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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

E.M. Pérez-Merino [a,*,1], J.M. Usón-Casaús [a,1], J. Duque-Carrasco [b], C. Zaragoza-Bayle [a], L. Mariñas-Pardo [c], M. Hermida-Prieto [c], M. Vilafranca-Compte [d], R. Barrera-Chacón [a], M. Gualtieri [e]

[a] Department of Animal Medicine and Surgery, Veterinary Faculty, University of Extremadura, 10003 Cáceres, Spain
[b] Veterinary Teaching Hospital, Veterinary Faculty, University of Extremadura, 10003 Cáceres, Spain
[c] Centauri Biotech Company, 15142 Arteixo, A Coruña, Spain
[d] Histovet. Avda. Països Catalans 12 D 08192 Sant Quirze del Vallès, Barcelona, Spain
[e] Department of Health Animal Science and Food Safety, Section of Surgery, Veterinary Faculty, University of Milano, Via Celoria, 10. 20133 Milan, Italy

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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.

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 lifelong 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).

* Corresponding author. Tel.: +34 927 257165.
E-mail address: evama@unex.es (E.M. Pérez-Merino).
[1] These authors contributed equally to this work.

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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 to 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. Each biopsy was repeated at the same place, moving deeper into the mucous membrane. This biopsy technique was repeated post treatment.

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 2×10^6 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