

Use of Mesenchymal Stem Cells in Corneal Ulcers in Dogs: A Case Report

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ABSTRACT

The development and complication of corneal ulcers (CU) in dogs has become a serious burden in pet health. Dogs with exaggerated juvenile-like craniofacial conformations, such as pug breed, are twenty times more prone to develop this condition. Although most of the cases are treated without surgical intervention, complicated and infected cases could lead to permanent blindness and other related complications. New approaches in treating this condition with mesenchymal stem cells (MSC) have been proposed with great results. Given their immunomodulation and regenerative properties, local administration of MSCs has proven to be safe and efficient in treating ocular related health conditions. This manuscript presents a complicated case of a canine CU treated with local infiltration of 1×10^6 canine dental pulp MSCs (cDMSCs). Stem cell therapy was performed at the Central Medica Veterinaria in Colima, Mexico with the consent of the owner, as it was hypothesized that the regenerative properties of MSCs could potentially alleviate the patient's condition. Notorious improvement was reported after 12 hours of infiltration, and neovascularization process was observed within the next few days. The patient was discharged on the 29th day of treatment, with minimal scar and fully regain normal vision. Therefore, using MSCs is a viable option for treating corneal ulcers in dogs.

Keywords: Corneal Ulcer, Dogs, Mesenchymal Stem Cells, Regenerative Medicine.

ABBREVIATIONS

CU: Corneal Ulcers; cDMSCs: Canine Dental Pulp MSCs; MSCs: Mesenchymal Stem Cells; cATMSCs: Canine Adipose Tissue-Derived Mesenchymal Stem Cells.

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INTRODUCTION

One of the most common conditions regarding canine ocular problems is the development of corneal ulcers. Along with corneal melanosis and the growth of cataracts, CU has become a serious burden in pet health [1]. These lesions are often accompanied by pain, redness sore in the ocular area, some degree of squinting and excessive tear production, affecting the overall wealth of the dog and even their domestic behavior [2]. Often called ulcerative keratitis, CU is keratopathy-associated lesions, in which loss of the epithelium and hyperactive inflammation processes are commonly related to corneal conditions [3]. Among the variety of causes leading to the development of CU, direct trauma in the eye during playtime is one of the most reported at veterinary consultation. In a similar way, entropion, distichiasis conditions, as well as viral and bacterial infections are frequently associated to this ocular condition [2].

Moreover, exaggerated juvenile-like craniofacial conformations, including small wide-open eyes, heightens the risk of presenting complicated cases of CU. Of special interest pugs are one of the most afflicted breed; given their craniofacial conformation pugs are twenty times more prone to present complicated cases of CU [4]. Although simple CU lesions are expected to heal with antibiotic prescriptions, antiinflammatory drugs or even without any veterinary intervention, severe complex cases often need surgical intervention. If not treated properly, complicated cases of CU could lead to permanent blindness as well as other health complications [1,5]. Therefore, currently innovative proposals in treating complicated and infected CU have been research. Tissue adhesives, conjunctival grafts, corneal transplants and the development of compatible biomaterials have been performed as an alternative with moderate successful results. [6]. In the need for safe and efficient alternatives, the use of mesenchymal stem cells (MSCs) both in human and animal related health problems has gained interest in the last decade. Previous studies have demonstrated the remarkable properties of MSCs to modulate the inflammatory response of the patient, along with their differentiation capacity. These mechanisms of action could accelerate and promote corneal recovery, due to their proven ability to differentiate into keratocytes *in vitro* and *in vivo*, while producing new collagen

within the host stroma [7, 8]. Given the MSCs properties within the scope of regenerative medicine, this manuscript aims to present the promising results obtained in a complicated case of canine CU.

CASE REPORT

A female pug with 5 years and 9 months old weighting 11kg was diagnosed with a severe case of CU in the right eye on September 2022. The patient has already been prescribed with combined ulcer kit (midriavet, proxi-flox, cloranfivet), along with an ophthalmologic nutraceutical 1 tab a day for 90 days, doxycycline half a tablet a day for 8 days, and ceftriaxone 1g/3mL every 24 hours trough intramuscular injection for 4 days as pharmacological treatment, which controlled the infection but failed to alleviate the CU. When the patient came to Central Medica Veterinaria in Colima, Mexico, it presented stitches in the area due to two failed tarsorrhaphy surgeries and stenosis. A revision fluorescein test was performed and notorious damage to the cornea was detected. The internal diagnosis confirmed the CU lesion, resulting in keratoconjunctivitis sicca previously diagnosed with entropion.

In order to promote fully regeneration of the ocular tissue, stitches of previous surgeries were removed and local administration of 1×10^6 canine dental pulp mesenchymal stem cells (cDMSCs) was carried out. The procedure was performed under general anesthesia, infiltrating at the conjunctival tissues in four different points as observed in Figure 1. Significant improvements in the inflamed region as well as increased vascularization in the corneal area were observed after 12 hours. Follow-up consultations were performed with good evolution signs; and saline solution cleanings along with the combined ulcer kit was prescribed for the following 7 days to prevent reinfection. 18 days after the cDMSCs infiltration, a fluorescein test showed no signs of lesion, outstanding close of the CU, improvement in the photosensitivity along with adequate ocular rotation. Complete healing of the CU was observed after 25 days of MSCs intervention, with minimum corneal scar. Combined ulcer kit dry eye treatment was prescribed for the following 4 days to prevent local dehydration, and on the 29th day medical discharge was made (Figure 1).

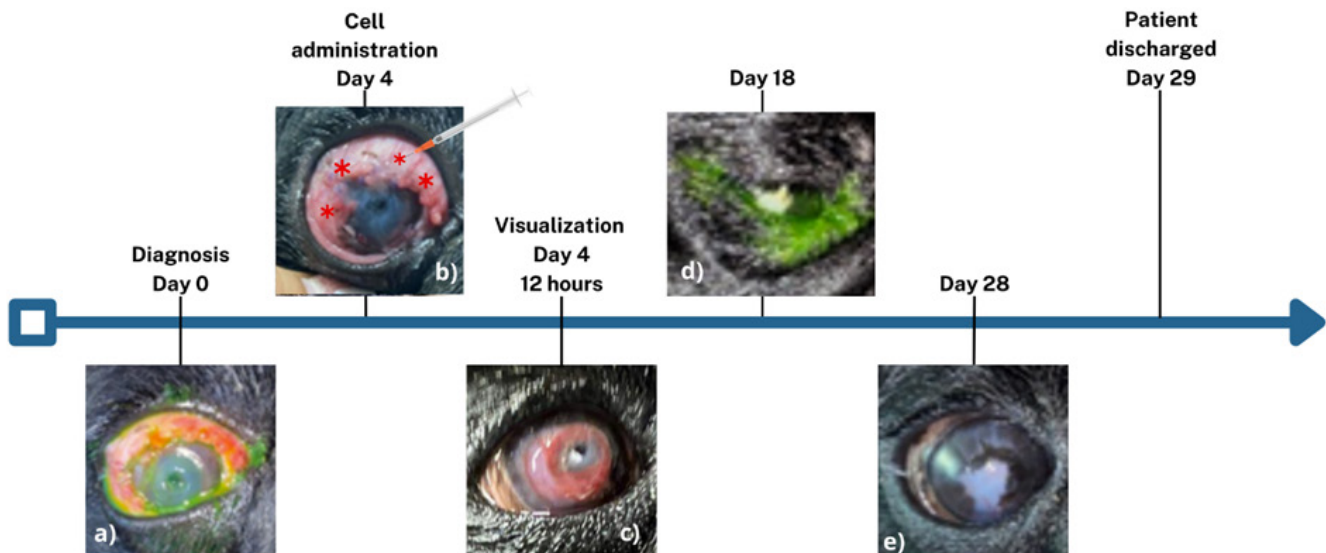


Figure 1. Clinical course and improvement after MSCs application. a) Positive fluorescence test for corneal injury, b) Corneal ulcer prior to application c) Vascularity increased after 12 hours of MSCs application, d) Negative fluorescence test for corneal injury, e) Corneal ulcer healed.

DISCUSSION AND CONCLUSION

CU is one of the most common ocular health concerns in dogs; which primary causes include trauma, foreign bodies, infection, inadequate lacrimal secretion, corneal endothelial dysfunction, spontaneous, toxic, and anatomical functional abnormalities in adnexa [9]. It has been hypothesized that the anatomical facial features of some dogs play a role in the development of severe cases of CU. Recently, a large population study reported that brachycephalic dogs with prominent eyes, pug breed included, have a higher overall susceptibility to CU [10]. Conventional CU medical management approaches differ in symptomatology; including proper antimicrobials, aqueous suppressants, anti-collagenases, anti-inflammatory therapy and promoting epithelial healing [11]. Nevertheless, severe and an infected case of this condition usually leads to the loss of more than half of the corneal thickness, as well as corneal perforation. These complications can have devastating visual consequences if not treated properly. Anatomical integrity of the cornea is desired but most of the time, surgical intervention failed to preserve corneal functionality [9].

Therefore, an urgent need to find new therapeutic approaches for CU, has gained the attention of veterinary clinics. Alternative treatments such as platelet-rich plasma [12], amniotic membrane graft [13], matrix regenerating agents [14,15] have been reported. Recently MSCs has been used as an alternative for CU in humans, with outstanding outcomes [16]. Likewise,

other animals such as cats [17], horses [18] and dogs [19, 20] have been documented with promising results. On a recent case report a Poodle diagnosed with CU was administered two doses of allogeneic stem cell through topical eye application; each dose containing 3×10^6 canine adipose tissue-derived mesenchymal stem cells (cATMSCs). Clinical evaluation, along with Fluorescein test, Schirmer test and ocular fundus exam showed remarkable improvement with lesion resolution [19]. In addition, on a very interesting study 26 breed-not-specified dogs were diagnosed with deep corneal ulcers and have been unsuccessfully treated for 15 days with allopathic drops. Dogs received an application of 3×10^6 allogeneic cATMSCs via subconjunctival in 12 instillations (one per hour) of 0.25 mL of transport medium containing 250,000 cells. After clinical evaluation, considering general discomfort and deepening of the CU, 84.6% of the cases reversed without the need of surgical intervention [20].

MSCs enhances an immunomodulation process via paracrine mechanisms, leading to an observable therapeutic action in corneal wound re-epithelialization [21]. Local administration of MSCs attenuates inflammation, stimulates neovascularization, accelerates corneal wound healing [22], and promotes endogenous regenerations [23]. These mechanisms of action place MSCs therapy as a safe and efficient approach in treating corneal conditions in the veterinary area. In this manuscript, we have presented an outstanding healing of

a CU in a canine patient using stem cell therapy. After the infection was controlled and MSCs infiltration was performed, the patient shown significant improvement in the first 12 hours. A notorious process of ulcer closure along with neovascularization process was observed within the first weeks of treatment. The patient was discharged on day 29th with no signs of infection. Our results are in accordance with has been previously reported [19,20], posing MSCs therapy as a therapeutic alternative in treating this condition.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

PP-H, YO-A have participated in the analyses and draft of the manuscript. RR-A, DR-V, DM-F, DM-D and MR-A has made significant contribution with the data and presentation of the case report. ST, DE has participated in the design and revision of the manuscript. All authors have read and approved the final manuscript.

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