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Therapeutic potential of Regener-Eyes® Ophthalmic Solution in the treatment of dry eye disease

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Abstract

Dry eye disease (DED) is a common and multifactorial disease of the ocular surface characterized by a deficiency in quality and/or quantity of the tear fluid. A detrimental immune response has an important role in the development and progression of DED. Dr. Harrell recently developed Regener-Eyes® (generic name "derived-Multiple Allogeneic Proteins Paracrine Signaling [d-MAPPS]"), an ophthalmic solution that contains a large number of immunoregulatory factors that are capable of penetrating the ocular surface and to efficiently attenuate the detrimental immune response in the eye, promoting repair and regeneration of injured tissue. Regener-Eyes® efficiently alleviated DED-related symptoms (dryness, grittiness, scratchiness, soreness, irritation, burning, watering, foreign body sensation, eye fatigue) and improved functional visual acuity in 131 DED patients, without causing any side effects.[12] Herewith, we described in detail the molecular mechanisms and signaling pathways that are responsible for the immunomodulatory effects of Regener-Eyes®, thereby exploring the therapeutic potential of Regener-Eyes® in the treatment of DED.

Introduction

Dry eye disease (DED) is a common and multifactorial inflammatory disease of the ocular surface characterized by a deficiency in quality and/or quantity of the tear fluid.[1] The multifactorial nature of DED involves several interrelated underlying pathologies, including the loss of homeostasis, instability and hyperosmolarity of the tears, and chronic eye inflammation that leads to the neurosensory dysfunction and visual disturbance. Accordingly, DED is usually manifested by dryness, grittiness, scratchiness, soreness, irritation, burning, watering, foreign body sensation, eye fatigue, and reduced functional visual acuity. Significantly impaired performance of vision dependent daily activities (reading, writing, driving) often diminishes the quality of life of DED patients.[2]

A detrimental immune response has played a crucially important role in the development and progression of DED. Accordingly, DED-related symptoms are often observed in patients who suffer from chronic inflammatory and systemic autoimmune diseases (Sjögren's syndrome, rheumatoid arthritis, systemic lupus erythematosus).[3–7] Considering the important role of inflammation in DED development, the main treatment strategy has shifted from hydration and lubrication of the dry ocular surface to the immunoregulation-based therapeutic approach, which is designed to break the vicious cycle of

continued on page 2 ▶▶

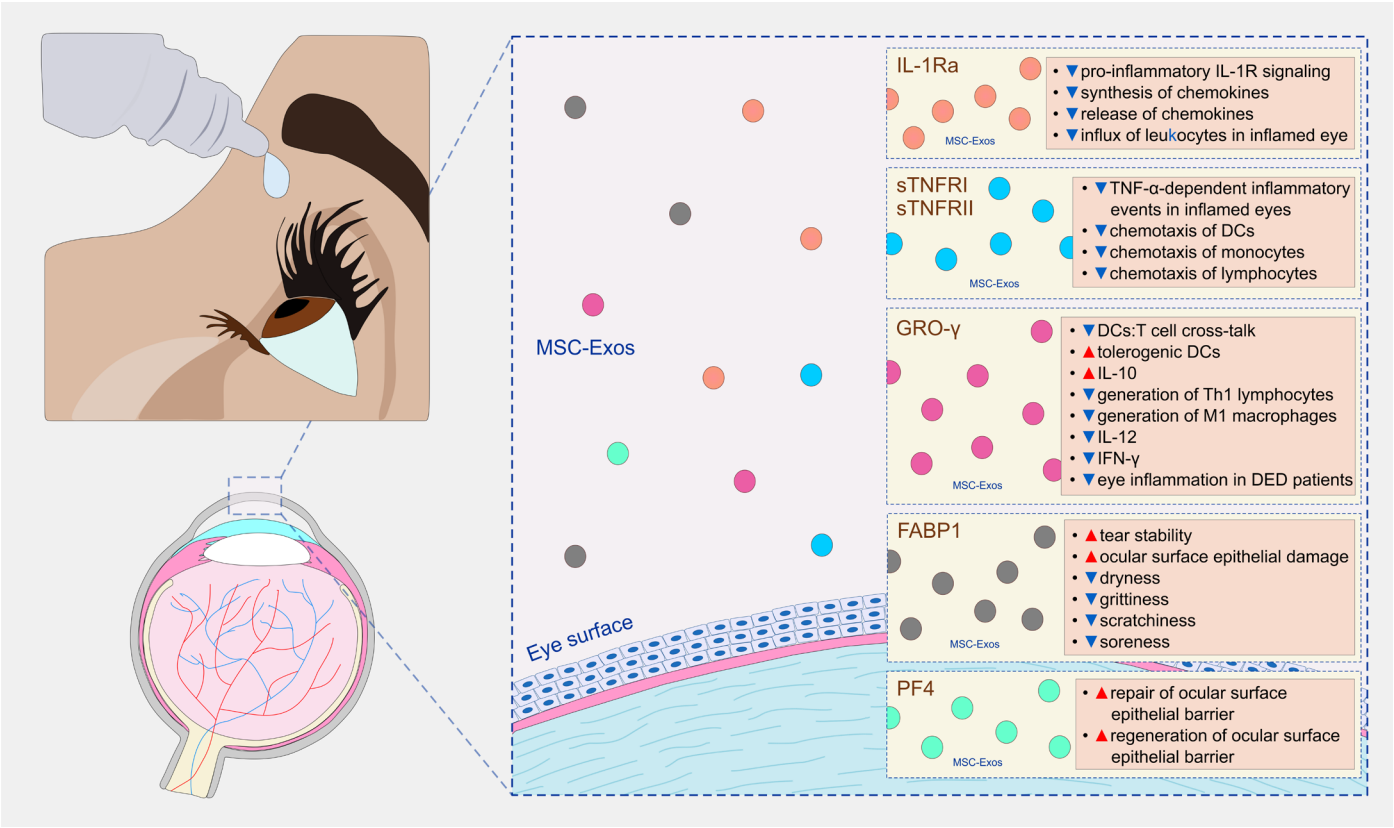
chronic inflammation in the eye.[3–6] The administration of immunosuppressive eye drops has the potential to attenuate the ongoing inflammation, resulting in the alleviation, as the well-developed protective mechanisms of the ocular surface ensure their rapid clearance from the pre-corneal tear film, thus limiting ocular penetration of the drug.[8] Therefore, there is a large unmet need for the development and clinical use of eye drops containing immunomodulatory factors that are able to bypass the ocular surface barrier and reach the target cells of the ocular surface and lacrimal system.[8]

In line with these findings, Dr. Harrell recently developed Regener-Eyes® (generic name "derived-Multiple Allogeneic Proteins Paracrine Signaling [d-MAPPS]"), an ophthalmic solution that contains a large number of immunoregulatory factors that are capable of penetrating the ocular surface to efficiently attenuate the eye's detrimental immune response, which may help to promote the repair and regeneration of damaged corneal tissue.[9] Herein, we described in detail the molecular mechanisms and signaling pathways that are responsible for the immunomodulatory effects of Regener-Eyes®, exploring the therapeutic potential of Regener-Eyes® in the treatment of DED.

Molecular mechanisms responsible for beneficial effects of Regener-Eyes® in DED treatment

Although Regener-Eyes® is acellular, it contains proteins, cytokines in addition to the water, glucose, lactates and electrolytes, and placental-derived biomaterials, which produce a large number of bioactive factors (lipids, proteins, enzymes, cytokines, chemokines, immunoregulatory proteins, trophic and growth factors), as well as microRNAs (miRNAs), which, due to their trophic and antimicrobial properties, support normal fetal growth and offer protection against pathogens and toxins.[10] Additionally, these placental biomaterials contain AF-MSC-sourced exosomes (AF-MSC-Exos), nano-sized extracellular vesicles that are enriched with AF-MSC-derived immunosuppressive molecules and growth factors.[10–11] Due to their nano-sized dimensions and lipid envelope, AF-MSC-Exos avoid biological barriers, and easily penetrate through the lipid-containing cell membranes of the ocular surface through direct fusion with the plasma membrane, thereby delivering their content to the cytosol of target cells.[10–12]

Regener-Eyes® is an engineered biological product derived from human placental-based biomaterials, manufactured under current Good Manufacturing Practices (cGMP), regulated and reviewed by the Food and Drug Administration (FDA).[9] Regener-Eyes® incorporates Regenerative Processing Plant's (RPP) proprietary patented sterilization process to provide for a safe, sterile product for clinical use.[9] Regener-Eyes® is enriched with AF-MSC-Exos containing AF-MSC-derived immunoregulatory, angio-modulatory and trophic factors capable of bypassing biological barriers to efficiently attenuate ongoing inflammation, promoting enhanced tissue repair and regeneration.[8] Specifically, Regener-Eyes® contains interleukin 1 receptor antagonist (IL-1Ra), soluble receptors of tumor necrosis factor alpha (sTNFRI, sTNFRII), growth-related oncogene gamma (GRO-γ), fatty acid-binding protein 1 (FABP1) and platelet factor 4 (PF4), which alleviate eye inflammation, support tear



Molecular mechanisms responsible for beneficial effects of Regener-Eyes®-containing eye drops in the management of DED. Regener-Eyes® contains a large number of immunoregulatory molecules, trophic and growth factors that attenuate ongoing inflammation and may help to promote repair and regenerate the epithelial barrier at the ocular surface of patients suffering from dry eye disease (DED). By delivering immunoregulatory molecules (interleukin 1 receptor antagonist [IL-1Ra], soluble TNF receptors [sTNFRI, sTNFRII], growth-related oncogene gamma [GRO-γ]), Regener-Eyes® inhibits detrimental immune response in inflamed eyes of DED patients, while, by delivering growth and trophic factors (fatty acid-binding protein [FABPI] and platelet factor 4 [PF4]), Regener-Eyes® supports tear stability and prevents injury of epithelial cells. This may contribute to the enhanced repair and regeneration of the epithelial barrier at the ocular surface of DED patients.
Source: Regener-Eyes®

stability, and prevent ocular surface epithelial damage, contributing to the enhanced repair and regeneration of ocular surface epithelial barrier in DED patients.[1,9,11–13]

IL-1Ra is a naturally occurring cytokine that acts as an inhibitor of inflammatory cytokine IL-1β that has a crucially important role in the recruitment of circulating leukocytes in inflamed eyes of DED patients.[5–6,12,14] Alterations in tear production and composition, particularly elevated osmolarity, activates c-Jun N-terminal kinase and NF-kβ signaling pathways in the epithelial cells of DED patients, which result in enhanced secretion of pro-inflammatory cytokine IL-1β.[11] IL-1β induces enhanced expression of adhesion molecules on endothelial cells, enabling the massive influx of antigen-presenting dendritic cells (DCs), macrophages and circulating lymphocytes in the lacrimal glands and ocular surfaces of DED patients.[5–6,12,15] When Regener-Eyes® containing IL-1Ra binds to the IL-1 receptor

continued on page 4 ▶▶

(IL-1R) on the endothelial cells of the eyes of DED patients, binding of IL-1β to IL-1R is blocked and the pro-inflammatory signals from IL-1R are stopped. Accordingly, various pro-inflammatory events, initiated by IL-1β:IL-1R binding, including the synthesis and releases of chemokines and the enhanced influx of leukocytes in inflamed eyes, are inhibited by Regener-Eyes® containing IL-1Ra.[1,9,15]

IL-1β acts synergistically with TNF-α to induce the enhanced recruitment of monocytes and lymphocytes in inflamed lacrimal glands and eyes of DED patients.[5–6] The binding of TNF-α to TNF-α receptors on endothelial cells attract circulating DCs, monocytes and lymphocytes, thereby creating an inflammatory loop within inflamed eyes.[5–6] Regener-Eyes® contains sTNFRI and sTNFRII, which bind to TNF-α and prevent TNF-α-dependent recruitment of circulating inflammatory immune cells in the eyes of DED patients.[1,9]

Cross-talk between IL-1β and TNF-α-recruited DCs, T cells, and macrophages on the ocular surface and inflamed lymph nodes is crucially important for the development and progression of DED.[4–5] DCs capture antigens and present them to the naive CD4+ T cells in regional lymph nodes. DCs, through the secretion of IL-12, induce differentiation of naive CD4+ T cells in effector, IFN-γ-producing Th1 cells, which, in turn, in an IFN-γ-dependent manner, promote polarization of resident macrophages in the inflammatory M1 phenotype.[5] In the eyes of DED patients, inflammatory (M1) macrophages produce large amounts of TNF-α, nitric oxide, and matrix metalloproteinases, which disrupt the epithelial barrier of the ocular surface.[5–6] Regener-Eyes® significantly attenuates the concentration of IL-12 in the supernatants of activated human peripheral blood mononuclear cells (pbMNCs) and alleviates production of IFN-γ in activated lymphocytes.[15] Regener-Eyes® contains GRO-γ, which is able to suppress DCs:T cell cross-talk and efficiently inhibits the DC-dependent generation of inflammatory Th1 cells.[9,15] GRO-γ-treated DCs had a tolerogenic phenotype characterized by increased secretion of immunosuppressive IL-10, and reduced production of inflammatory cytokines IL-12 and IFN-γ.[16] Accordingly, it is expected that topical administration of Regener-Eyes® affects the cross-talk between antigen-presenting, IL-12-producing DCs and naive CD4+ T cells in lymph nodes, thereby preventing DC-dependent generation of IFN-γ-producing Th1 lymphocytes. Since IFN-γ-producing Th1 lymphocytes enhance the inflammatory properties of macrophages and induce their polarization in pro-inflammatory (M1) cells, by delivering GRO-γ, Regener-Eyes® may prevent Th1 cell-dependent activation of intraocular M1 macrophages and attenuates M1 macrophage-driven eye inflammation in DED patients.[1,9,15]

Downregulated levels of FABP proteins were noticed in the tears of patients suffering from Sjögren's syndrome and DED.[17] FABP proteins regulate transepithelial water transport and maintain the epithelial barrier at the ocular surface.[17] Accordingly, the reduced expression and production of FABP proteins leads to disturbances in the epithelial barrier, causing increased tear evaporation and DED.[17] Regener-Eyes® contains a high concentration of FABP1 proteins, which are thought to regulate transepithelial water transport, support tear stability, and prevent ocular surface epithelial damage in the eyes of DED patients, resulting in the possible

alleviation of dryness, grittiness, scratchiness, and soreness.[1,9,17]

A topical administration of platelet-rich plasma eye drops that contains a large amount of PF4, epithelial growth factors, fibroblast growth factors, and vascular endothelial growth factor successfully treated moderate to severe DED. Regener-Eyes® contains a high concentration of PF4, which may promote the repair and regeneration of injured epithelial cells on the ocular surface.[1,9,18] Therefore, the beneficial effects of Regener-Eyes® may be partially explained by the regenerative and protective properties of PF4.[1,9]

Experimental and clinical evidence of Regener-Eyes®-based efficacy in DED treatment

We recently demonstrated that Regener-Eyes® may protect corneal epithelial cells from chemical injury.[12] While cytoplasm vacuolization and swelling, accompanied by the loss of cell-to-cell contact, were observed in benzalkonium chloride (BAC)-treated human corneal epithelial cells (HCEC) in vitro, these morphological and functional changes were not seen in BAC-treated HCEC that grew in the presence of Regener-Eyes®.[12] Additionally, Regener-Eyes® significantly improved viability of BAC-injured HCEC while protecting them from BAC-induced chemical injury.[12]

In line with these results are findings obtained in clinical settings.[12] Regener-Eyes® was shown to help efficiently alleviate ocular discomfort and pain in a study of 131 DED patients (27 males and 104 females with a median age of 62 years [range 19–85]) during a 12-month follow-up period.[12]

Decreases in VAS and SPEED scores in the Regener-Eyes®-treated DED patients were documented 3 months after the administration of Regener-Eyes®, while the highest reduction in VAS and SPEED scores in these patients were observed after 12 months of Regener-Eyes®-based therapy, indicating the increasingly beneficial effects of long-term use in alleviation of ocular symptoms in DED patients.[12] Importantly, Regener-Eyes® was well tolerated. None of 131 Regener-Eyes®-treated DED patients reported any side effects related to the Regener-Eyes® therapy, suggesting that topical application of Regener-Eyes® is a safe and effective therapeutic approach in DED treatment.[12]

Dry eye cases treated with Regener-Eyes®

(Courtesy of Marguerite McDonald, MD)

Case #1, M.R.

A 26-year-old female nurse presented 4 years ago with severe dry eye and epithelial basement membrane disease (EBMD) with recurrent erosion syndrome OU. She has undergone PTKs OU (2017 and 2019, respectively), though symptoms have returned, as new areas of EBMD have appeared outside the prior PTK treatment zones. She is reluctant to have more laser surgery, as she has already lost a lot of time to recurrent erosion episodes and fears the additional downtime will put her job at risk.

M.R. had a best corrected VA of 20/25 –2 OD and 20/30 –2 OS. After being on Regener-Eyes® Professional Strength QID OU for 4 weeks, her best corrected vision improved to 20/20 OU, and she has had

continued on page 6 ▶▶

no recurrent erosion symptoms. M.R. has now been on Regener-Eyes® Professional Strength for 4 months and is symptom-free.

Case #2, S.P.

A 46-year-old male with Sjögren's syndrome presented with extreme pain in both eyes and blurred vision. He was taking cyclosporine emulsion BID OU, preservative-free tears q 1 hour while awake OU, serum tears q 2 hours while awake, bland nighttime ointments OU, and omega-3 nutritional supplements, with minimal relief.

S.P.'s best corrected visual acuity was 20/100 OD and 20/70 OS. His most remarkable findings on slit lamp exam were 4+ corneal filaments OD, 2+–4+ OS, and a markedly decreased tear meniscus OU. His tear osmolarity readings were 391 mOsm/L OD and 359 OS.

Regener-Eyes® Professional Strength QID OU was added to the above regimen, with the exception of serum tears, which he was advised to discontinue. S.P. returned after 1 month with a best corrected visual acuity of 20/50 OD and 20/40 OS, with 2+ filaments OD and 1+–2+ OS. His pain is much improved. He is continuing his current regimen and will return in 2 months.

Case #3, T.R.

A 64-year-old female with a long history of Sjögren's syndrome presented with a non-healing epithelial defect OS. She had been given a course of valacyclovir 1 gram PO TID X 10 days, as well as Zirgan gel (ganciclovir, Bausch + Lomb) 5 X a day OS for 10 days, with no improvement. An amniotic membrane had been inserted but was immediately removed due to the patient's significant pain. T.R. was taking preservative-free tears q 2 hours while awake OU and bland ointment OU at night. On presentation, her best corrected acuities were 20/30 OD and 20/40 OS. A slit lamp exam revealed a 3 X 5 mm epithelial defect in the mid-peripheral cornea OS, in the supertemporal quadrant at 2 o'clock.

Regener-Eyes® Professional Strength QID OU was begun; preservative-free tears q 2 hours while awake OU and bland ointment QHS OU were continued.

On T.R.'s next visit 1 week later, the epithelial defect was healed for the first time in 4 months. The current regimen was continued.

Meibomian gland dysfunction (MGD)/meibomian gland regeneration (MGR)

Meibomian gland dropout and altered meibum secretion were usually seen in the patients suffering from DED.[19–20] Both congenital and acquired meibomian gland dysfunction (MGD) results in increased tear film osmolarity and leads to the development of evaporative DED.[19–20] We recently demonstrated the beneficial effects of Regener-Eyes® in the treatment of MGD-related DED.[13] In one case report, Regener-Eyes® promoted regeneration of injured meibomian glands and efficiently attenuated DED-related symptoms in a patient suffering from MGD.[13] Before the topical application of Regener-Eyes®, the meibomian ducts of this MGD patient were dilated, exhibiting enlargement and

tortuosity.[13] The morphology of the meibomian glands was significantly improved after 3 weeks of Regener-Eyes® therapy showing the hypo-illuminescent grape-like clusters. Similarly, hyper-illuminescent ducts tarsus indicated beneficial effects of Regener-Eyes® in restoration of meibomian gland and ducts morphology.[13] Additionally, Regener-Eyes® significantly improved DED-related symptoms in this MGD patient.[13] Before topical application of Regener-Eyes®, an MGD patient reported foreign body sensation and pain in the eyes, which were accompanied with grittiness, soreness, irritation, burning, and eye fatigue. Importantly, none of these DED-related symptoms were reported by the MGD patient after 3 weeks of Regener-Eyes® therapy.[13] Significantly improved tear film breakup time (TBUT) was noticed 3 weeks after Regener-Eyes®-based treatment, indicating restoration of meibomian gland function.[13] Complications such as ocular pain, persistent bleeding, and infections were not observed during or after the administration of Regener-Eyes®. This MGD patient did not report any adverse effects related to the Regener-Eyes®-based therapy, confirming that Regener-Eyes® is well tolerated and safe for topical application.[13]

Approximately 1 of 10 patients suffering from dry eye has underlying Sjögren's syndrome, an autoimmune disease characterized by immune cell-dependent destruction of lacrimal and salivary glands, ocular discomfort, and visual dysfunction.[21] Since Sjögren's syndrome-related dry eye is a progressive inflammatory condition, it may lead to corneal perforation, uveitis, scleritis, retinal vasculitis, and optic neuritis. Regener-Eyes® contains immunoregulatory, trophic and neuroprotective factors that could attenuate ongoing inflammation in the eye, promote epithelial cell proliferation, and prevent neural injury. Accordingly, significantly improved visual acuity, relieved ocular pain and complete healing of corneal epithelial defects were noticed in a Regener-Eyes®-treated patient with Sjögren's syndrome. Similarly, 4 weeks of Regener-Eyes®-based therapy remarkably improved visual acuity and significantly decreased ocular pain in a 26-year-old female who suffered from severe DED and epithelial basement membrane dystrophy (EBMD) with recurrent corneal erosion syndrome (RCES). Importantly, no recurrence of RCES symptoms were observed in this Regener-Eyes®-treated patient during a follow-up of 4 months, suggesting beneficial effects of Regener-Eyes® in the repair and regeneration of injured corneal epithelial cells.

Conclusions

Regener-Eyes® drops are a topical therapy for DED; they are an engineered biological product. The drops contain a large number of anti-inflammatory and trophic factors that attenuate the detrimental immune response in the eye and protect the epithelial cells of the ocular surface from injury and inflammation.[1,9,12–13,15] Topical administration of Regener-Eyes® may suppress ongoing ocular inflammation, may improve meibomian gland function, and may enhance the restoration of the ocular surface barrier in DED patients, without causing treatment-related adverse events.[1,13] Due to its potent immunosuppressive and regenerative properties, Regener-Eyes® should be considered as a powerful new therapeutic option in the management of DED.

continued on page 8 ▶▶

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