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Treatment of Indolent Ulcer with cultured canine mesenchymal stem cells

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Abstract

Background: *Indolent ulcers are noninfectious epithelial defects of the cornea, typically presenting as chronic, superficial lesions. These ulcers are most commonly observed in dogs and are the leading cause of ophthalmic consultation in the UVS Ophthalmology service. These ulcers, which are more common in older dogs, often have difficulty healing naturally. Underlying eye conditions and infections can further hinder the healing process, potentially causing vision impairment and eventually removal of the eye. In this manuscript, we present the success of using mesenchymal stem cells (MSC) in a preliminary pilot trial to treat indolent ulcers.*

Methods: *Companion animals with indolent eye ulcers that did not respond to traditional treatment protocols were treated with suspended canine bone marrow-derived MSCs (5 x 10^5 cells total) administered via an eyedropper and then monitored for a period of 2-3 hours post infusion before being released back to their owners. Dogs had a follow up visit after 48-72 hours by the veterinary team.*

Results: *Of the 12 adult canine pets with indolent ulcers, MSC treatment led to recovery of the ulcers within 5-6 weeks after administration.*

Conclusions: *This pilot trial showed the promise of the use of topical canine MSC for dogs with indolent ulcers. In the near future we are planning to conduct a randomized prospective trial. We consider the use of expanded stem cells to treat indolent ulcers represent a novel and potentially effective approach and it is our intention to further expand the use of MSCs in the indication of ocular indolent ulcers.*

Key words: Dog, Stem cell, Indolent, ulcers, companion pet.

Introduction

Indolent ulcers in companion dogs are common lesions of the cornea characterized by chronic, superficial damage and inflammation [1, 2]. These ulcers are typically spontaneous corneal defects and often result from superficial trauma such as abnormal eyelid development, mechanical injury, and infection. They are more prevalent in middle-aged and elderly dogs [3, 4] and are more frequent and harder to manage in brachiocephalic breeds including boxers, bull dogs, pugs and Boston terriers. Pathologically, indolent ulcers are marked by the loss of the corneal epithelium's basement membrane and the formation of superficial, acellular hyalinization in the stroma [4, 5]. Clinically, they often manifest as blepharospasm, epiphora, corneal edema, and fibrosis. Dogs with this condition may exhibit spastic entropion of the eyelid. The severity of the disease may vary, ranging from superficial to basal ulcers, Descemet's membrane ulcers, and even corneal perforation. Without timely intervention, the disease will progress. Diagnosis is typically achieved through clinical observation of typical manifestations and fluorescein staining. The presence of fluorescein staining indicates exposure of the corneal stroma.

Currently, the prevalent treatment for this condition involves the use of antibiotics in milder cases, conservative methods like debridement, and surgical procedures such as superficial keratectomy, which are currently regarded as the most efficacious [3, 6], but each strategy has limitations. Prior research has indicated that a combination of cotton-tip debridement, scalpel blade debridement, and superficial grid keratotomy is also effective for indolent ulcers, however, this approach is associated with a high surgical risk [7]. There is a demand for a relatively non-invasive yet effective treatment to address this disease which if left untreated can lead to visual impairment.

Mesenchymal stem cells (MSCs) are crucial agents in regenerative therapy and are presently being utilized for the treatment of diverse conditions, including degenerative diseases, trauma, and even tumors [8]. We have developed methods to expand canine MSC in *vitro* using defined protocols under GMP conditions. After cell expansion, MSC were cryopreserved in defined doses for further use. Vials were tested and released for use only after receiving quality control approval. The objective of this study is to assess the therapeutic potential of MSCs in managing indolent ulcers in a pilot series of companion pets which have previously failed conventional treatments.

Methods

Stem cell acquisition:

For this study, canine mesenchymal stem cells (MSCs) were obtained from bone marrow of healthy adult dogs. After bone marrow collection, the sample was washed multiple times with Dulbecco's phosphate-buffered saline (DPBS) contain-

ing 1% penicillin, streptomycin, and hygromycin B. Subsequently, the mixture of MSC was centrifuged at 1500 rpm for 5 minutes to remove the supernatant.

To the remaining tissue, 0.2% type II collagenase (Nordmark) was added and incubated for 5-10 minutes in a 37°C sterile water bath. The mixture was then centrifuged at 1500 rpm for 5 minutes, and the supernatant was discarded. To terminate enzymatic digestion, cold complete culture medium (DMEM) containing 10% fetal bovine serum (Corning) and 1% penicillin-streptomycin (Corning 30-004-CI) was added. Following this, the mixture was centrifuged at 1500 rpm for 5 minutes, and this rinsing process was repeated 3 times. The mixture was subsequently filtered through a 70 µm cell sieve and centrifuged at 1500 rpm/min for 5 minutes. The cell pellet was then re-suspended in complete culture medium and cultured in humidified 37°C tissue culture incubators.

Stem cell culture and storage

The MSC was expanded up to passage 8. MSC from passages 3-8 was used for subsequent experiments. For cell freezing, MSC were removed from the flask using trypsin-EDTA (Corning 25-051-CI), neutralized using complete culture media, and then the media was replaced by DMSO based freezing media (Bulldog Bio BB-01). Cells were aliquoted at 5 x 10^5 cells/vial and frozen in liquid nitrogen using a defined protocol developed and validated in our laboratory using controlled rate freezer using a validated freezing protocol (Fisher Scientific CryoMed).

Vials of canine MSC cells were labelled with specific lot number, date and identification. Lots were collected and quarantined and an aliquot of each lot was tested and maintained until the results were collected, analyzed, and reviewed by quality control. Approved lots were released from quarantine and place in the release inventory. Quality control testing which included aerobic, anaerobic, fungal testing with reports at two and seven days. Additionally, we tested for the presence of endotoxin and performed gram staining before the results were reviewed, approved, and released for use. The QC report was completed and reviewed and signed off before the vials were released from quarantine. One vial from each lot was retained as a long term archived sample and placed in liquid nitrogen vapor storage.

Vials of labelled canine MSC cells along with the quality control report were shipped via overnight courier to the Crestwood Veterinary clinic in Edmonton Canada in a dry shipper which maintained temperature at -140°C in the vapor phase of liquid nitrogen where they were placed in labelled cannister in a liquid nitrogen dewar for storage. The liquid nitrogen dewar was monitored daily and levels of liquid nitrogen were recorded and maintained to ensure safe storage of the MSC vials.

Treatment with MSC

Companion pets that attended the veterinary clinic with indolent ulcers were evaluated by the team and the owner was presented with a consent form for the inclusion in the pilot trial**.** Dogs with active infections were excluded from this study as well as those pet owners who choose not to be involved in this study and subsequent follow up.

The dogs included were those where previous treatments with debridement, grid teratotomy and/or topical antibiotics were ineffective (Figure 1). These dogs were then retreated with debridement,

followed by topical stem cells application and a third eyelid flap.

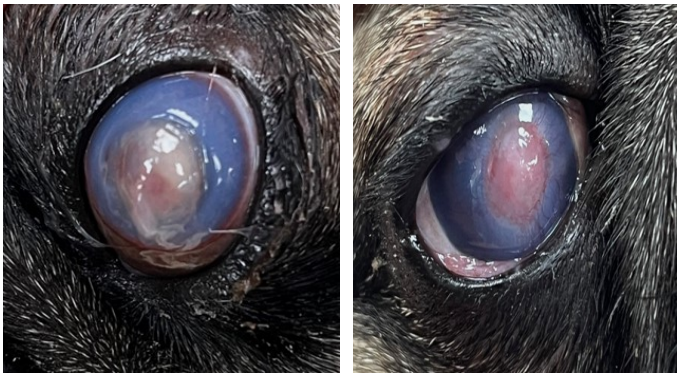

Figure 1. Photograph of the affected eye prior to MSC treatment. (A) and (B) representing 2 different patients.

For application, vials of MSC cells were collected from the liquid nitrogen storage dewar, and the vial was rapidly thawed in a 37°C sterile water bath within 40-60 seconds. The vial was then sprayed with 70% ethanol and the vial aseptically opened with the contents drawn into a 3 mL sterile syringe with 18-gauge needle and carefully transferred to a sterile eye dropper. The dogs received the cells via the eye dropper and the eyelid was sutured closed. Animals were monitored after treatment for a period of 2-3 hours post infusion in the clinic before being released back to their owners. Pets were followed up after 48 hours and 1 month by the veterinary team.

Results

Twelve (12) adult dogs were enrolled in this pilot trial. All 12 dogs had previously failed standard therapy, but they all saw their ulcers resolve when MSC was added to their treatment. No complication was observed after MSC therapy. Eye ulcers in our study typically healed within 2-4 weeks after administration of our stem cell product (Figure 2).

Figure 2: Photograph of the eye at 4 weeks following MSC treatment.

Discussion

Indolent ulcer is the most common canine ophthalmic disease and the most difficult type of corneal ulcer to treat in veterinary medicine [5], often caused by protracted course of disease after corneal injury. Currently, there are various treatment methods for indolent ulcers, including conservative treatment based on drug therapy and surgical treatment. Scholars used antibiotics combined with chondroitin sulfate to treat indolent ulcers in dogs. After four weeks of treatment, 81% of cases recovered [9]. However, due to the long treatment cycle, it is less commonly used. In comparison, corneal epithelial debridement is more commonly used, including cotton tip epithelial debridement and diamond burr debridement. It aims to continuously remove erosive epithelium, but previous reports have shown significant differences in cure rates, ranging from about 20% to 97.1%, and treatment duration varies from 2 to 3 weeks [3, 5, 7, 10]. In addition, thermal cautery, as a surgical procedure, is used to treat indolent ulcers. A retrospective analysis by Landrevie et al. showed that the cure rate after the first thermal cautery surgery was 65.1%, with an average cure time of about 15 days [12]. Superior keratectomy is a more invasive surgery, which has a reported success rate of up to 100% and can damage the basement membrane

and increase the contact between the epithelium and the underlying matrix [11]. However, due to the need for general anesthesia and the complexity of the procedures, they are generally not recommended as the first treatment [3].

MSCs have rapidly advanced in the field of regenerative therapy and are widely used in diseases such as joint diseases or trauma. They have also shown promise in treating eye diseases. MSC can differentiate into various types of corneal cells [12]. Previous studies indicate their effectiveness in managing dry eye disease, which is a contributing factor to indolent ulcers [13]. Additionally, vesicles derived from corneal MSC promotes corneal wound healing [14, 15]. Given that indolent ulcers are characterized by corneal injury and poor regeneration, MSC represent a highly promising treatment option. Multiple clinical trials have been conducted to treat corneal diseases with MSC.

In our study, all twelve dogs with indolent ulcers, including 11 adult French bull dogs and one boxer, were included in the study and were not cured after receiving antibiotics, debridement, or grid therapy. However, following MSC treatment, the indolent ulcers of all three dogs were cured. This indicates that MSCs have great potential in the treatment of indolent ulcers in dogs.

Indolent corneal ulcers in dogs can often lead to impaired visual function if not intervened in time. When deep ulcers occur, treatment is often challenging, leading to prolonged suffering of the dogs. We propose a new method for treating indolent ulcers, which has the advantage of short treatment cycle, simple method, and is more economical. The success rate of MSC treatment in our pilot study is 100%, however, the small sample size cannot fully reflect the therapeutic potential of MSC. In the very near future, more rigorous controlled trials will be conducted to improve this novel technology and attempt to understand the mechanism of action of the extraordinary outcomes of this technology.

Conclusion

Indolent ulcers represent a significant medical issue in companion dogs. This pilot study represents a novel and promising technology to treat indolent eye ulcers in companion pets.

It is our intention to further expand the use of MSC in indication and perform a randomized prospective trial in the very near future. This treatment of indolent ulcers with expanded stem cells represents a novel and potentially effective treatment.

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Conflicts of Interest:

All authors declare no conflict of interest in this study.

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