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

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ARTICLE INFO

Article history:
Accepted 4 August 2015

Keywords:
Canine
Inflammatory bowel disease
Clinical activity index
Albumin
Mesenchymal stem cell
Treatment

ABSTRACT

Mesenchymal stem cells (MSCs) have shown immunomodulatory and anti-inflammatory effects in experimental colitis, and promising clinical results have been obtained in humans with Crohn's disease and ulcerative colitis. The aim of this study was to determine the safety and feasibility of adipose tissue-derived MSC (ASC) therapy in dogs with inflammatory bowel disease (IBD). Eleven dogs with confirmed IBD received one ASC intravascular (IV) infusion (2 × 10^6 cells/kg bodyweight). The outcome measures were clinical response based on percentage reduction of the validated Clinical Inflammatory Bowel Disease Activity Index (CIBDAI) and Canine Chronic Enteropathy Clinical Activity Index (CCECAI), as well as normalisation of C-reactive protein (CRP), albumin, folate and cobalamin serum concentrations at day 42 post-treatment. The Wilcoxon test was used to compare variables before and after treatment.

No acute reaction to ASC infusion and no side effects were reported during follow-up in any dog. Six weeks post-treatment, the CIBDAI and CCECAI decreased significantly and albumin, cobalamin and folate concentrations increased substantially. Differences in CRP concentrations pre- and post-treatment were not significant (P = 0.050). Clinical remission (defined by a reduction of initial CIBDAI and CCECAI > 75%) occurred in 9/11 dogs at day 42. The two remaining dogs showed a partial response with reduction percentages of 69.2% and 71.4%. In conclusion, a single IV infusion of allogeneic ASCs was well tolerated and appeared to produce clinical benefits in dogs with severe IBD.

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Introduction

Idiopathic inflammatory bowel disease (IBD) is a common cause of chronic gastrointestinal disease in dogs. The pathogenesis of IBD involves multiple complex relationships including genetic predisposition, enteric bacteria, environmental factors and immunological abnormalities characterised by an inappropriate autoimmune reaction in the digestive tract (Jergens and Simpson, 2011; Simpson and Jergens, 2011). Current treatment protocols most often involve using immunosuppressive doses of corticosteroids (Dye et al., 2013) or cyclosporine to reduce intestinal mucosal inflammation and achieve clinical remission. However, a number of dogs treated these protocols have either no clinical response or relapse after weeks to months of treatment (Allenspach et al., 2006b). In addition, these drugs often induce adverse effects (Stroup et al., 2006).

While established IBD therapy has focused on inflammation control and immunosuppression, an optimal IBD therapy should also enhance epithelial proliferation and coordinate remodelling during the healing process. The application of mesenchymal stem cells (MSCs) as an alternative treatment for IBD to achieve these aims is a recent concept (Nagaishi et al., 2015). Successful preclinical studies using MSCs in animal models of colitis (Hayashi et al., 2008; Tanaka et al., 2008; Zhang et al., 2008, 2009) have paved the way for clinical trials. Since then, intravenous (IV). MSCs have demonstrated efficacy and safety in clinical trials with humans affected by a chronic and relapsing inflammatory, immune-related gastrointestinal disease also known as Crohn's disease (CD) (Duijvestein et al., 2010; Forbes et al., 2014). In veterinary medicine, a recent study of the use of IV MSC therapy in spontaneous feline enteropathy showed safety and a positive clinical response (Webb and Webb, 2014).
Based on these reports, our hypothesis was that allogeneic MSCs would also be beneficial in treating canine IBD. This study was designed to establish the safety and efficacy of an IV infusion of allogeneic adipose tissue-derived stem cells (ASCs) using several relevant clinical and laboratory end points. The endoscopy and histologic outcomes of the study were reported elsewhere (Perez-Merino et al., 2015).

Materials and methods

Dog selection

Owners of dogs with IBD presented at the Veterinary Teaching Hospital at the University of Extremadura (VTH-UEx) were offered study entry in compliance with the VTH Clinical Ethics Committee and the UEx Animal Care and Use Committee (protocol 13/070/03, 4 March 2013). All owners gave written informed consent.

Dogs had to meet previously published criteria for diagnosis of idiopathic IBD: persistent (>3 weeks duration) or recurrent gastrointestinal signs and an inadequate response to dietary and symptomatic therapies alone (Jergens et al., 2003). Dogs had received standard treatment (elimination diet, corticosteroids, antibiotics, and antidiarrhoeal and antiparasitic drugs) by the referring veterinarian which did not result in a full response to treatment or which resulted in recurrences. There was at least a 3-week washout period before entering the study. The dogs were re-evaluated at the VTH-UEx and the IBD diagnosis was confirmed, excluding other causes of chronic diarrhea on the basis of routine diagnostic tests. The minimum diagnostic evaluation for all dogs before admission to the study included a complete blood count (CBC), a serum biochemistry profile, a urinalysis, a faecal direct smear and zinc sulfate flotation for parasites, abdominal radiographs and ultrasound, as well as a histopathological review of mucosal biopsy specimens obtained via both gastroduodenoscopy and ileocolonoscopy (Pérez-Merino et al., 2015).

All dogs were given a clinical score using the Clinical Inflammatory Bowel Disease Activity Index (CIBDAI) scoring system established by Jergens et al. (2003), which is based on six gastrointestinal variables (scoring 0–3) that are routinely evaluated in affected dogs: attitude and activity, appetite, vomiting, stool consistency, stool frequency, and weight loss. A score of 0–3 indicated clinically insignificant disease, a score of 4–5 indicated mild IBD, a score of 6–8 indicated moderate IBD, and a score ≥9 indicated severe IBD. A second clinical scoring index, the Canine Chronic Enteropathy Clinical Activity Index (CCECAI) described by Allenspach et al. (2007) was also calculated. This index considers the six clinical signs included in the CIBDAI as well as serum albumin concentration and the presence of ascites, peripheral oedema and pruritus. Similar to the CIBDAI, four categories of severity were defined for the CCECAI: insignificant disease, 0–3; mild disease, 4–5; moderate disease, 6–8; severe disease, 9–11; very severe disease, ≥12. Criteria for patient inclusion were adult dogs (>1 year-old) that had histopathologically confirmed IBD, and a baseline CIBDAI score of at least 4. For adult dogs with confirmed IBD the exclusion criteria were pregnancy, sepsis and extreme physical impairment.

Baseline studies

Dogs with IBD were assigned a pre-treatment CIBDAI and CCECAI scores. Prior to treatment, a blood sample was collected for measurement of serum C-reactive protein (CRP), cobalamin (hypocobalaminaemia <200 ng/mL), folate (abnormal <7.7 μg/L) and albumin (hypoalbuminaemia <25 g/L).

Adipose tissue extraction and MSC culture

A male Bernese mountain dog, 2.5 years old and weighing 50 kg, was the donor of the adipose tissue. About 12 g of adipose tissue was aseptically collected from the abdominal region by surgical excision. MSCs were obtained as previously described (Yanez et al., 2006). Briefly, adipose tissue was digested with collagenase IV (Serva Electrophoresis) at 0.2 U/fat ml. over 1 h at 37 °C. The resultant vascular stromal fraction was cultured in Dulbecco’s Modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin-Glutamine (Biowest).

After 24 h, cultures were washed with phosphate buffer saline (PBS) to remove non-adherent cells and fresh medium was added. Cells were trypanosed when confluence was over 75% and subcultured until passage 3 was reached. Cultures were then trypanosed and frozen in FBS with 10% dimethyl sulfoxide (DMSO) (Sigma Aldrich). Several days before treatment, cells were defrosted and cultured until they were over 75% confluence. Cell cultures were then trypanosed and MSCs sent to the clinic. Viability was greater than 70% when arriving at the veterinary hospital 24 h after shipping (internal company tests). Random vials from passage 3 were used to test the MSC phenotype (Dominici et al., 2006). Photomicrographs of cultured ASCs and data from the flow cytometry analysis are shown in Fig. 1.

Dogs had received standard treatment (elimination diet, corticosteroids, antibiotics, and antidiarrhoeal and antiparasitic drugs) by the referring veterinarian which did not result in a full response to treatment or which resulted in recurrences. From diagnosis, dogs were followed up weekly for general physical condition and possible post-treatment adverse events. Six weeks after ASC therapy, clinical assessment was conducted at the VTH-UEx by calculation of the post-treatment CIBDAI and CCECAI scores, as well as CRP, folate, cobalamin and albumin post-treatment blood concentrations. Remission was defined as a ≥75% reduction of the post-treatment CIBDAI and CCECAI scores compared with their corresponding pretreatment values. Partial clinical remission was defined as a post-treatment reduction of the CIBDAI and CCECAI scores of <75% but ≥25% compared with their corresponding pre-treatment values.

Statistics

Since values were not normally distributed, clinical scores and albumin, cobalamin, folate and CRP concentrations before and after treatment in the same animal were compared by non-parametric Wilcoxon rank sum tests. Statistical analyses were performed using the R 3.0.1 software package for Windows. Statistical significance was set at P < 0.05.

Results

Study population

Eleven dogs with moderate to severe IBD were included in the study (Table 1). Histology at the time of the first endoscopy showed severe lymphocytic-plasmacytic infiltrates in 10 dogs and histiocytic ulcerative colitis in one dog (Pérez-Merino et al., 2015).

The group included five dogs with moderate and six dogs with severe IBD based on the CIBDAI, and five dogs with moderate IBD, one dog with severe IBD and five dogs with very severe disease based on the CCECAI. Low serum albumin levels were present in three dogs and ascites was noted in two of them. Pre-treatment, hypocobalaminaemia was observed in eight dogs and low levels of folate were observed in seven dogs. Low levels of cobalamin and folate were both present in six dogs, combined with hypoalbuminaemia in three of them. All dogs had normal values of CRP. Table 2 shows the pre-treatment values of the clinical and selected biochemical parameters.

Outcome evaluations

No acute reactions to ASC infusion were observed in any dog during infusion and no adverse reactions or side effects were reported during follow-up.

After 2 weeks of ASC therapy, a clinical response occurred in all dogs. Digestive symptoms disappeared (vomiting, diarrhoea, soft stools) and all animals increased activity and appetite and started gaining weight. At the end of the follow-up period, the median population weight was significantly increased (P = 0.003); the percentage of weight gain oscillated between 4% and 15% and was higher in the largest breeds. Ultrasound studies showed that the ascites that was present in two dogs gradually improved by the second and fourth weeks and had almost disappeared at day 42.

Six weeks post-treatment, the median CIBDAI and CCECAI were both decreased significantly. After treatment, the group comprised 10 animals with clinically insignificant disease and one dog with mild disease based on the CIBDAI and CCECAI. The median population serum albumin concentration was significantly increased (P = 0.004). The initially hypoalbuminaemic dogs had normal levels
after treatment, although the increase was not significant for this subgroup (P = 0.250). The median population serum cobalamin and folate concentrations increased significantly post-treatment (P = 0.001). This increase was significant for the subgroup of dogs with low pre-treatment values (P = 0.008 for cobalamin and P = 0.016 for folate), but not for the subgroup of dogs with normal pre-treatment values (P = 0.250 for cobalamin and P = 0.125 for folate).

CRP serum concentrations started and remained low and within the normal range, declining slightly in eight dogs and increasing in three dogs. Differences between the pre- and post-treatment values were not significant (P = 0.050).

Table 2 and Fig. 2 summarise the changes in bodyweight, clinical indices and laboratory parameters.

According to the clinical activity indices, clinical remission occurred in 9/11 dogs at day 42, with reductions of entry indices.
between 83.3% and 100%. The two remaining dogs showed a partial response with reduction percentages of 69.2% and 71.4%.

Discussion

Different animal species have been used as in vivo models for testing the efficacy of stem cells in treating various human pathologies. However, the application of this therapy in veterinary practice is very limited. Several reports have explored the local use of MSCs for the treatment of orthopaedic diseases in horses (Frisbie and Smith, 2010) and dogs (Black et al., 2007, 2008), and a recent study reported the safety and potential efficacy of the systemic administration of ASCs for cats suffering from chronic enteropathy (Webb and Webb, 2014).

Although MSCs have been shown to represent a promising immunomodulatory therapeutic strategy for human autoimmune diseases, and in spite of the experimental research and clinical trials suggesting the efficacy and safety of MSC treatment for ulcerative colitis or CD (Ben-Ami et al., 2011), no other studies have investigated the clinical utility of stem cells for the treatment of systemic immunological diseases in dogs.

In humans, IV administration of bone marrow-derived MSCs (either autologous or allogeneic) has been shown to be safe and feasible for the treatment of refractory CD, obtaining a clinical response based on the CD activity index (CDAI) in 3/11 patients (Duijvestein et al., 2010), and in 12/16 patients in a second more recent phase II clinical trial (Forbes et al., 2014). A similar phase II study also reported a significant reduction in CDAI in all 10 patients treated, and an apparent positive correlation between the dose of cells infused and the clinical response (Taupin, 2006). The results obtained in our study are similar to those conducted in humans and suggest the safety and efficacy of IV allogeneic MSCs for severe canine IBD. In laboratory animals, MSCs have also shown therapeutic effects in induced colitis, reducing bloody stools and weight loss and suppressing the overall disease activity in mice (Tanaka et al., 2008; Zhang et al., 2009). A recent study, in which IV ASCs were used to treat seven cats with spontaneous diarrhoea of not less than 3 months duration, reported a significant improvement or the

Table 2
Comparison of clinical activity indices (CIBDAI and CCECAI), weight, and selected laboratory variables before and after infusion of adipose tissue-derived stem cells (ASCs).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIBDAI</td>
<td>10.00 (6.50–12.00)</td>
<td>1.00 (0.00–1.00)</td>
<td>0.004</td>
</tr>
<tr>
<td>CCECAI</td>
<td>11.00 (7.00–12.50)</td>
<td>1.00 (0.00–1.00)</td>
<td>0.004</td>
</tr>
<tr>
<td>Weight</td>
<td>7.1 (5.7–14.9)</td>
<td>7.5 (6.2–171)</td>
<td>0.003</td>
</tr>
<tr>
<td>Albumin (g/L) (n = 3)</td>
<td>29.2 (25.5–35.2)</td>
<td>32.0 (29.8–37.3)</td>
<td>0.004</td>
</tr>
<tr>
<td>Albumin &gt;25 g/L (n = 8)</td>
<td>31.4 (18.3–22.7)</td>
<td>31.0 (30.6–31.5)</td>
<td>0.250</td>
</tr>
<tr>
<td>Cobalamin (ng/L) (n = 12)</td>
<td>192.00 (166.50–213.00)</td>
<td>276.60 (222.50–391.00)</td>
<td>0.001</td>
</tr>
<tr>
<td>Cobalamin &gt;200 ng/L (n = 6)</td>
<td>181.50 (157.50–192.75)</td>
<td>251.50 (209.50–287.70)</td>
<td>0.008</td>
</tr>
<tr>
<td>Cobalamin &gt;200 ng/L (n = 3)</td>
<td>288.20 (258.10–314.10)</td>
<td>448.00 (401.00–454.00)</td>
<td>0.250</td>
</tr>
<tr>
<td>Folate (ng/mL) (n = 3)</td>
<td>4.12 (3.35–10.75)</td>
<td>12.90 (10.60–20.10)</td>
<td>0.001</td>
</tr>
<tr>
<td>Folate &gt;77 ng/mL (n = 7)</td>
<td>3.60 (2.90–4.04)</td>
<td>12.20 (9.10–17.90)</td>
<td>0.016</td>
</tr>
<tr>
<td>Folate &gt;77 ng/mL (n = 4)</td>
<td>15.20 (11.77–19.10)</td>
<td>15.55 (12.65–19.60)</td>
<td>0.125</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>0.90 (0.55–1.20)</td>
<td>0.60 (0.25–0.80)</td>
<td>0.050</td>
</tr>
</tbody>
</table>

Variables are expressed as the median and interquartile range 25–75% [P25–P75]. P values were calculated using the Wilcoxon test.

CIBDAI, Clinical Inflammatory Bowel Disease Activity Index; CCECAI, Canine Chronic Enteropathy Clinical Activity Index.

Fig. 2. Effect of drug therapy on the Clinical Inflammatory Bowel Disease Activity Index (CIBDAI), Canine Chronic Enteropathy Clinical Activity Index (CCECAI), weight, albumin, cobalamin, folate and C-reactive protein (CRP) concentrations. Significant differences between baseline and 42 days after the therapy were observed for all parameters except for CRP concentrations.
complete resolution of clinical signs in 5/7 cats based on an owner’s questionnaire (Webb and Webb, 2014).

The reduction in the CIBDAI and CCECAI scores could (in most cases) be related to solid stools and a quick decrease in soft stool frequency, which was practically normal by the second week in most cases. Previous studies have reported the speed of the response to treatment in dogs with lymphoplasmacytic enteropathies, which can be observed during the first 10 days after treatment initiation (Rodríguez-Franco et al., 1999). The dog diagnosed with histiocytic colitis showed the lowest reduction in CIBDAI and CCECAI entry scores. Since studies have demonstrated the direct causal role of Escherichia coli in canine histiocytic colitis and a clear correlation between E. coli eradication using enrofloxacin and clinical remission (Mansfield et al., 2009; Craven et al., 2011), enrofloxacin was allowed for this dog, and clinical improvement for this dog cannot be attributed to the stem cell therapy.

Corticosteroids and various immunosuppressive agents are commonly prescribed for the treatment of IBD in dogs, achieving significant reductions in CIBDAI scores. Several studies report response rates to prednisolone or prednisone of 50% (Allenspach et al., 2007), 66% (Allenspach et al., 2006a), 69% (Dye et al., 2013) and 83% (Jergens et al., 2010), and of between 75% (Garcia-Sancho et al., 2007) and 80% (Jergens et al., 2003, 2010) when combined with metronidazole. When using either budesonide as an induction therapy (Dye et al., 2013) or cyclosporine as a rescue agent in steroid-refractory dogs (Allenspach et al., 2006b), remission rates of 78% have been reported.

Although our study population was more severely affected than those with previous reports (Jergens et al., 2003, 2010; Allenspach et al., 2006b; Garcia-Sancho et al., 2007), the remission percentage obtained in the current study (81%) is similar to the best results reported. Whereas systemic corticosteroids are often associated with adverse effects (Pietra et al., 2013), ASC infusion was shown not to have damaging side effects in dogs during follow-up, as already shown in cats (Quimby et al., 2013; Webb and Webb, 2014) and humans (Duijvestein et al., 2010; Forbes et al., 2014).

ASC therapy has also been shown to be effective in increasing low serum albumin and cobalamin values. Although hypoalbuminaemia and low serum cobalamin concentrations have been associated with negative outcome in canine chronic enteropathies (Allenspach et al., 2007), a more recent study reported that IBD remission rates in prednisone-treated dogs exceeded 83%. This was explained by the fact that the hypoalbuminemia was not too severe and was not associated with ascites (Jergens et al., 2010). In our study, hypoalbuminemia was also not severe, but an association between ascites and hypocobalaminemia was observed in two animals, where a 90% reduction in CIBDAI was noted. Although cobalamin supplementation associated with immunosuppressive treatment has been described (Jergens et al., 2010), the hypocobalaminemic dogs in the current study had serum cobalamin concentrations in the normal range post-treatment without vitamin supplements. It is possible that ASCs may enhance the digestive and absorptive functions of the small intestine in addition to their anti-inflammatory activity (Semont et al., 2010), thereby facilitating the normalisation of cobalamin and folate levels without any other supplementary source.

Unlike other IBD studies (McCann et al., 2007; Jergens et al., 2010), no increased CRP values were observed in our population pre-treatment despite high CIBDAIs. Many human IBD patients have CRP concentrations within the normal range at diagnosis and it has been suggested that this marker may be the most useful to assess the response of individual patients to treatment (Vermire et al., 2006). Although ASCs exhibit multilineage differentiation capacity (Kono et al., 2014), a growing body of evidence suggests that MSC differentiation into the target cell type makes little contribution to healing. Rather, MSCs appear to function primarily through signalling via cellular mediators and 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 modulating the healing environment and favouring tissue repair (Hackett, 2013; Nagaishi et al., 2015). The exact mechanism by which MSCs suppress the immune system is not fully understood. It is known, however, that MSCs have immunosuppressive features in common with regulatory T lymphocytes (Kramer et al., 2003; Beyth et al., 2005), and a likely role for nitric oxide (NO) in MSC-mediated immunosuppression has been identified (Ren et al., 2008). In addition to modulating immune activity, MSCs may also regulate wound healing through direct or indirect interactions with resident tissue-committed stem cells (Stappenbeck and Miyoshi, 2009), and they also improve integrity of the small intestine through the regulation of endogenous epithelial cell homeostasis. The effects of MSCs are a consequence of their ability to improve the renewal capability of the small intestinal epithelium and to promote fast recovery of dysfunctional secretory responses (electrolyte and water transport, such as chloride secretion across epithelial cells) (Semont et al., 2010).

Our study is an unblinded, non-comparator study of a new therapy and has therefore limitations. However, the efficacy and adverse effect of conventional IBD treatments with corticosteroids or other immunosuppressive drugs are well known. Although the dogs had undergone previous therapies at their referring veterinarians before being included in the study, some medical records were incomplete making it difficult to assess the adequacy of these previous therapies. Therefore, it is not possible to assert that these animals were entirely refractory to conventional treatments. For that reason, we have avoided the term ‘refractory IBD’, as the term ‘refractory CD’ is usually employed for human trials. Subsequent evaluations of long-term safety and efficacy, dose ranging and dose interval evaluations are also required. With these limitations in mind, allogeneic MSCs may nonetheless represent a significant therapeutic alternative or advance for treating canine IBD. In general, their convenient isolation procedure, the lack of significant immunogenicity (which allows for allogeneic transplantation without using immunosuppressive drugs), the absence of ethical controversies and their ability to modulate the immune response and to protect injured tissues make MSCs an interesting therapy for canine IBD.

Conclusions

A single infusion of allogeneic ASCs was well tolerated and appeared to produce clinical benefits in dogs with confirmed IBD. Further studies are required to confirm the long-term safety and efficacy of this therapeutic approach and to refine dose and frequency of administration.

Conflict of interest statement

Drs. Mariñas-Pardo and Hermida-Prieto are employees of Centauri Biotech. 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.

Acknowledgements

Preliminary results were presented as an abstract at the 54th Annual Conference of the Associazione Italiana Veterinari Piccoli Animali, Bentivoglio (Bologna), 11–12 April 2015.
References


