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(RESEARCH ARTICLE)

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The impact of adipose-derived mesenchymal stem cells on corneal neovascularization after alkali burn in a rabbit model: A clinical and histological investigation

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Abstract

The cornea, the transparent outer layer of the eye, is essential for vision, as it refracts the light to focus images. As the function is vital, corneal inflammation can cause visual disturbances and even blindness. A large number of studies have confirmed that multiple inflammatory diseases involving corneal lead to visual damage and long-term inflammation, which may result in loss of sight. Purpose: To determine effects of adipose derived mesenchymal stem cells (ADMS) on healing of alkali-induced corneal wounds in the rabbit. The protocol included 20 rabbits with induced corneal alkali injuries in both eyes. Subconjunctival injections were administered to half of the rabbits (MSC group, n=10), while the other half (the control group, n=10) were left untreated. The rabbits were subjected to slit-lamp examination and photography for evaluation of corneal neovascularization. Histological studies showed enhanced healing in MSC-treated eyes. Interestingly, significant differences between MSC and control group were evident in corneal neovascularisation and re-epithelialisation as well as in the formation of neovascular tissue at weeks two and four post-surgery.

Keywords: AD-MSCs; Alkali burn; Corneal neovascularization; Rabbit model

1. Introduction

In essence, the cornea, a clear part of the eye, is necessary to focus light so that you can see. As a result of corneal inflammation the vision may be compromised and even blindness may ensue as the cornea is a vital requisite for seeing. There are several reports indicating that a variety of corneal inflammatory diseases lead to chronic corneal inflammation and visual impairment, both of which can result in blindness (1). Since it is an avascular tissue, the cornea is an immunologically privileged site (2). Its unique organization of stromal collagen fibrils and lack of vascularization, at least in the center, are what make it transparent. Between pro- and antiangiogenic mechanisms a dynamic balance can maintain corneal transparency (3). A way to protect against pathogens is corneal neovascularization (NV) (4).

Ocular alkali burn is a common injury worldwide, particularly in developing countries. Caustics often lead to permanent impairment of vision due to extensive corneal destruction (5). Clinically, secondary corneal NV is the most detrimental condition for vision, except recurrent epithelial erosions, corneal ulceration and severe stromal inflammation (6). The transparent, nonvascularized cornea acts as the refractive surface of the eye (7). Numerous leukocytes and macrophages invade the cornea after alkali burn (8). In addition, there was a significant elevation in the concentration of various cytokines in the alkali-burned cornea, including the inflammatory proteins IL-1β, IL-6, and vascular endothelial growth factor A (VEGF-A) (9). The imbalance of proangiogenic and anti-angiogenic factors will induce corneal neovascularization (10).

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Mesenchymal stem cells (MSCs) are a type of multipotent cell originally derived from the bone marrow but have now been isolated from the adipose tissue (11), heart tissu (e12), cord blood (13) and oral tissue. Much of the recent literature has demonstrated multifaceted applications for MSCs such as immunomodulatory, anti-inflammatory, tissue regeneration and tissue repair (14). It has been a subject of recent investigation with encouraging results for the treatment of ocular chemical burns (15). In consideration of the above issues, present study was designed to evaluate the effect of subconjunctival MSCs injection on the early stage of corneal wound healing after alkali burn in a rabbit.

2. Materials and Methods

2.1. Isolation and Management of Mesenchymal Cells Derived from Adipose Tissue

MSC were isolated from rabbit adipose tissue as described previously (16). Overall, ketamine/xylazine mix; consisting of intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg) was applied to anesthetize the animals (17). The inguinal fat pad was rinsed with phosphate-buffered saline (PBS). Subsequently, it was sliced and digested with collagenase type 1 for 1 h at 37°C under shaking. Three distinct layers appeared, as the mixture was dropped at room temperature for 15 min after equal volume of PBS added.

The MSC-laden middle layer was removed and centrifuged at 600×g for 10 min. The cell pellet was resuspended in Dulbecco's modified Eagle's medium containing 5% fetal bovine serum, 100 IU penicillin per millilitre and 100 µg streptomycin per millilitre. Then it was grown in a 37°C, 5% CO2 humidified incubator.

2.2. Animal Model

The study included 20 New Zealand rabbits (n=10 eyes). Both rabbits were 10 months old and weighed approximately 2.5 kilograms. The rabbits were kept in a standard 12-h light-dark cycle room. The study protocol was approved by both the Baghdad University Ethics Committee and the Baghdad Department of Veterinary Medicine Committee. The rabbits were randomly assigned to two groups.. Group 2 received none of these treatments (n = 10), Group 1 was the MSC group (n = 10). Topical Proparacaine hydrochloride 0.5% was applied, then ketamine (50 mg/kg) and xylazine (5 mg/kg) were given intramuscularly to all rabbits in each group to induce anesthesia. A 5% povidone-iodine solution was instilled into the conjunctival sac. A Sodium hydroxide (NaOH)-soaked (1 N) Whatman Filter Paper (Whatman, Maidstone, Kent, UK) with a diameter of 3 mm was put on the upper cornea for 30 seconds to induce alkali injury. This was then chased by a 1-min washoff with a balanced salt solution. In the first week, the animals received the 0.1% dexamethasone and 0.3% tobramycin solution three times a day. 2×106 MSCs in 0.5 ml PBS were injected into the subconjunctival space of the injured eyes immediately after the alkali injury induction and eye drops were administered. The subjects in the wait-list group received no treatment.

2.3. Clinical Evaluation

The animals' eyes were examined for opacification and neovascularization. Every rabbit was examined under a slit light. Following the formation of the alkali injury, as well as during the second and fourth weeks following surgery, pictures were taken as a follow-up.

2.4. Histology

Samples of eye tissue were embedded in paraffin after being fixed in 10% formalin. Hematoxylin and eosin (H&E) was used to stain eye sections (2.5 µm) for connective tissue staining and morphological assessment. A pathologist assessed each sample.

2.5. Statistical Analysis

SAS software (version 9.1) was used to analyze the data. To evaluate significant differences between means, two-way ANOVA and the least significant difference (LSD) post hoc test were used. A P-value of less than 0.05 was deemed statistically significant.

3. Results

3.1. Effect of ADMSCs on Corneal Neovascularization

In contrast to the ADMSC group, which showed decreased NV, representative photos showed that the area of NV significantly expanded with time in the control group. Both the control and ADMSC groups' corneas had neovascularization. Photographs

obtained two weeks after corneal alkali burn in both groups (acute and chronic) showed that the corneal surface was covered with neovascular tissue in both the ADMSC and control groups. This showed that new vessels erupted from the nearby limbus and seemed fine and dense. At this point, most of the arteries were growing around the limbus in the direction of the wounded corneal region (Figures 1 and 2, taken at four weeks). Neovascularization increased in both the control and ADMSC groups following corneal alkali burn (acute and chronic), although neovascular tissue vanished in the ADMSC group in contrast to the control group (Figures 3 and 4).

Figure 1 Two weeks after the alkali burn, the acutely wounded cornea's morphological appearance showed grade 2 moderate opacity (yellow arrow) and conjunctival and perilimbal neovascularization (black arrow)

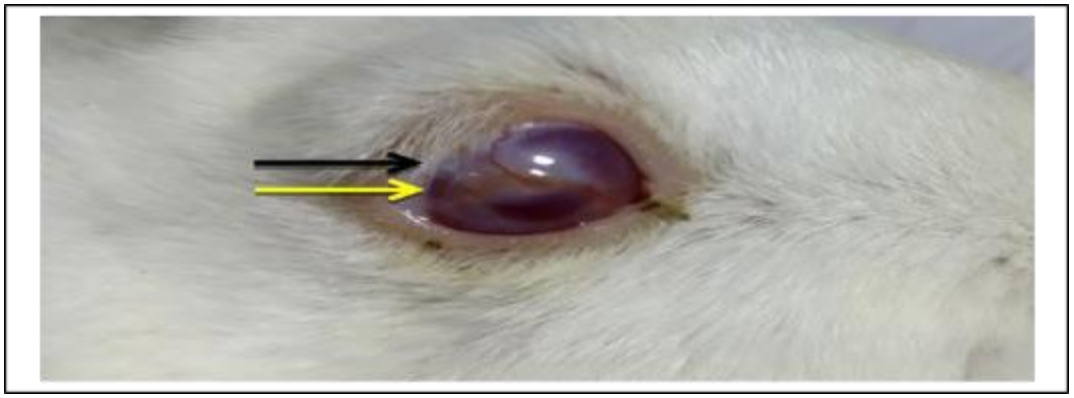

Figure 2 The critically wounded cornea's morphological appearance two weeks after therapy, showing grade 2 moderate opacity (yellow arrow) and extensive corneal neovascularization (black arrow)

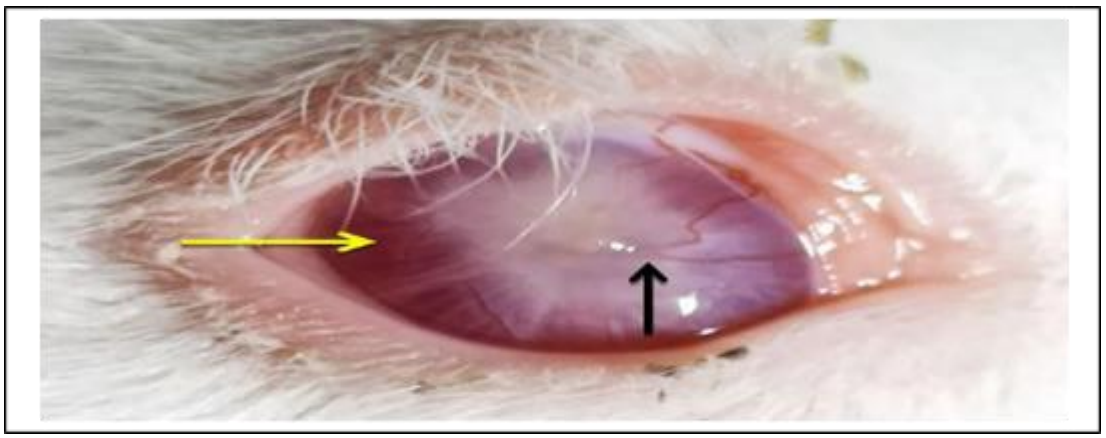

Figure 3 Four weeks after alkali, the damaged cornea's morphological appearance showed grade 2 moderate opacity (yellow arrow) and moderate corneal neovascularization (black arrow).

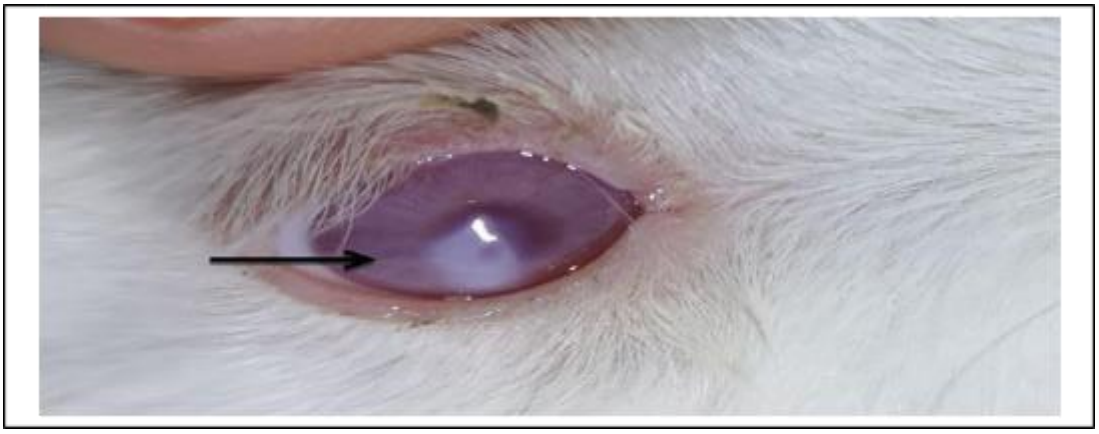

Figure 4 Four weeks after therapy, the acutely wounded cornea's morphological morphology showed the moderate corneal opacity grade 1 (arrow)

3.2. Histopathologically

A cellular fibrotic deposition, a focal increase in basal epithelium, a marked irregular thinning of corneal epithelium with a long finger-like projection, and a severe disruption of corneal stromal structure, primarily in the interior portion with evidence of cyst formation and corneal edema, are all present in Figure 5. The corneal epithelium seemed thin and wrinkled with goblet cells, as seen in figure 6. Bubbles are seen, and the Bowman layer and basal epithelium have completely disappeared..

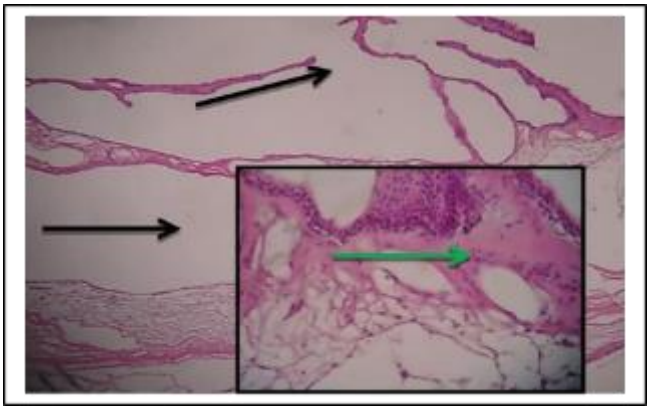

Figure 5 Two weeks after the injury, a histological segment of the rabbit cornea (acute control group) showed a significant disruption of the corneal stroma and epithelium, with substantial stromal edema (black arrow), localized epithelial hyperplasia, and subepithelium fibrosis deposition (green arrow) (H and E X10, 40)

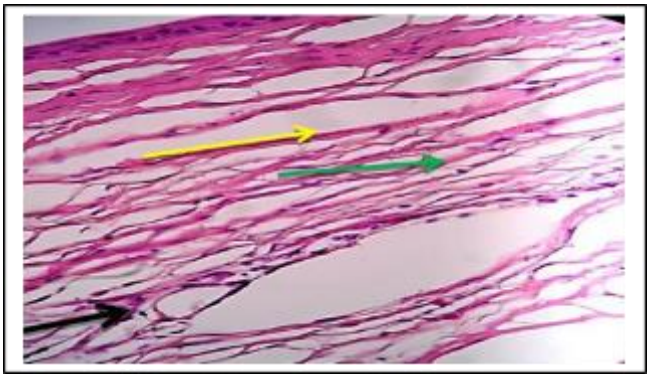

Figure 6 Two weeks after the injury, a histological slice of the rabbit cornea from the acute control group showed modest stromal vascularization (black arrow), mild inflammatory infiltration (yellow arrow), and irregular collagen bundles (green arrow) (H and E X40).

Anteriorly, there is noticeable stromal edema with necrotic stromal patches.

Based on figures 7 and 8, corneal examination showed sparsely dispersed keratocytes, a highly vascular collagenous stroma with heterogeneous cellular infiltration, and moderate irregular epithelialization with no basal epithelium or Bowman layer. The corneal surface lining was shown in Figure 5 with a multilayer of epithelium and prominent basal epithelium, as well as a regular anterior stromal with many keratocytes and few arteries and a bowman layer that was comparable to normal.

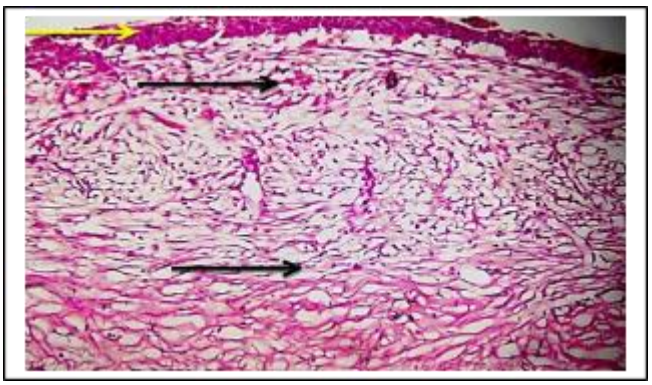

Figure 7 Histological slice of rabbit cornea two weeks after MSC implantation (acute treatment group), showing vascular collagen stromal with mixed cellular infiltration (plasma cells and lymphocytes) (black arrow) and modest irregular epithelialization (yellow arrow) (H and E X10)

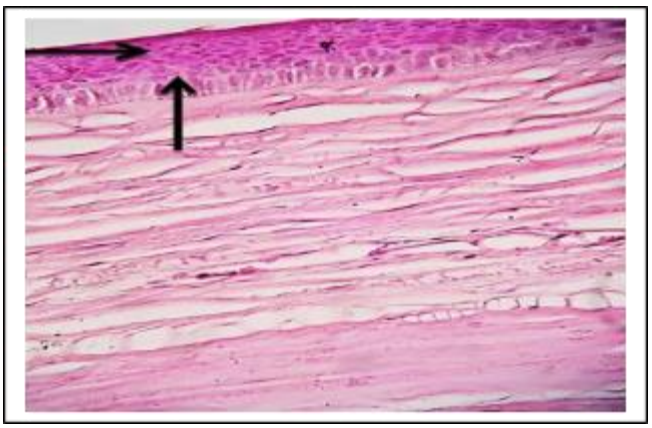

Figure 8 Histological section of rabbit cornea at four weeks post MSC transplantation (acute treatment group) demonstrating a multilayer epithelium covering the surface of the cornea, basal epithelium and Bowman's layers are distinctly apparent (black arrow) (H and E X40)

4. Discussion

MSCs are a viable therapeutic approach for corneal restoration because of their many advantages. First of all, they are multipotent progenitor cells that are readily extracted from a variety of tissues, including bone marrow (18), adipose tissue, peripheral blood (19), and the limbal stroma of the human eye (20) (21). According to reports, MSCs may self-renew as undifferentiated cells before differentiating into various mesenchymal tissues (22). Most significantly, MSCs may inject into an allogeneic host without being rejected and evade immune system surveillance (23).

The current study's findings showed that using allogeneic MSCs in a rabbit model of corneal damage resulted in a more notable decrease in corneal NV. This result is consistent with a prior work by Pirounides, Komnenou (24), who showed that MSC provided by three distinct methods successfully blocked the creation of new vasculature when given to the rabbit right after incision. MSC transplantation has been administered by a variety of methods, including intrastromal injection (25), subconjunctival administration (26), systemic administration (6), and nanofiber scaffolds (27).

Ghazaryan, Zhang (28) proposed that subconjunctival injection overcomes the challenges of amniotic membrane-associated MSC transplantation and lowers VEGF levels, leading to superior results, in line with the current study's findings. By sub-conjunctivally administering 2x106 MSCs in 0.5ml PBS, we assessed the therapeutic effectiveness of MSCs. An image was obtained after two and four weeks. Following the injury, the MSC had a smaller neo-vascularized region than the control group. In a 28-day postoperative follow-up, the MSC group's corneas recovered more quickly than those of the control group. Through a paracrine signaling pathway, mesenchymal stem/stromal cells produce soluble chemicals that suppress angiogenesis and lower VEGF levels (15), while simultaneously upregulating TNF-stimulated gene-6 (TSG-6) (29). Exosome secretion may mediate MSC paracrine action, according to a number of studies examining vascular and arterial disorders (30).

5. Conclusion

Allogeneic mesenchymal stem cells that use topically (subconjunctival) showed good repair of cornea MSC showed good result that regarded with inhibited of neovascularization.

Compliance with ethical standards

Disclosure of conflict of interest

The writers affirm that they have no competing interests.

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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