



Model Classification Methods in the Treatment of Corneal Ulcers with Mesenchymal Stem Cells

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Authors' contributions

This work was carried out in collaboration among all authors. Author RSDS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MCF and MBP managed the analyses of the study. Author MBP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study was carried out to classify the difficulty in treating corneal ulcers using mesenchymal stem cells as cell therapy. Seven studies were used to carry out the research. It was carried out by the Department of Animal Morphology and Physiology (DMFA) and the Laboratory of Physiology

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and Experimental Surgery (LAFICE). The research was carried out through a bibliographic survey, highlighting the following topics: routes of application of Mesenchymal Stem Cells (MSCs), tissue origin of MSCs, sex of the animal, species of the animal, depth of the corneal ulcer, comorbidity and time required for re-epithelialization. The results showed that the study that came closest to the set of topics related to increased treatment difficulty took 270 days to recover. The study that came closest to the set of topics related to the least difficulty of treatment took 21 days to recover, demonstrating the efficiency of the proposed classification of difficulty of treatment. The study concludes the efficiency of the method for classifying the difficulty of treating corneal ulcers using mesenchymal stem cells.

Keywords: Stem cells; mesenchymal stem cells; ophthalmology; cell therapy and corneal ulcer.

1. INTRODUCTION

Corneal ulcer is a major public health problem in the developing world as it causes prolonged morbidity, loss of vision and, many a time, loss of an eye (Ibanga et al., 2016). It is defined as a lesion or loss of integrity of the layers of the cornea. The cornea is the most superficial part of the eye and is subdivided into five layers: epithelium, Bowman's layer, stroma, Descemet's membrane and endothelium (COBO et al., 2024). Corneal ulcer is a potentially blinding disease if left untreated and rural communities in developing countries are often unable to access appropriate treatment (Ibanga et al., 2016). Infected corneal ulcers that don't show any healing within two weeks despite proper medical treatment are considered resistant and need surgical intervention. The amniotic membrane promotes epithelialization, reduces the formation of scars and new vessels, and reduces inflammation. It contains nerve growth factor and is believed to be non-immunogenic. Also, it reduces the pain caused by friction of eyelids over the ulcer surface and decreases the risk of infection. Interrupted and running sutures are types of suturing techniques that have been used to fixate the amniotic membrane to the cornea (Elghany et al., 2023). Trauma, infection, inflammation, immunity, and other factors can cause corneal damage, and severe cases can affect vision and appearance. Statistics show that corneal disease is the second most common blinding eye disease after cataracts. (Liu, X et al., 2021). Corneal ulcers can be classified according to the depth of the affected layer, as follows: 1 - superficial corneal ulcers, which damage the epithelium, the first layer of the cornea; 2 - deep corneal ulcers, which damage the stroma, the third layer of the cornea (Byrd et al., 2024; Falcão et al., 2020; Iqbal et al., 2022).

Stem cells can be categorized into two types depending on their source: embryonic and adult

stem cells. Adult stem cells are present in a multitude of organs and tissues within the body, such as skeletal muscles, brain, bone marrow, dental pulp, liver, spinal cord, cornea, adipose tissue, and more. Among these, Mesenchymal Stem Cells (MSC), regarded as multipotent progenitor cells, can arise from either embryonic or adult sources (Bhujel, et al., 2023). Several studies have demonstrated the beneficial effects of using MSC in the treatment of corneal ulcers, both superficial and deep (Freitas, 2018; Kawata et al., 2019; Pereira, et al., 2024). In addition, a significant number of studies have presented treatments that associate the use of mesenchymal stem cells with the treatment of corneal ulcers. These treatments associated with mesenchymal stem cells can be conventional or unconventional.

The International Society for Cellular Therapy establishes three basic criteria for classifying a stem cell as a MSC. The first criterion states that mesenchymal stem cells must be adherent to plastic when subjected to basic culture conditions. The second requires them to be positive (more than 95%) for CD105 (endoglin or SH2), CD73 (SH3 or SH4) and CD90 (Thy-1) and negative (less than 2%) for CD45, CD34, CD14 and CD11b. The third and final feature is the cells' ability to differentiate into bone, fat and cartilage tissue when in vitro (ALMALKI and AGRAWAL, 2016; BIANCO, ROBEY and SIMMONS, 2008). The mesenchymal stem cell was first described in 1867 by Cohnheim, who suggested the first existence of non-hematopoietic stem cells in the bone marrow, which had been unknown until then (STEFANŠKA et al., 2020). In addition to bone marrow, other sources of isolation were subsequently discovered (BIANCO, ROBEY and SIMMONS, 2008).

Various studies have demonstrated the importance of establishing and following a

protocol or model in clinical treatment, as can be seen in the works by Mamme, Mastell and Lemley (2014) and Tingle (2023). The standardization of clinical procedures has become increasingly common in the clinical environment, both in human and veterinary medicine. However, this standardization is still limited when it comes to the use of mesenchymal stem cells in the treatment of corneal ulcers.

This study aimed to analyze research into cell therapy using mesenchymal stem cells in the treatment of corneal ulcers. If a pattern is identified in the results of specific studies, it will be possible to describe the most efficient treatment model. The model will vary according to the species treated (human or non-human animal) and the level of ulcer (superficial or deep), which are the only two factors that cannot be altered in the clinical strategy since the patient's previous condition cannot be chosen. Therefore, these factors become standard settings for the creation of treatment models.

2. MATERIALS AND METHODS

The work consists of a bibliographical survey, using the keywords mesenchymal stem cells, cell therapy and corneal ulcer and the combinations 1- mesenchymal stem cells AND corneal ulcer and 2- cell therapy AND corneal ulcer, searched on Google Scholar and the Scielo and Pubmed databases. A survey of the studies used was carried out. To compose the tables presented in Results and Discussion, the inclusion criteria were documents that used mesenchymal stem cells to treat corneal ulcers; the exclusion criteria were review studies and studies that used non-conventional techniques associated with mesenchymal stem cells.

To create the tables, due to the small number of articles that met the inclusion and exclusion criteria, no specific time interval was set for selecting the studies. However, the oldest article identified was published in 2008, while the most recent was published in 2020. The papers used in the introduction and discussion of the research, on the other hand, had a time frame from 2000 to 2024.

The study analyzed the following aspects in the selected articles: routes of application, the origin of the mesenchymal stem cells, depth of the corneal ulcer, sex of the animals, associated comorbidities, citations of the studies analyzed and the time required for re-epithelialization. To identify an order of difficulty in treatment,

considering the topics mentioned above, and, based on this analysis, propose a treatment model, the topics reported in more than one study were evaluated based on their medians. Those that appeared in only one study had their data used directly as a reference. The choice to use the median is because this measure is not influenced by extreme values, making it possible to integrate variations.

3. RESULTS AND DISCUSSION

The literature review resulted in the identification of seven (7) research (Table 1), in which the following topics were analyzed: routes of application, origins of the mesenchymal stem cells, levels of depth of the corneal ulcer, sex of the animals, related comorbidities, citations of the studies analyzed and the time required for re-epithelialization/healing of the ulcer.

3.1 Classification of Study Topics According to their Difficulty of Treatment

The results of the medians (in the case of recurrent topicals) and total days of the isolated studies of the healing/reepithelialization time of the corneal ulcer, based on the selected studies, were organized as follows:

Routes: in terms of routes of application, the subconjunctival route had a median of 21 days for corneal ulcer healing (the most positive result); the topical route had a median of 100.5 days of treatment (intermediate result); and implant application with injection, with a single representative, had 270 days (the most negative result). In a study published by Shukla et al. in 2019, the subconjunctival route proved superior in the treatment of corneal ulcers with mesenchymal stem cells. In this study, the authors evaluated the efficacy of the different routes of application, which corroborated the result presented in this research.

Origins: in terms of origins, mesenchymal stem cells (MSCs) from allogeneic dental pulp and allogeneic bone marrow (the most positive results), which had only one study for each origin, showed values of 28 and 21 days for ulcer healing, respectively, with these studies determining the final results. On the other hand, MSCs originating from allogeneic adipose tissue showed a median of 35 days for ulcer healing, which was the most negative result.

Levels: in terms of levels, the superficial ulcer had a median of 17.5 days to complete recovery

Table 1. Results of the classification of topics: routes of application, origins of mesenchymal stem cells, levels of depth of the corneal ulcer, sex of the animals, their comorbidities, citations of the studies analyzed and days for re-epithelialization/healing of the ulcer

Research	Routes	Origins	Ulcer level	Species	Sex	Comorbidities	Days	Citation
1	Subcon uncivil	Allogeneic adipose tissue	Deep	Dogs	male	Type 1 diabetes	14	Falcão et al. (2020)
2	Implanted with injection	Allogeneic adipose tissue	-	Dogs	-	Keratoconjunctivitis sicca	270	Villatoro et al., (2015)
3	Subconjunctival	Allogeneic adipose tissue	Superficial	Dogs	Female	-	14	Amorim, et al. (2022)
4	Topic	Allogenic adipose tissue	Deep	Dogs	-	Keratoconjunctivitis sicca	180	Sgrignoli, et al. (2018)
5	Subconjunctival	Dental pulp allogenic	-	Dogs	Female	Keratoconjunctivitis sicca	28	Palafox-Herrera et al. (2023)
6	Subconjunctival	Allogeneic adipose tissue	Deep	Dogs	-	-	35	Valeeva et al., (2024)
7	Topic	Bone marrow allogeneic	Superficial	Sprague-Dawley rats	male	-	21	Oh et al., (2008)

Table 2. Comparative analysis of most positive and negative results of the studies surveyed

Comparison	routes	origins	ulcer level	Species	Sex	comorbidities	Days	citation
More positive	Subconjunctival route	Bone marrow allogeneic	superficial ulcer	Sprague-Dawley rats	Males	-	-	-
Research 7	Topic	Bone marrow allogeneic	superficial ulcer	Sprague-Dawley rats	Males	-	21	Oh et al., (2008)
More negative	Implanted with injection	Allogeneic adipose tissue	deep ulcer	Dogs	females	Keratoconjunctivitis sicca	-	-
Research 2	Implanted with injection	Allogeneic adipose tissue	-	Dogs	-	Keratoconjunctivitis sicca	270	Villatoro et al., (2015)

(the most positive result), while the deep corneal ulcer had a median of 180 days to complete healing (the most negative result).

Species: in terms of levels, superficial ulcers had a median of 17.5 days for complete recovery (the most positive result), while deep corneal ulcers had a median of 180 days for complete healing (the most negative result).

Sex: sex, males had a median of 17.5 days for the ulcer to heal (the most positive result), while females had a median of 21 days (the most negative result). Studies comparing the haematological and biochemical parameters between Wistar rats and dogs (*Canis lupus familiaris*), both male and female, indicate that there are small differences in these configurations depending on gender (DE LACERDA et al., 2017). This tendency towards sexual dimorphism in the physiology of these animals probably also applies to other mammals, which may explain the difference in results.

Comorbidities: concerning comorbidities, in the studies that mentioned keratoconjunctivitis sicca (CCS), the median recovery time was 180 days (the most negative result). For diabetes, in the only study in which this comorbidity was mentioned, the recovery time was 14 days (the most positive result). The studies that did not report comorbidities showed a shorter median time for corneal ulcer healing.

3.2 Treatment Difficulty Classification Model

By grouping the topics with the most positive results and comparing them with the study that most closely resembles them (the most positive topics), and following the same pattern for the most negative topics, comparing them with the study that most closely resembles them (the most negative topics) (Table 2). This allowed us to analyze which pattern of characteristics/topics analyzed may present a more or less complex situation for treating corneal ulcers. "Most negative" would be the sequence of combined factors that make treatment most difficult. On the other hand, "more positive" would be the sequence of combined factors that would make treatment less difficult.

Research 7, which came closest to the "most positive" details, showed a total recovery time of 21 days. On the other hand, research 2, which most closely resembled the "most negative" topics, recorded a period of 270 days for complete recovery of the corneal ulcer. This fact

reinforces the efficiency of the treatment difficulty classification model.

This study needs to be replicated with more research and in a more specific way in relation to the species used, requiring a different model for each one. This is of great importance both for pre-clinical and clinical research and for medical practice, both human and veterinary.

3.3 Applying the Model to Make Treatment More Efficient

Table 2 shows details that can be modified, such as the choice of the route of application of the MSCs and their tissue origin. On the other hand, factors such as species, gender, comorbidities and ulcer level cannot be altered. As an example of the application of the model that seeks to improve treatment results, Study 2 can be considered, addressing the details that can be adjusted (route of application and tissue origin of the MSCs), which fit into the most negative to those that fit into the most positive (Table 3).

When replacing the changeable topics, the route of application can be adjusted, swapping the topical route for the subconjunctival route. Similarly, the tissue origin of the MSCs can be modified, changing the MSCs from allogeneic adipose tissue to MSCs from allogeneic bone marrow. This change represents the substitution of topics described as more negative (injection and allogeneic adipose tissue) for those considered more positive (subconjunctival route and allogeneic bone marrow tissue). Therefore, an improvement in treatment results is expected. To prove its superiority, the new treatment should have a healing and/or re-epithelialization recovery time of less than 270 days.

The study that most closely followed the most positive descriptions (most positive model) recorded a recovery time of just 21 days, while the study that most closely resembled the most negative descriptions (most negative model) showed a recovery time of 270 days, resulting in a difference of 249 days. This data reinforces the efficiency of the classification model for the treatment of corneal ulcers.

As is the case in hospital, clinic and health centre environments, where resources are scarce, substitutions will not always be for the most positive model (route of application and tissue origin of the MSCs) for the species. In some cases, you can opt for special treatments as intermediaries for those with fewer negative topics or comorbidities.

Table 3. Comparative table between the set of most negative topics and the application of the model

Comparison	Routes	Origins	Ulcer level	Species	Sex	Comorbidities	Days	Citation
More negative	Implanted with injection	Allogeneic adipose tissue	deep ulcer	Dogs	females	Keratoconjunctivitis sicca	-	-
Research 2	Implanted with injection	Allogeneic adipose tissue	-	Dogs	-	Keratoconjunctivitis sicca	270	Villatoro et al., (2015)
Replacement for research 2	Subconjunctival	Allogeneic bone marrow	-	Dogs	-	Keratoconjunctivitis sicca	-	-

4. CONCLUSION

The research observed patterns that facilitate or hinder the treatment of corneal ulcers using mesenchymal stem cells as cell therapy. In other words, a model was developed to classify this difficulty. It was also possible to classify two treatment models: a more positive one for ulcer recovery, lasting 21 days, and a more negative one, lasting 270 days. There was therefore a difference of 249 days between the most positive and most negative models.

This procedure needs to be evaluated individually for each species. Furthermore, it is important that the number of studies on the treatment of corneal ulcers using MSCs as a therapy increases. With more research that reproduces the methodology applied here and considers the individualization of species, the understanding of this classification model and its applicability. It should be proven to be efficient or not, as a way of classifying difficulty models in the treatment of corneal ulcers.

It is also necessary to compare studies in which only one of the topics analyzed varies, in order to understand how each topic, as a variant, influences treatment. As it is a classification model applicable to any species, the study is of great importance for human and veterinary medicine.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The ChatGpt artificial intelligence was only used for grammar correction and was not used to generate texts, data or figures. The prompt used to correct grammar and spelling was: correct the grammar and spelling of text.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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