Insights in Stem Cells

Extended Abstract
Vol. 5, Iss. 3
2019

Stem Cell Research 2018-Comparative Light and Electron Microscopic Study on the Therapeutic Efficacy of Adipose Derived Stem Cells Versus Exosomes for Experimentally Induced Acute Corneal Injuries in Rats-Nahla El-Eraky El-Azab- Benha University

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Introduction

Cornea is a specialized transparent avascular tissue that has a dome-shaped structure with smooth surface and it is formed of three functional layers. The location of the cornea is over the pupil, the iris, and the anterior chamber of the eye. It provides a clear vision by refracting light onto the lens and centralizing on the retina. Cornea protects the eye against infections, abrasions, and structural damage to deeper tissues. It contains self-renewal cells that have massive regeneration capacity. After minor scratches, the cornea can improve quickly; otherwise the deeper scars can lose their transparency and disturb the vision. Corneal damage can be induced through many factors such as chemical, mechanical, or thermal injury. Chemical injuries, especially those produced by alkaline agents, are a worldwide common cause of ophthalmologic emergency especially in developing countries in young age groups.

The approaches to the treatment of corneal alkali burn are based on using one member of the following medical and operative therapeutic strategies such as steroids, non-steroidal anti-inflammatory agents, citrate, argon laser photocoagulation, photodynamic therapy, artificial tears, collagenase inhibitors, therapeutic contact lenses, topical fibronectin, conjunctival transplantation, amniotic membrane repairing and limbal transplantation. Occasionally, these therapies are ineffective, particularly for large inflammatory Corneal Neovascularization (CNV) and thus insight is directed towards alternative treatment modalities for eliminating those dangerous drawbacks.

ADSCs are a new type of MSCs that are plentiful in individuals. They include a type of multipotent adult stem cells isolated from adipose tissue and have the capacities of self-regeneration and differentiation into various cell lineages. Both BMSCs and ADSCs have common biological properties such as cell surface markers, gene expression profile, immunosuppressive features, and differentiation capacity, but ADSCs have more proliferative capacity than that of BMSCs. ADSCs have been used in a recent technique for wound repair, regeneration, and tissue engineering procedures because the adipose tissue is easy to gain and process; in addition, the isolated cells expand rapidly and differentiate into different cell types in vitro. Exosomes are products of endocytosis. They are small vesicles with a bilipid membrane, their diameter ranges from 30-150 nm, and they have abundant proteins, lipids, mRNAs, and microRNAs for transmitting hereditary information. They are released by all cell categories and found in blood, urine, breast milk and play valuable role in intercellular communication by protein and RNA delivery. They are released physiologically under ordinary circumstances, but their rate of release is increased under pathological states such as degeneration and tumors. Nowadays, exosomes have a vital role in treatment of many diseases such as liver fibrosis, acute kidney injury, myocardial infarction, ischemia induced neural degeneration and wound repair. The aim of the study is to evaluate the efficacy of ADSCs versus MSCs-EX on experimentally alkali-induced corneal injuries in rats.

Materials and Methods

40 adult male rats of average weight 150-200 g were utilized in this study. Rats were held in the laboratory animal house unit of Kasr Al-Ainy Faculty of Medicine, Cairo University, Cairo, Egypt. Strict maintenance and cleaning measures were applied to keep the animals in

This work is partly presented at 10th World Congress and Expo on Cell & Stem Cell Research March 19-21, 2018 held in New York, USA

a normal healthy state. They were housed in animal cages at room temperature (25 ± 1°C) and relative humidity (55 ± 5) with 12 hrs light/12 hrs dark cycle and nourished balanced diet and water ad libitum. All ethical rules for animal treatment were followed and were managed via the animal facilities. The experimental protocol was approved by the Institutional Animal Care Committee of Cairo University, Cairo, Egypt.

Isolation, phenotype and features of ADSCs

ADSCs were prepared from adipose tissue gained from flanks and inguinal areas of healthy 9 week-old rats (n=10). Adipose tissue was hydrolysed by collagenase type II (Sigma, USA) liquefied in Phosphate Buffer Saline (PBS); Gibco/Invitrogen, Grand Island, New York, USA) for 2 hrs at 37°C. 2 µm filters were used to eliminate all tissue debris, followed by centrifugation at 1,000 rounds per minute (rpm) for 5 min to form a cell pellet which was cultured with a RPMI medium (Gibco BRL, USA) and 10% fetal bovine serum (FBS, Gibco BRL, USA). The culture was humidified in a cell culture incubator containing 5% carbon dioxide at 37°C. At 70-90% adipose MSCs confluence, it was separated with 0.25% trypsin-EDTA (Gibco BRL, USA) and resuspended in other flasks. Fourth-passage ADSCs were applied in the whole experiments. The ADSCs were characterized in culture by their morphology, spindleshaped cells. Additional identification of adipose MSCs was established by identification of CD29 and CD44 surface markers using flow cytometry (Beckman Coulter).

Transmission Electron Microscope (TEM) of MSCs-EX:

Exosomes were gently placed on Formvar-coated copper grids. They were allowed to be adsorbed for 45 min and then handled for standard uranyl acetate staining. The grids were irrigated with PBS three times and left to semidry at room temperature prior to examining in TEM. Images were obtained by using TEM JEOL (JEM-2100; Akishima, Tokyo, Japan) at an accelerating voltage of 80 kV [19].

Labelling of ADSCs with PKH26 dye: ADSCs were harvested in the fourth passage and labelled with the PKH26 (Sigma-Aldrich, St. Louis, MO, USA) [5]. Fluorescent linker dye PKH26 is a red fluorochrome having 551 nm excitation and 567 nm emission that binds irreversibly to cell membranes. Cells were centrifuged and washed twice in a serum-free medium. They were pelleted and suspended in stain solution.

Conclusion

ADSCs and exosomes can improve corneal alkali burn injuries and prevent their complications through anti-inflammatory and antiangiogenic roles. MSCs-EX are a very promising approach and are considered better than ADSCs as they are safer than stem cells and their nano-dimension can easily cross through biological barriers and enter target organs.

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This work is partly presented at 10th World Congress and Expo on Cell & Stem Cell Research March 19-21, 2018 held in New York, USA

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