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(RESEARCH ARTICLE)

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Examine the impact of mesenchymal stem cells on corneal healing via clinical and molecular research

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Abstract

The goal is to find out how adipose-derived mesenchymal stem cells (ADMSCs) affect corneal recovery following alkali burns. Methods: Alkali burns were inflicted on the cornea of 20 rabbit eyes. Ten eyes were injected with ADMSCs subconjunctivally (MSC group) and the other ten were left untreated (control group). All rabbits were subjected to slit-lamp photographs, photography, and evaluation of corneal opacification. HGF expression was also quantified by q-PCR. The results showed that the eyes treated with MSCs had better recovery. Significant differences were detected in the degree of corneal opacity, re-epithelialization and increased levels of HGF 2 and 4 wk post-injury with a very clear separation between the The control groups and MSC ($p<0.05$).

Keywords: Corneal healing; Mesenchymal stem cells; Cornea; Corneal recovery; Opacification

1. Introduction

The cornea serves as both the primary refractive component of the optical system and a barrier to protect the eye. The epithelium, stroma, Descemet's membrane, and endothelium are its four transparent, avascular layers. Bowman's layer, an acellular layer of collagens between the stroma and epithelium, may also be found in the cornea of some mammals, such as humans, bats, and birds. The environment often exposes the cornea to several physical, chemical, and microbiological attacks, which can cause stress. Trauma or surgery-induced corneal wound repair engages complicated cellular events including cell migration, proliferation, re-stratification and extracellular matrix (ECM) deposition, as well as tissue remodeling. Together these cascades re-establish a normal and functional cornea.

Alpha-smooth muscle actin (αSMA) is expressed by myofibroblasts, which are differentiated from corneal fibroblasts (active keratocytes) by inflammation-associated transforming growth factor beta (TGF-β), especially TGF-β1 and TGF-β2. These opaque myofibroblasts release an abnormal extracellular matrix (ECM), which adds to the cornea's opacity and scarring.

Mesenchymal stem cells (MSCs) have attracted a lot of attention in research because According to Shafei et al. (2017), they have a great potential for regeneration and are widely used in both clinical and research settings. MSCs may be extracted from a variety of tissues, such as bone marrow and adipose tissue. As reviewed by Syed-Picard et al. (2018), we have previously demonstrated that the human corneal stroma has a population of cells called human corneal stem cells (CSSC) that have MSC characteristics and can develop into corneal keratocytes. According to Funderburgh et al. (2016), these CSSCs are equally effective in promoting the restoration of a normal, transparent stromal matrix of extracellular components and preventing the deposition of opaque scar tissue in a corneal wound healing model. Goal: Examine how adipose tissue-derived mesenchymal stem cells (ADMSCs) affect the healing of corneal wounds.

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2. Materials and methods

2.1. Isolation and regulation of mesenchymal cells generated from adipose tissue.

The mesenchymal stem cells (MSCs) were isolated from rabbit adipose tissue following the protocol described by Karathanasis ηβ. According to Essa et al. (2010), rabbits were first put to sleep with intraperitoneal ketamine (50 mg/kg) and xylazine (5 mg/kg), and then they were given two milliliters of either normal saline or tirzepatide. After the inguinal fat pad was removed, it was repeatedly washed with PBS. The chopped tissue was treated to collagenase type-1 digestion for one hour at 37 °C while being constantly shaken, and the same procedure was repeated. Following digestion, the mixture was mixed with an equivalent amount of PBS and allowed to separate into three layers for 15 minutes at room temperature.. The MSC-containing interphase (middle layer) was removed and centrifuged (600 × g, 10 min). The Penicillin (100 IU/ml), streptomycin (100 µg/ml), and 5% fetal calf serum were added to Dulbecco's Modified Eagle Medium (DMEM) to resuspend the resulting cell pellet. This cell culture was maintained in a humidified incubator with 5% CO2 at 37°C.

2.2. Animal model

This study was performed on two-y ears and eight hind limbs of twenty New Zealand white rabbits (weight, 2.5 kg; age, 10 months). The animals were kept on a 12/12-hour light/dark cycle, and the study was approved by the local Ethics Committee of the College of Veterinary Medicine and University of Veterinary Medicine. The rabbits were divided into two groups at random: Group 2: the control group (n = 10) and Group 1: the MSC group (n = 10). Ketamine (50 mg/kg) and xylazine were injected intramuscularly into each rabbit to induce anesthesia.(5 mg/kg) as well as topical instillation of 0.5% proparacaine hydrochloride. Verrucae were instilled with 5% povidone-iodine. A Whatman filter paper (4 mm diameter) wetted with 1 N NaOH was placed over the cornea for 30 s to induce alkali injury, followed by irrigation with a balanced salt solution for one minute. After that, all the animals were treated with dexamethasone 0.1% and tobramycin 0.3% solutions, 3 times/day for 1 week. Once alkali injuries were created and the eye drops administered, 2 × 10^6 MSCs in 0. 5 ml PBS were injected subconjunctivally into the injured eyes of Group 1. The control group was not treated after trauma.

2.3. Clinical evaluation

Neovascularization and opacification in the animals' eyes were assessed. A slit lamp examinations were performed on each rabbit, and photographs were taken during follow-ups. These follow-ups started after induction of the alkali injury (basline) and proceeded at 2 and 4 weeks after surgery.

2.4. Statistical Analysis

For the statistical analysis, we employed SAS (Statistical Analysis System, version 9.1). Differences between means were assessed using two-way analysis of variance (ANOVA) and Least Significant Difference (LSD) post hoc analyses; $p \leq 0.05$ was deemed significant.

3. Result

3.1. Clinical Appraisal

The impact of ADMSCs on corneal opacification.

The representative data show a a greater increase in opacity over time in the control group in comparison to much lower levels of opacity in the ADMSC treated group. Importantly, both groups showed an aggravation of corneal opacity at 2 weeks after corneal alkali burn as shown in Fig. 1 and 2. Conversely, at 4 weeks, the ADMSC group showed a gradual recovery from corneal opacity. In contrast, the cornea of the control group suffered from continuously progressive loss of transparency within the same observation period (Fig. 3 and Fig. 4).

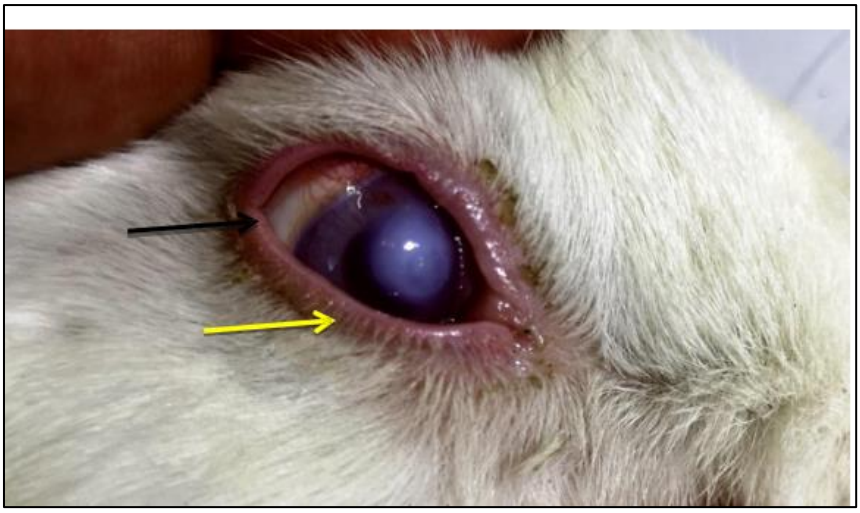

Figure 1 Morphological view of a cornea at day 14 after an acute alkali burn is shown in Figure 1. Grade 2 significant corneal opacity (yellow arrows) and conjunctival and perilimbal neovascularization (black arrows) are present

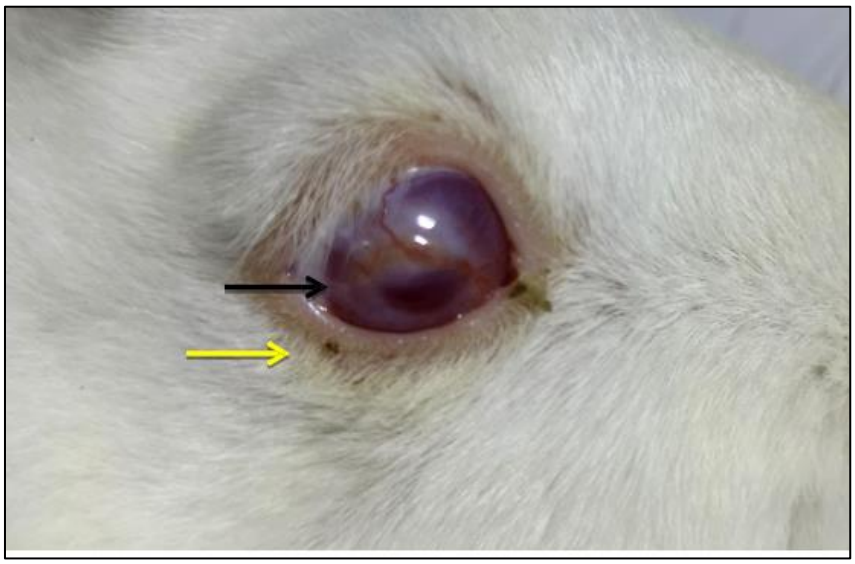

Figure 2 Two weeks after the treatment, the acutely injured cornea showed remarkable morphological alteration. There was intense corneal neovascularization (black arrows) and a grade 2 mild opacity (yellow arrows)

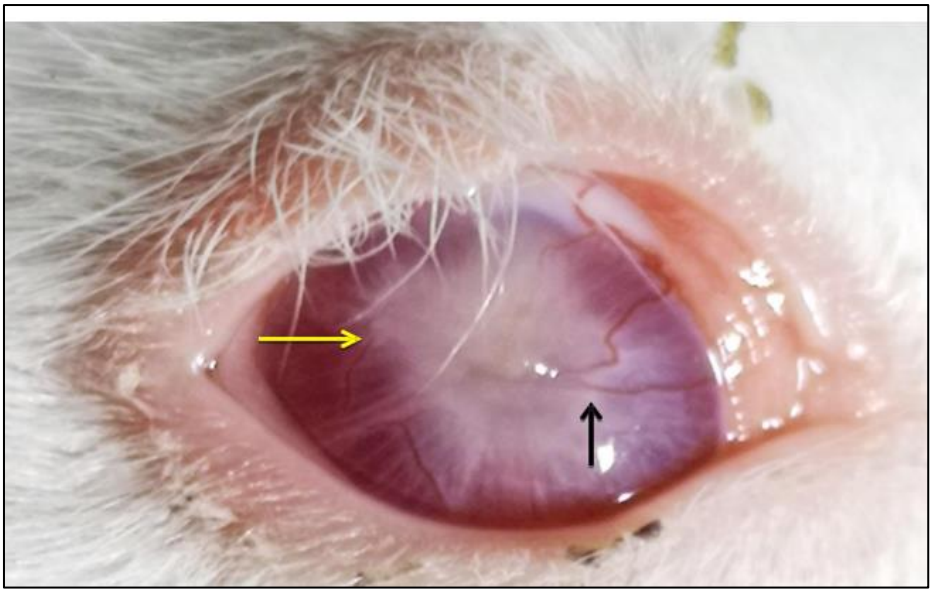

Figure 3 Four weeks after the alkali burn, the damaged cornea's morphological appearance showed grade 2 moderate opacity (yellow arrow) and moderate corneal neovascularization (black arrow)

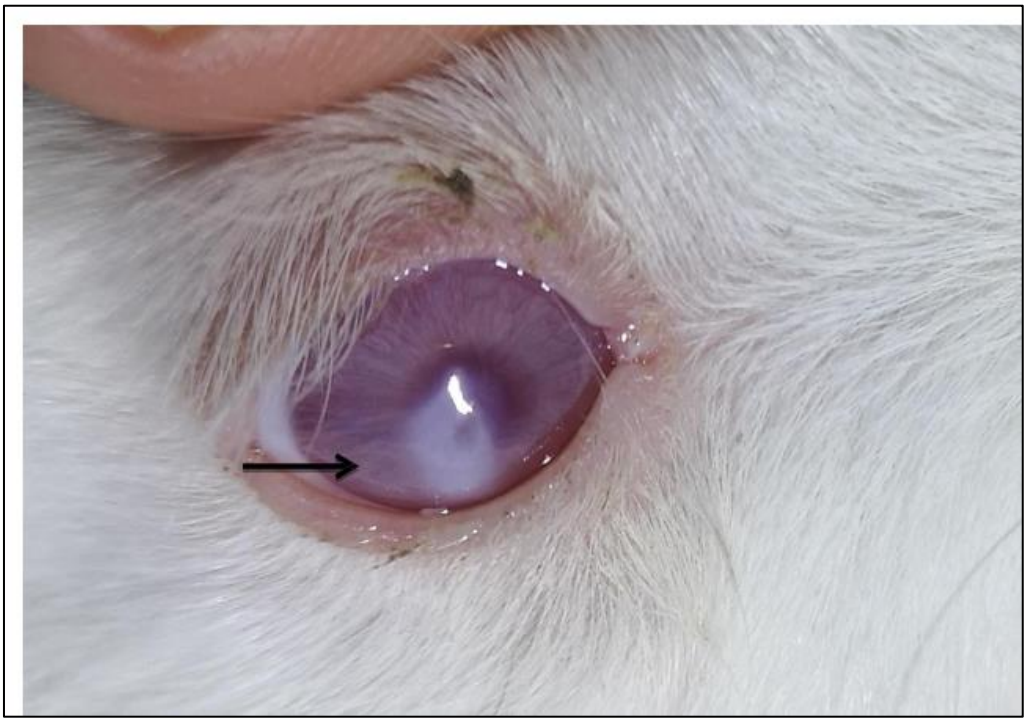

Figure 4 The morphological investigation of the acutely damaged cornea at 4 weeks after treatment revealed a faint cornea opacity equivalent to a Grade 1, as indicated by the arrow

3.2. HGF Expression and ADMSCs' Impact

Our findings show that MSCs have an increased expression of the hepatocyte growth factor (HGF) under inflammatory conditions. Corneas were harvested at 2, 4, and 8 weeks post-injury for HGF detection by quantitative PCR. Remarkably, MSCs corneas showed much higher expression of HGF than control ones. The IOD of HGF in the MSC group was significantly higher than that in the control group ($P<0.05$), as shown in Figure 3 and described in Table 1.

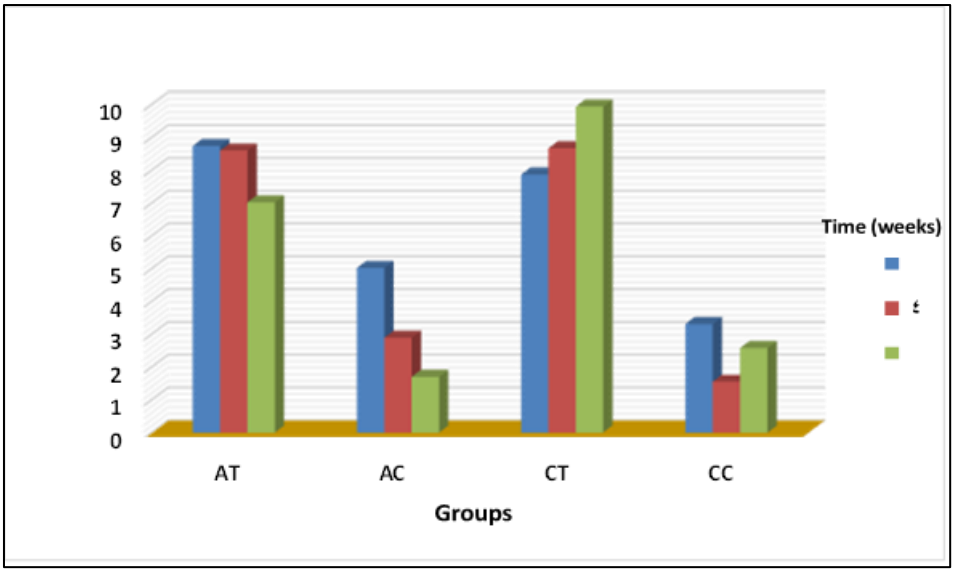

Figure 5 The MSCs promoted the expression of HGF in the corneas of alkali-burned rabbits. Samples of the cornea were taken two, four, and eight weeks following the damage. Real-time q-PCR was used to determine the mRNA expression levels of HGF after total RNA was extracted from these tissues. When compared to the control group, the groups receiving MSC injections had considerably higher HGF expression, and the changes were statistically significant (P<0.05)

Table 1 Expression of the HGF gene

Experimental Groups	Week 2	Week 4	Week 8	Overall Mean ± SD
Group AT	8.74 ± 2.97 Aa	8.60 ± 4.79 Aa	7.02 ± 2.49 Aa	8.12 ± 3.39 A
Group AC	5.02 ± 2.69 Ba	2.89 ± 3.01 Ba	1.70 ± 1.14 Ba	2.90 ± 2.86 B
Group CT	7.87 ± 1.83 Aa	8.67 ± 1.68 Aa	9.94 ± 4.22 Aa	8.83 ± 2.76 A
Group CC	3.31 ± 0.71 Ba	1.55 ± 0.40 Ba	2.58 ± 3.80 Ba	2.48 ± 2.20 B
Time Period Mean ± SD	6.23 ± 3.03 a	5.43 ± 4.29 a	5.09 ± 4.69 a	5.58 ± 4.02

Comparisons in the vertical direction are indicated by capital letters, and in the horizontal direction by lowercase letters. Different letters indicate significant differences at P<0.05.

4. Discussion

Corneal wound healing is necessary to recover with clear vision, as any defect can cause corneal opacification. In the past decade, various growth factors have gained attention for their positive influence on corneal epithelium. We show from our study that adipose-derived mesenchymal stem cells Hepatocyte Growth Factor (HGF) is expressed at a higher level by ADMSCs in the treated group of inflamed corneas than in the control group.. This result is in line with the results of Sharad K. et al 2016.

In the present study, the authors identified shows MSCs generated from human bone marrow (BM) have elevated HGF expression naturally. To mimic the therapeutic implications, the researchers created an eye wound in mice by removing the corneal epithelium and anterior stroma mechanically. MSCs were given intravenously one hour after this injury. The MSCs were labeled with GFP (a fluorescent protein marker) and their targeted migration to the injured eye regions was demonstrated.

Control groups were included in the study for comparison: uninjured corneas and injured corneas not treated with MSCs. The corneas were harvested 3 days post-lesion. The HGF contents in these samples was determined using qPCR and ELISA techniques. These findings demonstrated that injured corneas treated with MSCs had significantly elevated

HGF levels, both in transcripts and proteins, compared to those from injured corneas without treatment and normal corneas. Which of the HGF-mediating effects may be responsible for the MSC-promoted tissue repair.

Those MSC corneas are more transparent than the controls due to the anti-fibrotic properties of HGF. This is in agreement with the results of Liang et al., 1998 that HGF acts as a paracrine growth factor in the corneal epithelium wound healing that promotes mesenchymal–epithelial interaction. In human corneal epithelial cells, activation of HGF on its receptor c-Met triggers mitogen-activated protein kinase (MAPK) cascades via either protein kinase C or the receptor-Grb2/Sos complex to the Ras pathway. Our results are similarly consistent with those of Yong et al. (2016), who found that hepatocyte growth factor is a crucial antifibrotic molecule that works against TGF-β1 to reduce fibrosis in several organs (Okayama et al., 2012). More specifically, HGF stimulates Smad7, which inhibits TGF-β signaling and Smad2 phosphorylation.

5. Conclusion

- Allogeneic mesenchymal stem cells that use topically (subconjunctival) give good repair of corneal healing after alkali burn.
- MSC elicited good result that regarded with inhibited of fibrosis.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The ethics committee of the University of Baghdad, Iraq's College of Veterinary Medicine assessed every technique used in this study. The committee inspector examined the process during the experiment.

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