dose of stem cells decreased the ratio of spermatogenesis impairment induced by colitis.

Testicular function-related parameters. Figure 3 shows testicular function-related parameters in the control, PBS, and SC groups. The mean epididymal sperm counts in the control, PBS2, and SC2 groups were higher than those in the PBS1 and SC1 groups on days 8 and 25 (*P*<.001) (Figure 3a). The mean weights of testes in the control and SC2 groups were higher than that in the PBS1 group on days 8 (*P*=.009, .015, respectively) and 25 (*P*=.014, .047, respectively) (Figure 3b). The mean Johnsen scores in the control, SC1, and SC2 groups were higher than that in the PBS1 group on days 8 (*P*<.001, <.001, <.001, respectively) and 25 (*P*<.001, <.001, <.001, respectively). The mean Johnsen scores in the control, SC1, and SC2 groups were higher than that in the PBS2 group on days 8 (*P*<.001, =.001, <.001, respectively) and 25 (*P*<.001, <.001, <.001, respectively) (Figure 3c, 3e). The mean testosterone levels in the control and SC2 groups were higher than that in the PBS1 group on day 25 (*P*=.041, .048, respectively) (Figure 3d). The mean 3β-HSD expressions in the PBS1 and PBS2 groups were relatively lower than those in the control, SC1, and SC2 groups on days 8 and 25 (Figure 3f).

Comparisons of testicular function between groups. Table 1 shows the comparison of parameters related to testicular function between the PBS1 and PBS2 groups and between the SC1 and SC2 groups on days 8 and 25. Although most parameters compared directly between the PBS1 and PBS2 groups and between the SC1 and SC2 groups did not differ statistically, except for the epididymal sperm count, we compared all these parameters together between the PBS1 and PBS2 groups and between the SC1 and SC2 groups using the sign and binomial test. On days 8 and 25, the overall testicular function impairment in the PBS2 group was lower than in the PBS1 group (*P*=.031, .031, respectively). On days 8 and 25, the overall testicular function impairment in the SC2 group was lower than in the SC1 group (*P*=.031, .031, respectively). Colitis can induce testicular function impairment in some mice, and the epididymal sperm count may be an indicator of testicular function impairment. Stem cell treatment decreased the ratio of testicular function impairment induced by colitis.

Severity of colitis-related parameters. Figure 4 shows the severity of colitis-related parameters in the control, PBS, and stem cell groups. The DAI in the PBS1 group (8.48 ± 0.28) was higher than that in the PBS2 group (7.13 ± 0.48) on day 8 (*P*=.007), and the DAI in the SC1 group (6.05 ± 0.42) was higher than that in the SC2 group (4.14 ± 0.45) on day 7 (*P*=.028) (Figure 4a). The overall DAI of the entire experimental course in the PBS1, PBS2, SC1, and SC2 groups was analyzed with the sign and binomial test. The results showed that the overall DAI in the PBS2 group was significantly lower than that in the PBS1 group (*P*=.022), and the overall DAI in the SC2 group was significantly lower than that in the SC1 group (*P*<.001). The results show that more severe clinical symptoms in mice happened in the acute stage of colitis, and more spermatogenesis impairment in mice happened in the acute and chronic stages of colitis.

The mean colon length in the control and SC2 groups was longer than that in the PBS1 group on days 8 (*P*<.001, =.002, respectively) and 25 (*P*=.001, .025, respectively). The comparisons of colon length in the PBS1 and PBS2 groups on days 8 and 25, respectively, did not differ statistically nor did the corresponding comparisons in the SC1 and SC2 groups (Figure 4b). The mean colon weights in the control and SC2 groups were lighter than that in the PBS1 group on days 8 (*P*<.001, =.048, respectively) and 25 (*P*<.001, <.001, respectively). The comparison of mean colon weights in the PBS1 and PBS2 groups did not differ statistically on day 8. The comparison of colon weights in the SC1 and SC2 groups on day 8 did not differ statistically (Figure 4c). Additionally, the mean colon weights in the PBS2 group were lighter than that in the PBS1 group on day 25 (*P*=.029). The comparison of colon weights in the SC1 and SC2 groups on day 25 did not differ statistically (Figure 4c).

The mean inflammation scores of the colon in the control and SC2 groups were lower than that in the PBS1 group on days 8 (P<.001, =.015, respectively) and 25 (P<.001, <.001, respectively). The comparison of mean inflammation scores of the colon in the PBS1 and PBS2 groups did not differ statistically on days 8 and 25. The comparison of mean inflammation scores of the colon in the SC1 and SC2 groups did not differ statistically on days 8 and 25 (Figure 4d). Figure 4e shows greater inflammatory cell infiltration and mucosal erosion in the PBS1 and PBS2 groups than those in the control, SC1, and SC2 groups.

Comparisons of parameters related to colitis severity. Table 2 shows the comparison of parameters related to colitis severity between the PBS1 and PBS2 groups and between the SC1 and SC2 groups on days 8 and 25. All the parameters related to colitis severity in the PBS2 group were relatively better than those in the PBS1 group on days 8 and 25. All the parameters related to colitis severity in the SC2 group were relatively better than that in the SC1 group on days 8 and 25. Although most parameters related to colitis severity compared between the PBS1 and PBS2 groups and between the SC1 and SC2 groups did not differ statistically, we compared all the parameters related to colitis severity together between the PBS1 and PBS2 groups and between the SC1 and SC2 groups using the sign and binomial test. The results showed that colitis in the PBS1 group was more severe than that in the PBS2 group on days 8 and 25 (P=.016, .016, respectively), and colitis in the SC1 group was more severe than that in the SC2 group on days 8 and 25 (P=.016, .016, respectively).

Discussion

We found that hUCMSCs reduced the ratio of testicular function impairment in acute and chronic colitis in the mouse model (Fig. 2). More severe colitis in the acute stage induced more testicular function impairment in acute and chronic colitis even with stem cell treatment (Fig. 4 and Table 2).

Some studies in men have revealed that abnormal semen quality in IBD is associated with disease activity and poor nutritional status.[37–39] In a mouse study, DSS-induced UC was found to be associated with testicular injury.[5] We found that the overall testicular function impairment in the PBS2 group was lower than in the PBS1 group on days 8 and 25 (P = .031, .031, respectively) (Fig. 3 and Table 1). The statistics significance proved that the methodology rationale about testis function which regrouping “vehicle impaired”and"vehicle normal" by using the lower limit of sperm count. Colitis induced testicular function impairment in some mice, and the epididymal sperm count might be an indicator of testicular function impairment (Fig. 3a). Additionally, colitis can impair steroidogenesis in the testes, decrease the expression of 3βHSD in Leydig cells of the testes, and can decrease the serum testosterone level (Fig. 3d, 3e and 3f).

In this study, regardless of acute or chronic colitis, the DAI was higher in mice with lower epididymal sperm counts than higher epididymal sperm counts, including that in the PBS and SC groups, especially in the acute stage of colitis (Fig. 4a). The overall testicular function impairment in the SC2 group was lower than in the SC1 group on days 8 and 25 (P = .031, .031, respectively). This finding means that more severe clinical symptoms were noted in the acute stage of colitis, and more testicular function impairment was noted in the acute and chronic stages of colitis even with stem cell treatment (Table 2). Stem cells can decrease the ratio of testicular function impairment induced by colitis (Fig. 2). According to the results of the study, we propose that if the severity of colitis can be reduced in the acute stage, the ratio of testicular function impairment induced by colitis may be decreased in the acute and chronic stages. hUCMSCs could be an option for the treatment of UC and UC-related testicular function impairment.

According to the literature and our previous study, BMMSCs, hUCMSCs, and human umbilical cord blood-derived MSCs have been proven to decrease the severity of colitis in mice.[24–26] Recently, a study showed that local injection of allogeneic MSCs could protect acute testicular torsion–induced germ-cell injury.[40] However, no study about the effects of MSC treatment on DSS-induced colitis-related testicular injury is reported. Besides, few articles are published on therapies for improving colitis-related testicular function impairment, and 6-gingerol, a phenolic compound isolated from *Zingiber officinale*, exerted protective influence against chronic ulcerative colitis-induced testicular damage via mechanisms involving its antioxidant and anti-inflammatory properties.[17] 6-Gingerol may be more beneficial than sulfasalazine in ameliorating testicular toxicity, especially when administered for a long time (63 days).[17] According to our study, a single injection of hUCMSCs can reduce the ratio of testicular function impairment in acute and chronic colitis. For clinical use, a single injection of hUCMSCs may be convenient for patients.

Some studies revealed the presence of antisperm antibodies in both male and female with IBD, and these antibodies might play a part in infertility.[41,42] Antisperm antibodies could be a result of the increased immunological response caused by increased intestinal

permeability; these antibodies might be the result of immunization against antigens of the common intestinal flora own common antigenicity with spermatozoa.[41] Another study proposed that the possible mechanism could involve UC-induced testicular toxicity due to damaged inflammatory cells migrating into the systemic circulation and entering the testes via the testicular artery to cause testicular damage.[5] These IBD autoantibodies, pro-inflammatory cytokines, and damaged inflammatory cells may disrupt the blood-testis barrier and decrease the functional integrity of testes. This situation may provide an environment good for the damaged inflammatory cells to enter the seminiferous tubules of the testis and then cause testicular damage.[5,43]

DSS causes disruption of the epithelial layer of the colon, followed by an acute inflammatory response marked by massive infiltration of neutrophils and macrophages into the mucosa,[44] with the secretion of many cytokines and chemokines.[45] Our previous study showed that hUCMSCs could reduce acute colitis and could decrease the progression of acute to chronic colitis owing to their paracrine effects and interactions with immune cells, which subsequentially reduced the level of IL17A, Gro-α, MIP-1α, MIP-2, and eotaxin.[28] hUCMSCs, recruited by chemokines,[46,47] helped to repair the epithelial layer of the colon, decreased the secretion of chemokines by epithelial and inflammatory cells, and directly influenced inflammatory cell proliferation.[26,30,46] We propose that when the severity of acute colitis was alleviated, the numbers of damaged inflammatory cells migrating into the testes may decline, and the severity of testicular injury may decrease in acute and chronic colitis. Additionally, the hUCMSCs can migrate to the testes and survive there at least for 25 days.[28] hUCMSCs may exert anti-inflammatory and immunomodulatory properties in the testes directly. The details of this mechanism require further investigation and verification in future studies.

In spite of advancements in the treatment of IBD, approximately one-third of patients remain unresponsive to existing therapies.[48] Although hUCMSCs could not make all mice with colitis attain disease remission, hUCMSCs may be a candidate for the treatment of UC and colitis-related testicular function impairment. After treatment with single injection of stem cells, there were a few mice in the clinically severe group which the degree of testicular damage was still significantly higher than that in the mild group. Further research is needed to elucidate the mechanisms of action, minimum effective dosing, and the optimal frequency of stem cells injection. Clinical studies in patients are a future research requirement.

Conclusions

We found that hUCMSCs could reduce the ratio of testicular function impairment in acute and chronic colitis. More severe colitis in acute stage might induce more testicular function impairment in acute and chronic colitis even with stem cell treatment.

List Of Abbreviations

BMMSCs: bone marrow mesenchymal stem cells

DAB: 3, 3'diaminobenzidine

DAI: disease activity index

DSS: dextran sulfate sodium

hUCMSCs: human umbilical cord mesenchymal stem cells

IBD: irritable bowel disease

MSCs: mesenchymal stem cells

3β-HSD: 3β-hydroxysteroid dehydrogenase

Declarations

Ethics approval and consent to participate

This work was approved by the institutional review board of Taoyuan General Hospital, Ministry of Health and Welfare, Taoyuan, Taiwan (IRB Number: TYGH104043). All women provided written informed consent. The research was conducted in accordance with the Helsinki Declaration. All applicable international, national, and institutional guidelines for the care and use of animals were

followed. All animal experiments were performed in accordance with relevant guidelines and regulations of Animal Ethics Committee of Chung Yuan Christian University (IACUC Approval Number: 106019) accredited for laboratory animal care by Ministry of Health and Welfare of Taiwan, Republic of China.

Consent for publication

'Not applicable' for that section.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding

This work was supported by grants from Taoyuan General Hospital, Taiwan (PTH10545, PTH10708, PTH10709 and PTH108069).

Authors' contributions

Y-L C, H-Y L and C-K C designed the research and wrote the article. X-F L and K-T C performed the animal studies. S-P C, C-K C, H-Y L, and J-L H provided the study materials. K-S L performed histology assistance in this study. M-F H participated in the study design. M-F H and C-K C participated as leaders of the study design. All authors read and approved the final manuscript.

Competing Interests

“The authors declare that they have no competing interests.”

Acknowledgements

"Not applicable" in this section.

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Tables

Table 1 Comparison of parameters related to testicular function between the PBS1 and PBS2 groups and between the SC1 and SC2 groups on days 8 and 25

Day 8	Control	PBS1	PBS2	*P*	SC1	SC2	*P*
Epididymal sperm count (10^6/mL)	21.54±1.22	12.48±0.85	23.12±1.10	<.001[a]	14.32±0.66	24.27±0.73	<.001[a]
Testes weight (mg)	168.45±2.98	150.90±5.79	160.48±4.45	.260[a]	168.18±6.85	169.44±3.94	.881[a]
Johnsen score	9.52±0.04	8.58±0.09	8.62±0.09	>.999[a]	9.27±0.12	9.30±0.04	.790[a]
Serum testosterone (ng/mL)	1.80±1.29	0.47±0.17	0.58±0.19	.377[a]	1.23±0.69	1.29±0.57	.978[a]
Ratio of spermatogenesis impairment		57.1%			29.0%		
Overall *P*				.031[b]			.031[b]
Day 25							
Epididymal sperm count (10^6/mL)	25.59±2.20	10.90±1.43	25.60±1.70	<.001[a]	13.67±0.73	26.14±1.08	<.001[a]
Testes weight (mg)	182.89±4.76	163.76±7.92	165.12±10.51	.920[a]	171.38±7.21	177.68±4.33	.244[a]
Johnsen score	9.41±0.04	8.41±0.16	8.45±0.10	.973[a]	9.10±0.11	9.33±0.04	.338[a]
Serum testosterone (ng/mL)	2.05±1.07	0.24±0.03	0.60±0.36	>.999[a]	1.78±0.95	1.91±0.68	.685[a]
Ratio of spermatogenesis impairment		63.6%			35.5%		
Overall *P*				.031[b]			.031[b]

[a]Mann-Whitney U test (PBS1 vs. PBS2) (SC1 vs. SC2)

[b]Sign and binomial test (PBS1 vs. PBS2) (SC1 vs. SC2)

Table 2 Comparison of parameters related to colitis severity between the PBS1 and PBS2 groups and between the SC1 and SC2 groups on days 8 and 25

Day 8	Control	PBS1	PBS2	*P*	SC1	SC2	*P*
Colon length (cm)	6.85±0.23	4.99±0.23	5.14±0.21	.664[a]	5.69±0.23	6.01±0.17	.356[a]
Colon weight (mg)	41.75±2.96	85.88±8.44	78.92±9.66	.599[a]	66.62±9.69	64.92±6.40	.848[a]
Inflammation score	0.58±0.18	4.86±0.20	4.83±0.20	.899[a]	3.00±0.65	2.81±0.48	.722[a]
Sum stool score	0.69±0.38	19.33±1.23	19.00±1.27	.867[a]	15.00±2.18	14.31±1.16	.653[a]
Sum posture score	0.00±0.00	4.58±0.71	3.07±0.50	.126[a]	1.75±0.63	0.81±0.33	.452[a]
Sum body weight loss score	1.92±0.83	17.83±1.83	14.93±1.46	.300[a]	17.50±2.59	13.19±1.32	.417[a]
Overall *P*				.016[b]			.016[b]
Day 25							
Colon length (cm)	7.27±0.24	5.99±0.23	6.14±0.15	.482[a]	6.58±0.29	6.78±0.20	.559[a]
Colon weight (mg)	40.87±2.90	116.51±16.93	66.70±10.12	.029[a]	62.04±10.16	54.71±6.26	.307[a]
Inflammation score	0.33±0.11	3.93±0.42	2.75±0.52	.145[a]	2.09±0.51	1.50±0.34	.261[a]
Sum stool score	3.95±1.08	71.27±3.33	60.44±7.32	.370[a]	48.18±5.19	38.00±4.35	.123[a]
Sum posture score	0.16±0.11	11.27±1.75	7.00±1.94	.131[a]	4.36±1.31	1.62±0.45	.144[a]
Sum body weight loss score	2.00±0.41	40.45±6.90	34.67±6.93	.370[a]	29.18±4.70	23.81±2.83	.389[a]
Overall *P*				.016[b]			.016[b]

[a]Mann-Whitney U test (PBS1 vs. PBS2) (SC1 vs. SC2)

[b]Sign and binomial test (PBS1 vs. PBS2) (SC1 vs. SC2)

Figures

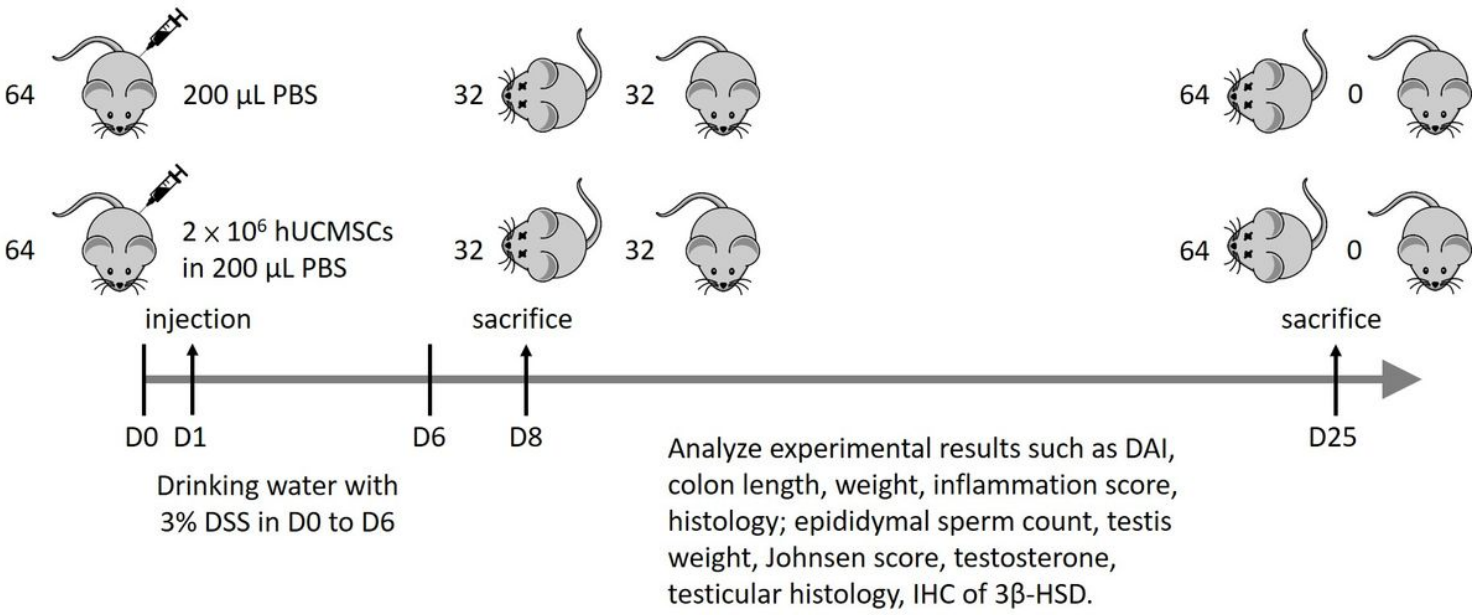

Figure 1

Flow chart of therapeutic effects of a single dose of human umbilical mesenchymal stem cells on testicular injury in mice with colitis. The mice in the PBS and SC groups were given 3% DSS (45 kDa; TdB Consultancy, AB, Uppsala, Sweden) ad libitum in their drinking water for first 6 days and plain water for the remainder of the study. The mice in the SC group were via intraperitoneal injected with 2.0 × 106/200 µL hUCMSCs on day 1 after receiving DSS. The mice in the PBS group received PBS 200 µL injected on day 1 after receiving DSS. The mice in each group were anesthetized and sacrificed on days 8 and 25.

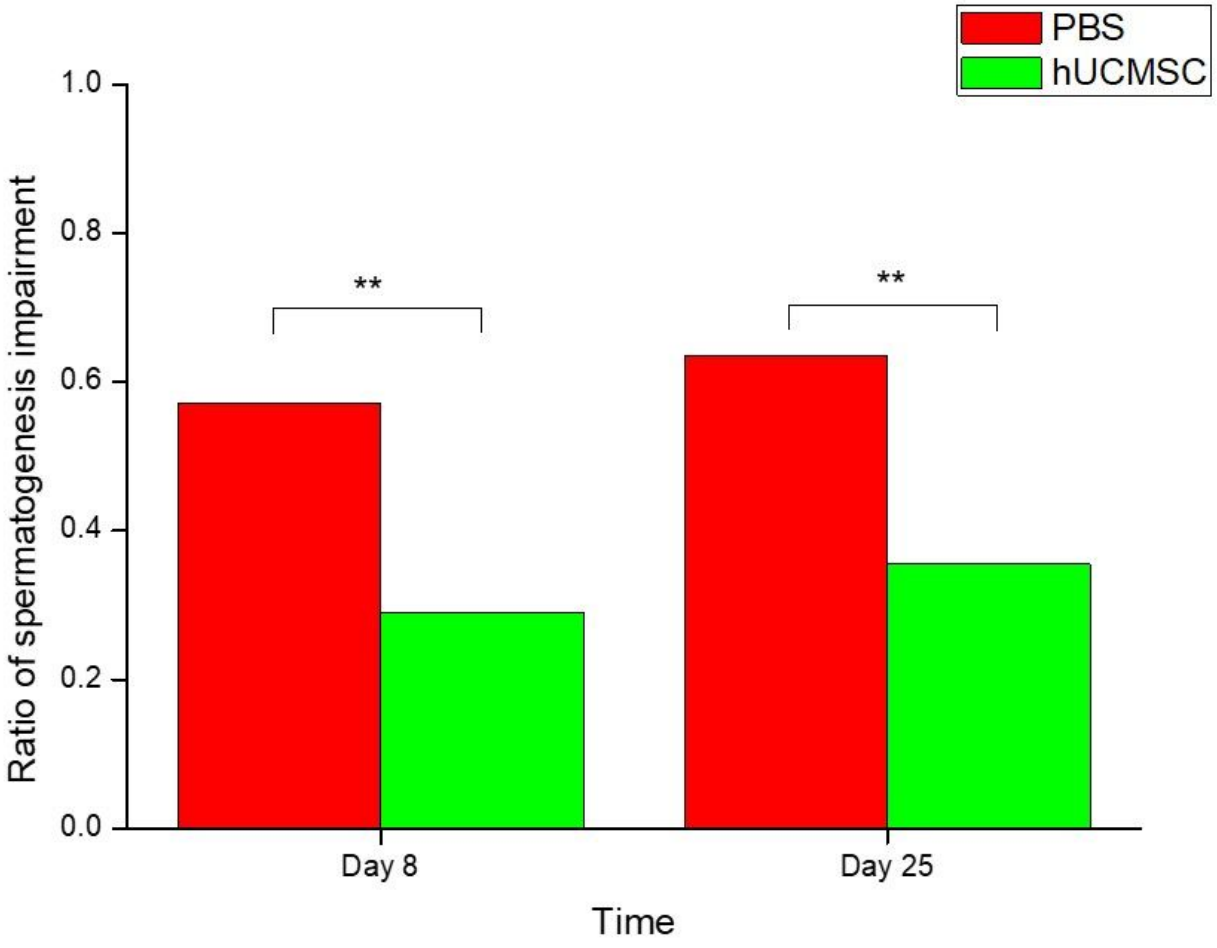

Figure 2

The comparison of spermatogenesis impairment ratio between the PBS groups and stem cells groups. Numbers of mice in the PBS1, PBS2, SC1, and SC2 groups on day 8 were 16, 12, 9, and 22, respectively. The mice numbers in the PBS1, PBS2, SC1, and SC2 groups on day 25 were 14, 8, 11, and 20, respectively. The ratio of spermatogenesis impairment in the stem cell groups was statistically lower than that in the PBS groups on days 8 and 25. The data was analyzed by Chi square test. **P<.05.

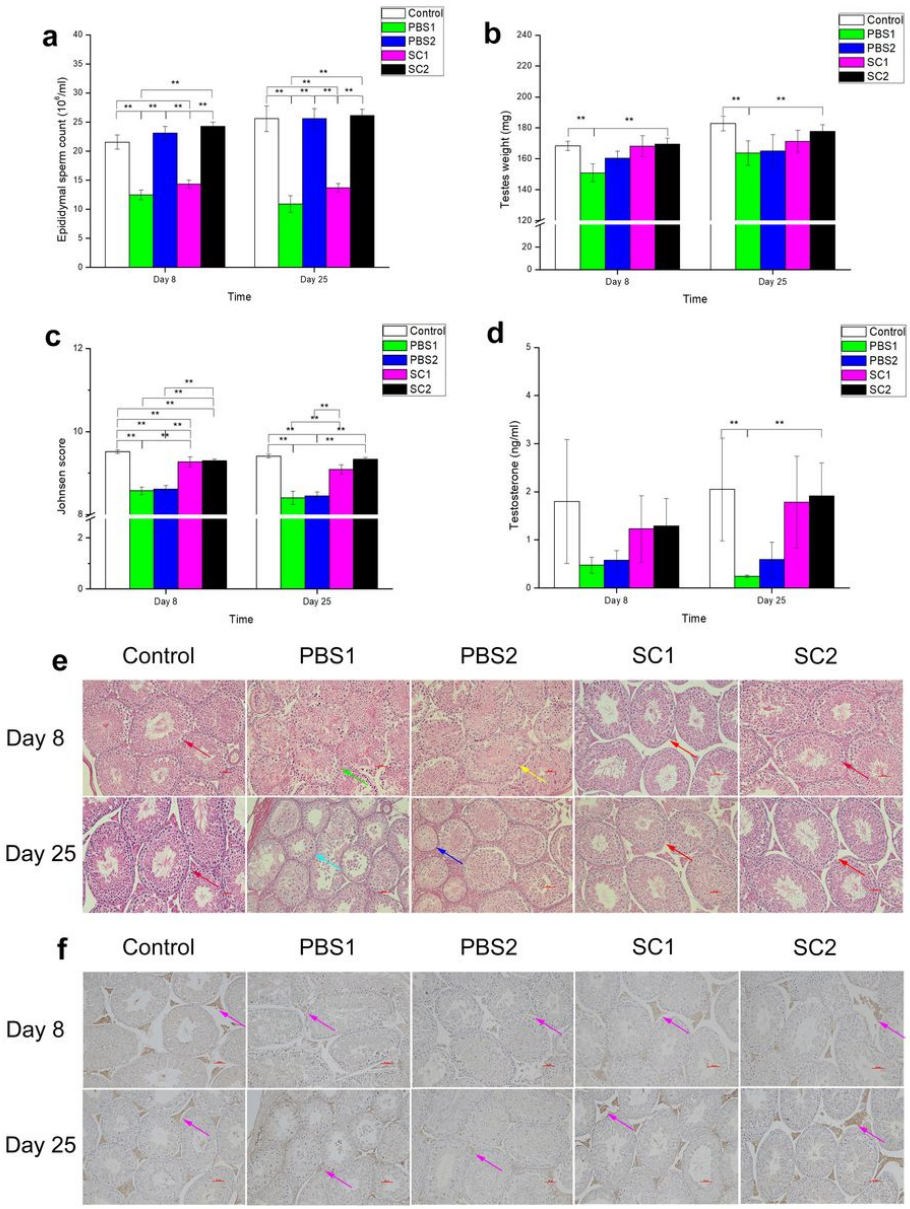

Figure 3

The comparison of testicular function related parameters between the control, PBS, and stem cells groups. (a-d) Epididymal sperm count, testes weight, Johnsen score, and serum testosterone level in each group. (e) Testicular histology in each group. The sections were stained with hematoxylin and eosin (original magnification × 200, scale bar is 50 μm). The red arrow means a Johnsen score of 10 indicates full spermatogenesis. The yellow arrow means a Johnsen score of 8 indicates few late spermatids. The green arrow means a Johnsen score of 7 indicates no late spermatids. The light blue arrow means a Johnsen score of 4 indicates few spermatocytes. The deep blue arrow means a Johnsen score of 3 indicates spermatogonia only. (f) Immunohistochemistry for the detection of 3β-hydroxysteroid dehydrogenase in Leydig cells of testes. The purple arrow means the location of Leydig cells of testes. The deep brown color means higher expression of 3β-hydroxysteroid dehydrogenase in Leydig cells and the light brown color means lower expression of 3β-hydroxysteroid dehydrogenase in Leydig cells. The results are shown as mean ± SEM. The statistical method was Mann-Whitney U test. **P<.05.

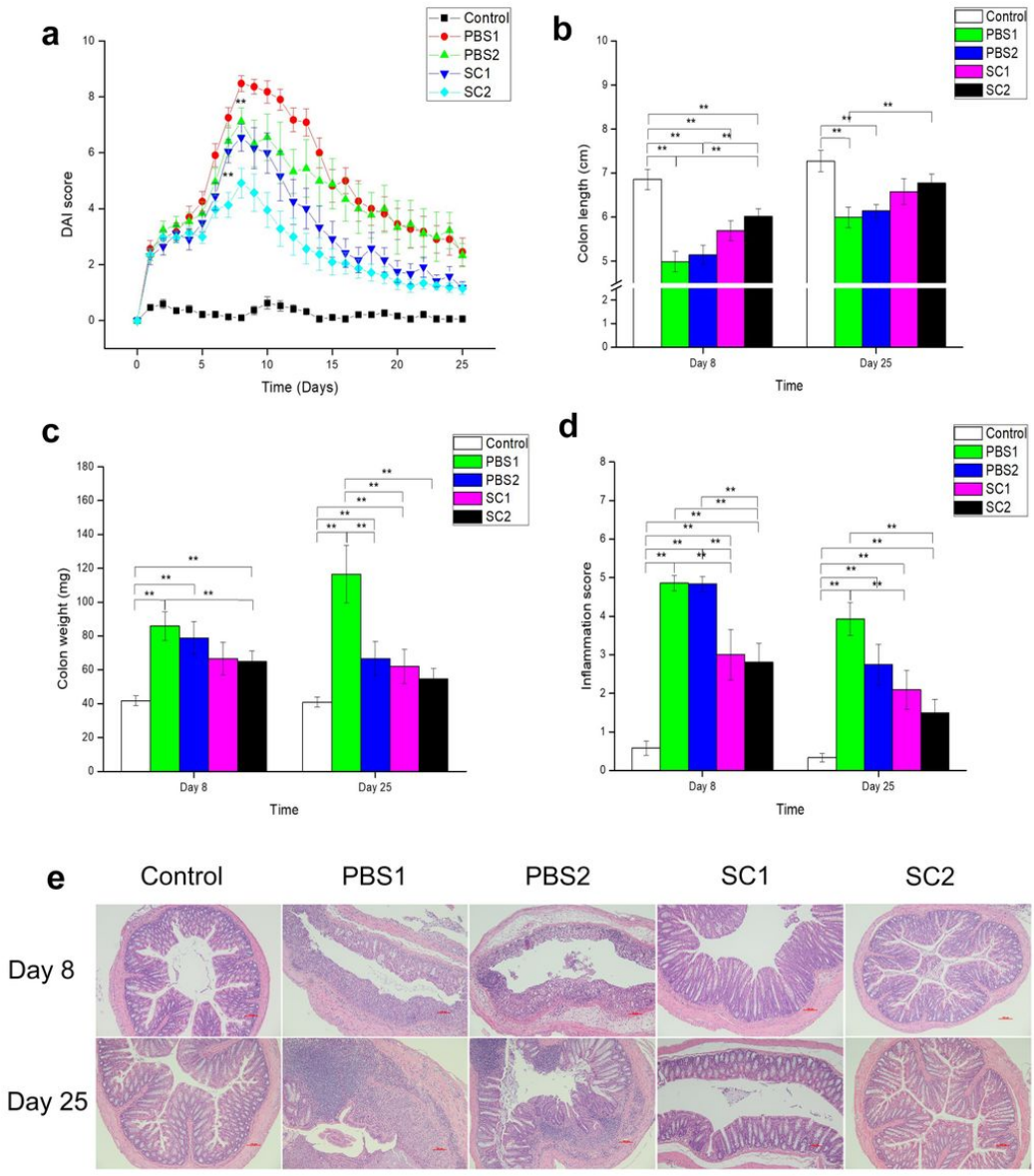

Figure 4

Comparison of clinical symptoms and severity of colitis between the control, PBS, and stem cells groups. (a) The DAI score includes scales for stool consistency, body weight loss, fur texture, and animal posture. The DAI (mean ± SEM) in the PBS2 group was statistically lower than that in the PBS1 group on day 8 (P=.007). The DAI (mean ± SEM) in the SC2 group was statistically lower than that in the SC1 group on day 7 (P=.028). The DAI of entire experimental course in the PBS1, PBS2, SC1, and SC2 groups was analyzed with the sign and binomial test. The DAI of entire course in the PBS2 group was significantly lower than that in the PBS1 group (P=.022) and the DAI of entire course in the SC2 group was significantly lower than that in the SC1 group (P<.001). (b-d) Colon length, colon weight, and inflammation score in each group. (e) Colon histology in each group. The sections were stained with hematoxylin and eosin (original magnification × 100, scale bar is 100 μm). The results are shown as mean ± SEM. The statistical method was Mann-Whitney U test. **P<.05.