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Safety and efficacy of allogeneic adipose tissue-derived mesenchymal stem cells for treatment of dogs with inflammatory bowel disease: Endoscopic and histological outcomes.

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Highlights

- Adipose-derived stem cells were tested as therapy for canine inflammatory bowel disease.
- Endoscopic and histological scales were used to assess macroscopic and microscopic changes.
- Endoscopic and histological scores decreased significantly after therapy.
- Endoscopic remission was achieved in 4/11 patients.
- Histological remission was not achieved in any patient

Abstract

Systemic administration of mesenchymal stem cells (MSCs) has been shown to be safe and efficacious in humans with Crohn's disease. The aim of this study was to evaluate the safety of an intravenous (IV) infusion of adipose tissue-derived mesenchymal stem cells (ASCs) and to assess macroscopic and histological effects in the digestive tract of dogs with inflammatory bowel disease (IBD). Eleven dogs with confirmed IBD received a single ASC infusion (2×10^6 cells/kg bodyweight). Full digestive endoscopic evaluation was performed pre-treatment and between 90 and 120 days post-treatment with mucosal changes being assessed using a fit-for-purpose endoscopic scale. Endoscopic biopsies from each digestive section were evaluated histologically according to the World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group criteria. The pre- and post-treatment canine IBD endoscopic index (CIBDEI) and histological score (HS) were calculated and compared using the Wilcoxon test. Remission was defined as a reduction of >75% of the CIBDEI and HS compared with pre-treatment.

No acute reactions to ASC infusion or side effects were reported in any dog.

Significant differences between pre- and post-treatment were found in both the CIBDEI (P = 0.004) and HS (P = 0.004). Endoscopic remission occurred in 4/11 dogs with the remaining dogs showing decreased CIBDEI (44.8% to 73.3%). Histological remission was not achieved in any dog, with an average reduction of the pre-treatment HS of 27.2%. In conclusion, a single IV infusion of allogeneic ASCs improved gastrointestinal lesions as assessed macroscopically and slightly reduced gastrointestinal inflammation as evaluated by histopathology in dogs with IBD.

Keywords: Canine; Inflammatory bowel disease; Gastrointestinal endoscopy; Endoscopic biopsy; Mesenchymal stem cell; Treatment.

Introduction

Idiopathic inflammatory bowel disease (IBD) in dogs can be a significant challenge for veterinarians. Some dogs are refractory to traditional treatments using cyclosporine or steroids, and this life-long medication is not always effective for maintaining remission of IBD and is associated with adverse side effects (Jergens and Simpson, 2011). Endoscopy and histological evaluation of endoscopic intestinal biopsies are necessary for the diagnosis of IBD, and are also useful to measure severity and evaluate the efficacy of treatment in clinical trials (Slovak et al., 2014).

The immunomodulatory, anti-inflammatory and reparative properties of mesenchymal stem cells (MSCs) make them a promising tool for treating immune-mediated and inflammatory disorders. Encouraging results obtained with experimental animal models of colitis have supported clinical trials in humans evaluating the systemic administration of autologous or allogeneic MSCs for the treatment of refractory luminal Crohn's disease (CD) with promising results in terms of efficacy (Swenson and Theise, 2010). The aim of this study was therefore to evaluate the safety of an intravenous (IV) infusion of adipose tissue-derived mesenchymal cells (ASCs) and to assess their macroscopic and histologic effects on the digestive tract of dogs with confirmed IBD. The clinical and laboratory outcomes of the study have been reported elsewhere (Pérez-Merino et al., 2015).

Materials and methods

Dogs

The trial was conducted at the Veterinary Teaching Hospital of the University of Extremadura (VTH-UEx). The protocol was approved by the VTH Clinical Ethics Committee and the UEx Animal Care and Use Committee (protocol 13/H07/03, 4 March 2013). All

clients gave written informed consent.

All dogs were diagnosed with idiopathic IBD according to previously published clinical criteria (Jergens et al., 2003). Furthermore, no dog showed evidence of extra-alimentary tract inflammation. Inclusion criteria included: (1) moderate to severe IBD, as defined by the Clinical Inflammatory Bowel Disease Activity Index (CIBDAI) (Jergens et al., 2003) and the Canine Chronic Enteropathy Clinical Activity Index (CCECAI) (Allenspach et al., 2007); (2) the absence of any immunomodulating drug therapy (e.g. corticosteroids, metronidazole or cyclosporine) within 21 days prior to referral. For adults with confirmed IBD, the exclusion criteria were pregnancy, sepsis and extreme physical impairment.

Diagnostic criteria for IBD included: persistent (>3 weeks) gastrointestinal signs; failed responses to dietary (hydrolysate or commercial intact protein elimination diet) or symptomatic therapies (anthelmintics, antibiotics, anticholinergics, gastrointestinal protectants) alone; and histopathological evidence of intestinal inflammation. The minimum diagnostic evaluation for all dogs included a complete blood count (CBC), a serum biochemistry profile, urinalysis, a direct (wet mount) and indirect (flotation) examination of faeces for endoparasites, abdominal radiographs and ultrasound.

Endoscopic examination

Gastroduodenoscopy and ileocolonoscopy were performed after a 36-48 h fasting period, and no liquid was allowed 6 h before the examination. For colonoscopy preparation the dogs received bisacodyl orally (5-20 mg every night from 72 h prior to the colonoscopy) and two enemas were performed 12 h and 4 h before examination.

The examination was conducted under inhalation anaesthesia with the dog positioned on the left side. The endoscopic examination was carried out using a Fuji 2200 flexible videoendoscope with a working length of 1010 mm and diameter of 9.0 mm. Endoscopic mucosal changes were assessed using a canine IBD endoscopic index (CIBDEI) developed for this purpose. Dogs were assigned an endoscopy score for stomach (S-CIBDEI), duodenum (D-CIBDEI), ileum (I-CIBDEI) and colon (C-CIBDEI) by the authors performing the endoscopies (JMU-C and EMP-M). For each dog the global CIBDEI score (G-CIBDEI) was obtained by adding together the independent organ CIBDEIs (Table 1). Finally, if lymphangiectasia (white spot presence) was detected during the duodenoscopy, it was scored separately from 0-3 according to published criteria (Larson et al., 2012).

Sample collection and histological examination

Flexible through-the-endoscope biopsy forceps with 2.5 mm smooth-edged oval cups were used to collect 10 mucous membrane specimens from the stomach, duodenum, ileum and colon from all dogs for histopathological evaluation. Biopsies were repeated post-treatment at the same place, moving deeper into the mucous membrane.

Biopsy samples were fixed in 10% neutral buffered formalin, embedded in paraffin blocks and cut perpendicular to the mucosa. The 6 µm-thick sections were stained routinely with haematoxylin and eosin (HE). Slides were examined by a European College of Veterinary Pathologists Board-certified pathologist (MV). Histopathological evaluation of all biopsies was performed according to the criteria proposed by the World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group for diagnosing gastrointestinal inflammation in dogs and cats (Day et al., 2008).

The numerical addition of the grades of histopathological change (where normal = 0, mild = 1, moderate = 2 and marked = 3) for each histological parameter (eight for the stomach and colon and nine for the duodenum) provided a histological score (HS) for the stomach (S-HS), duodenum (D-HS) and colon (C-HS). Because specific templates for the interpretation of ileal biopsies are not provided by the guidelines of the WSAVA Gastrointestinal Standardization Group, the ileal histological scores (I-HS) were obtained by following the guidelines provided for duodenal biopsies. For each dog, a global histopathological score (G-HS) was calculated by adding partial histological scores (Table 2).

Treatment protocol

ASCs were produced in Centauri Biotech laboratories under internal standard operating procedures. The adipose tissue was obtained from a single donor, meeting strict criteria of negative testing for prevalent infectious disease markers as previously described (Pérez-Merino et al., 2015). The MSC phenotype of the adherent cells was verified in accordance with the International Society for Cellular Therapy (Dominici et al., 2006). The cells were cryopreserved and underwent a last culture passage just before release for treatment.

Following histological confirmation of IBD, dogs received a single dose of ASCs. Thawed cells were infused (volume, 250 mL) over 15 to 20 min through a peripheral IV cannula placed in the cephalic vein, at a target dose of 2 x 10^6 cells/kg bodyweight. The dogs were monitored during infusion and for 60 min prior to being discharged.

Outcome measures

Dogs were re-evaluated by endoscopy at the VTH-UEx between 90 and 120 days after

ASC infusion, and histopathologic analysis of mucosal samples was repeated to obtain post-treatment global and partial CIBDEIs and HSs.

Remission was defined as a ≥ 75% reduction in post-treatment G-CIBDEI and G-HS compared to the corresponding pre-treatment values. A partial response was considered if the post-treatment reduction was <75% but >25% of the pre-treatment value.

Statistics

Since values were not normally distributed, non-parametric Wilcoxon rank sum tests were used to assess differences between pre- and post-treatment. Variables are provided as the median and interquartile range 25%–75% (P_{50} [P_{25}-P_{75}]). Statistical significance was set at $P < 0.05$. Statistical analyses were performed using the commercially available R-3.0.1 software system for Windows.

Results

Baseline characteristics at inclusion

Eleven dogs were enrolled in this study. Breeds included American Staffordshire terrier, French Bulldog, English Beagle, Boxer, Yorkshire terrier, mixed breed, Staffordshire Bull terrier, Bichon Frise, and West Highland White terrier. There were six males (including one neutered), and five intact females. The median age was 45.6 months (10 dogs were aged between 1 and 4 years, and one was 12 years old).

The animals had shown clinical signs between 5 months and 1 year before presentation. The initial presenting complaints predominantly involved the small intestine in six dogs, with small bowel diarrhoea, accompanied by vomiting in five of them. One dog

showed clear signs of colitis with haematochezia and mucoid faeces. In the remaining four dogs, the disease affected both the small and large intestines resulting in mixed gastrointestinal signs. All dogs suffered from loss of appetite, weight loss (particularly severe in six dogs) and deterioration of coat quality. Ascites was recorded in 2/11 dogs.

Endoscopic findings

Upper and lower endoscopies and endoscopic biopsies were performed in all dogs prior to treatment. The most important pre-treatment findings in the stomach included mild mucosal oedema with mucosal thickening (9/11) and increased granularity (5/11). One animal presented with very severe ulcerative gastritis. The most common macroscopic duodenal alterations included moderate increased granularity and friable mucosa (11/11), mucosal erythema (6/11) and narrowed lumen (4/11). Three dogs showed lymphangiectasia (two dogs to a moderate degree, scoring 2 points on the Larson endoscopic scale, and one dog to a severe degree, scoring 3 points), which was previously diagnosed by ultrasounds and confirmed by endoscopy (Fig. 1A). Although the surface of the mucous membrane of the colon was clearly plicated and reddened in most animals (10/11), mild injuries were predominant. Only one moderate form of colonic alteration (Fig. 2A), one severe form with a few colonic ulcers, and one very severe form of ulcerative colitis were observed. From an endoscopic perspective, the ileum was moderately affected in only three dogs, coinciding with dogs affected by duodenal lymphangiectasia.

Disease severity based on G-CIBDEI scoring showed that the group included one mild, seven moderate and three severe IBD dogs. Partial pre-treatment CIBDEIs showed that the duodenum was the area most severely and commonly affected. The colon obtained the second-highest score, though this was mostly due to the high scores of the ulcerative forms.

The stomach and ileum obtained the lowest scores, corresponding to mild forms (Table 3).

A second round of upper and lower endoscopies was performed after an average of 108 days (range: 90-125 days) in eight animals, 140 days in two animals and 150 days in one animal, following ASC infusion. Statistical analysis of the CIBDEIs identified significant differences between the pre- and post-treatment macroscopic endoscopic lesions (Figs. 1B and 2B), both in global (P = 0.004) and partial (P = 0.003) indices. Post-treatment, the group included ten mild forms and one moderate form (corresponding to histiocytic colitis) according to the G-CIBDEI. The decrease in partial CIBDEIs was greater for the stomach and ileum than for the colon and duodenum (Table 3).

Endoscopic remission occurred in 4/11 dogs. The seven remaining dogs showed a partial response with G-CIBDEI reduction of between 44.8% and 73.3%.

Histological findings

Pre-treatment, histological evaluation showed predominant lymphocytic-plasmacytic infiltrates in all cases, and severe histiocytic colitis in one dog. All dogs had histopathological lesions of IBD in both the small and large intestines. Based on G-HS, eight dogs had mild and three dogs had moderate intestinal inflammation. Partial pre-treatment scores were higher and more uniform for the duodenum and colon than for the stomach and ileum.

Treatment significantly decreased the gastric (P = 0.004), duodenal (P = 0.003), ileal (P = 0.007), colonic (P = 0.003) and global (P = 0.004) HSs (Table 4). Post-treatment, the group included 10 mild and one moderate (the histiocytic colitis, which remained moderate) forms according to the G-HS. The magnitude of the decrease in partial scores was similar in

all cases. Histological remission was not achieved in any dog. A partial response was seen in nine dogs with an average percentage reduction in the G-HS of 32.6% (range, 26%-38%). The two non-responsive dogs had reduced G-HS of by 22% and 23%, respectively.

Discussion

A robust rationale based on experimental studies and human clinical trials was the basis for evaluating MSCs for the treatment of dogs with IBD. This study represents the first clinical trial evaluating the safety and efficacy of MSCs in dogs with IBD.

The utility of endoscopy for diagnosing and assessing IBD evolution is clear in human and veterinary patients. However, limited clinical trials in dogs with IBD have used endoscopy, and those endoscopic evaluations were limited to the upper digestive tract (Garcia-Sancho et al., 2007) or resulted in a global assessment of the endoscopic appearance of the digestive tract (Allenspach et al., 2007). A validated endoscopic activity score for canine IBD has recently been reported (Slovak et al., 2015). This useful tool was however published after the completion of the current study, so we based our endoscopic index on pre-existing descriptions from other authors (Garcia-Sancho et al., 2007), on the standardised report forms for lower gastrointestinal endoscopy described by the WSAVA Gastrointestinal Standardization Group, and on our own experience. Nevertheless, the usefulness of some parameters that we scored (such as hyperaemia) is quite questionable (Slovak et al., 2014) and the use of similar parameters (e.g. mucosal irregularities and mucosal thickening) can result in a reiterative assessment and a repetitive scoring of the same lesion.

In the current study, the duodenum was more and the stomach less severely affected than the samples from the study of García-Sancho et al. (2007). Both studies showed a

significant decrease in the severity of lesions, but the study of García-Sancho et al. (2007) reported 25% of dogs with no endoscopic improvement whereas we observed a 100% response. Our study also showed higher improvement in less affected parts, such as the stomach and ileum, and more persistence of endoscopic damage in the most severely affected sections.

Allenspach et al. (2007) assigned a global endoscopy score from 0 to 3 (based on the degree to which the duodenum and colon were affected) to dogs with chronic enteropathies, and found no difference in the endoscopy score before and after treatment with diet and/or prednisolone and/or cyclosporine. It is possible that using a more detailed scale that scores each lesion and digestive portion separately may increase the detection of differences pre- and post-treatment. Endoscopic scoring is also limited by the lack of well-defined lesions (both qualitatively and quantitatively) and the lack of uniformity between observers. Slovak et al. (2014) found lower inter-observer variation in identifying endoscopic abnormalities between experienced endoscopists compared to less experienced ones. In our study, inter-observer agreement was optimal, since both endoscopists had the same training and experience and have performed endoscopic procedures at the same hospital for 20 years.

In two phase I and II studies in humans affected by CD, endoscopic improvement occurred in 2/7 patients (Duijvestein et al., 2010) and in 7/15 patients (Forbes et al., 2014), respectively, at day 42 after IV infusions of MSCs. Our endoscopic scores showed a response in all dogs, but unlike the human scale, which focused on the most affected gastrointestinal portion (such as the colon), we also scored the less affected areas such as the stomach and ileum, which improved following treatment.

The significant histological improvement in our dogs contrasts with the results of other researchers who were unable to find an improvement in the histopathology of intestinal samples post-treatment using traditional therapies based on a standard combination of prednisone (Garcia-Sancho et al., 2007; Willard and Mansell, 2011), budesonide (Dye et al., 2013) and cyclosporine (Allenspach et al., 2007). However, our results are consistent with one of the more recent studies, which showed that WSAVA histology scores were significantly reduced in dogs with IBD at day 90 after receiving either a probiotic strain (VSL3) or a combination therapy of prednisone and metronidazole (Rossi et al., 2014).

Although most authors employ the histopathological guidelines described by WSAVA to assess IBD histological changes, few calculate the histological score as the sum of the eight or nine histological parameter scores described for each digestive portion, as we did. Procoli et al. (2013), who used our approach, found a median duodenal and ileal WSAVA score of 4 in dogs with chronic small intestinal enteropathies. Our results were similar for the ileal scores, but indicated more severely affected duodena. Jergens and Simpson (2011) suggested that simply adding together unweighted numerical severity scores does not reflect the real severity of the disease. However, at present, this is the most widely accepted tool for evaluating treatment efficacy histologically.

We attempted to minimise the limitations of our evaluation by using high quality biopsy specimens that were always analysed by the same histopathologist. With regards to the single case of histiocytic colitis, its global scores decreased mainly due to the improvement in the associated small intestinal inflammation, but no significant colonic improvement, either endoscopic or histological, was observed. Although stem cell therapy can contribute to mucosal healing, enrofloxacin therapy appear still mandatory in colitis cases (Craven et al.,

2011).

Administering ASCs has been shown to reduce inflammation (Stappenbeck and Miyoshi, 2009; English and Mahon, 2011; Singer and Caplan, 2011). Our histological results agree with the results of various studies using MSCs to treat experimentally induced enterocolitis. Systemic administration of MSCs significantly improved histological colitis scores, restored epithelial barrier integrity, reduced crypt damage and the extent of inflamed areas, and decreased infiltration of inflammatory cells (Tanaka et al., 2008; Zhang et al., 2009). Treatment may also result in faster recovery of small intestinal structure and function (Semont et al., 2010).

MSCs are known for their active proliferation, plastic differentiation, strong immunomodulation, low immunogenicity and abundant trophic factor production (Nagaishi et al., 2015). Surprisingly, their therapeutic effects do not depend on their full engraftment, but rely on their capacity to inhibit pathogenic immune responses and to release trophic factors favouring tissue repair (Hackett, 2013). Previous studies have found that the conditioned medium of MSCs, including pleiotropic gut trophic factors, promoted intestinal epithelial repair (Watanabe et al., 2014), and MSC therapy increases and accelerates the recovery of the small intestine with reversible alterations by accelerating structural restoration and re-epithelisation (Semont et al., 2010). This combination of anti-inflammatory and regenerative effects could explain the benefits of MSC therapy compared to purely anti-inflammatory therapies.

One limitation of this study is the use of non-validated scales to quantify histological and endoscopic damage, which makes the numerical and statistical results only indicative of

the possibilities of the treatment. Moreover, the small number of dogs involved limits the statistical analysis. Discrepancy between the degree of endoscopic and histological improvement, and the short follow-up period are two additional limitations. Many other significant issues remain for the appropriate design and interpretation of studies with MSCs, including patient selection, disease stage, disease activity, MSC source, dosage, and long-term safety and efficacy. However, the outcomes of this study offer a promising perspective for testing ASC treatment in dogs with IBD.

Conclusions

A single infusion of allogeneic ASCs was safe in dogs with confirmed IBD, improved gastrointestinal lesions as assessed by endoscopic evaluation, and reduced microscopic gastrointestinal inflammation.

Conflict of interest statement

Drs. Mariñas-Pardo and Hermida-Prieto are employees of Centauri Biothech. This company supplied the ASCs used in this study, but played no role in the study design, collection, analysis and interpretation of data, or in the content of the manuscript or submission for publication. The other authors have not received any consultancy fees, and no other funding has been received from Centauri Biotech. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Figure legends

Fig. 1. Pre- and post-treatment endoscopic images of duodenum. (A) Duodenum with severe granularity and severe lymphangiectasia pre-treatment; (B) Duodenum at 105 days after adipose tissue-derived stem cell (ASC) infusion, with rare pinpoint white foci.

Fig. 2. Pre- and post-treatment endoscopic images of colon. (A) Moderate colitis with erosions, granularity and friability. (B) Normal colon at 140 days after adipose tissue-derived stem cell (ASC) infusion.

Table 1

Canine IBD endoscopic activity index (CIBDEI) developed for the quantitative assessment of macroscopic changes.

Organ/Index	Item	Scoring	Severity degree
Stomach/S-CIBDEI	Hyperaemia/vascularity Mucosal thickening	0 = none 1 = mild 2 = moderate 3 = severe	Normal 0 Mild 1-3 Moderate 4-6 Severe 7-9 Very severe 10-11
	Erosion /ulcer	0 = none 1 = <2 2= 3-5 3= >6	
	Bile presence	0 = absence 1 = presence	
	Gastric folds	0 = normal 1 = increased	
Duodenum/D-CIBDEI	Lumen	0 = normal 1 = narrowed	Normal 0 Mild 1-4 Moderate 5-8 Severe 9-12 Very severe 13-16
	Hyperaemia/vascularity Mucosal irregularities Mucosal thickening	0 = none 1 = mild 2 = moderate 3 = severe	
	Friability	0 = None 1 = Contact bleeding 2 = Spontaneous bleeding 3 = Massive bleeding	
	Erosion/ulcer	0 = none 1 = <2 2= 3-5 3= >6	
Ileum/I-CIBDEI	Hyperaemia/vascularity Texture of mucosa	0 = none 1 = mild 2 = moderate 3 = severe	Normal 0 Mild 1-3 Moderate 4-6 Severe 7-9 Very severe 10-12
	Friability/haemorrhage	0 = None 1 = Contact bleeding 2 = Spontaneous bleeding 3 = Massive bleeding	
	Erosion/ulcer	0 = none 1 = <2 2= 3-5 3= >6	
Colon/C-CIBDEI	Hyperaemia/vascularity Mucosal oedema	0 = none 1 = mild 2 = moderate 3 = severe	Normal 0 Mild 1-3 Moderate 4-6 Severe 7-9 Very severe 10-12
	Friability/haemorrhage	0 = None 1 = Contact bleeding 2 = Spontaneous bleeding 3 = Massive bleeding	
	Erosion /ulcer	0 = none 1 = <2 2= 3-5 3= >6	
Global/G-CIBDEI	Sum of S-CIBDEI, D-CIBDEI, I-CIBDEI and C-CIBDEI		Normal 0 Mild 1-12 Moderate 13-25 Severe 26-38 Very severe 39-51

500 **Table 2**

501 Criteria used for the histopathological grading of intestinal biopsies and obtaining histological

502 scores (HSs).

Organ/HS	Morphological features	Scoring	Severity degree
Stomach/S-HS	Surface epithelial injury Gastric pit epithelial injury Fibrosis/glandular nesting/mucosal atrophy Gastric lymphofollicular hyperplasia Intraepithelial lymphocytes Lamina propria lymphocytes and plasma cells Lamina propria eosinophils Lamina propria neutrophils	0 = none 1 = mild 2 = moderate 3 = severe	Normal 0 Mild 1-3 Moderate 4-6 Severe 7-9 Very severe 10-11
Duodenum/D-HS	Villous stunting Epithelial injury Crypt distension Lacteal dilatation Mucosal fibrosis Intraepithelial lymphocytes Lamina propria lymphocytes and plasma cells Lamina propria eosinophils Lamina propria neutrophils	0 = none 1 = mild 2 = moderate 3 = severe	Normal 0 Mild 1-6 Moderate 7-13 Severe 14-20 Very severe > 20
Ileum/I-HS	Villous stunting Epithelial injury Crypt distension Lacteal dilatation Mucosal fibrosis Intraepithelial lymphocytes Lamina propria lymphocytes and plasma cells Lamina propria eosinophils Lamina propria neutrophils	0 = none 1 = mild 2 = moderate 3 = severe	Normal 0 Mild 1-6 Moderate 7-13 Severe 14-20 Very severe >20
Colon/C-HS	Surface epithelial injury Crypt hyperplasia Crypt dilatation/distortion Fibrosis/atrophy Lamina propria lymphocytes and plasma cells Lamina propria eosinophils Lamina propria neutrophils Lamina propria macrophages	0 = none 1 = mild 2 = moderate 3 = severe	Normal 0 Mild 1-6 Moderate 7-12 Severe 13-18 Very severe > 19
Global/G-HS	Sum of S-HS, D-HS, I-HS and C-HS		Normal 0 Mild 1-25 Moderate 26-51 Severe 52-77 Very severe 78-102

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Table 3

Comparison of canine IBD endoscopic activity index (CIBDEI) for stomach (S-CIBDEI), duodenum (D-CIBDEI), ileum (I-CIBDEI), colon (C-CIBDEI) and global (G-CIBDEI) before and after infusion of adipose tissue-derived stem cells (ASCs).

Index	Pre-treatment	Post-treatment	*P*
S-CIBDEI	2.00 [2.00-3.50]	0.00 (0.00-0.50)	0.003
D-CIBDEI	9.00 [8.00-10.00]	3.00 (2.00-5.00)	0.003
I-CIBDEI	4.00 [2.50-6.00]	1.00 (0.00-1.50)	0.003
C-CIBDEI	6.00 [4.50-7.00]	2.00 (1.00-2.50)	0.003
G-CIBDAI	22.00 [19.00-25.50]	6.00 (4.50-10.0)	0.004

Variables are expressed as the median and interquartile range 25–75% (P_{50} [P_{25}-P_{75}]).

P values were calculated using Wilcoxon test.

Table 4

Comparison of histological scores (HS) for stomach (S-HS), duodenum (D-HS), ileum (I-HS), colon (C-HS) and global (G-HS) before and after infusion of adipose tissue-derived stem cells (ASCs).

Index	Pre-treatment	Post-treatment	*P*
S-HS	3.00 (2.50-3.50)	2.00 (1.50-2.00)	0.004
D-HS	7.00 (7.00-8.50)	5.00 (4.00-6.00)	0.003
I-HS	4.00 (3.00-5.00)	3.00 (2.00-3.50)	0.007
C-HS	8.00 (6.00-9.50)	5.00 (5.00-7.00)	0.003
G-HS	22.00 (21.00-25.50)	16.00 (13.50-18.00)	0.004

Variables are expressed as the median and interquartile range 25–75% (P_{50} [P_{25}-P_{75}]).

P values were calculated using Wilcoxon test.

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