International Journal of Retina and Vitreous

COMMENTARY Open Access

External limiting membrane: retinal structural barrier in diabetic macular edema

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Abstract

Advances in spectral-domain optical coherence tomography (SD-OCT) technology have enhanced the understanding of external limiting membrane (ELM) and ellipsoid zone (EZ) in diabetic macular edema. An increase in VEGF has been demonstrated to be associated with sequential ELM and EZ disruption on SD-OCT. An intact ELM is a prerequisite for an intact EZ in DME. Anti-VEGF therapy leads to restoration of barrier effect of ELM. The ELM restores first followed by EZ restoration.

Diabetic retinopathy (DR) is a major complication of diabetes mellitus [1]. Diabetic retinopathy affects 93 million people worldwide. Among them, 21 million have treatable form of diabetic macula edema (DME) [2]. In DR, vascular endothelial growth factor (VEGF) leads to early blood- retinal barrier breakdown, capillary non-perfusion, and endothelial cell injury and death [3]. VEGF is involved in the initiation of retinal vascular leakage and capillary non-perfusion [4]. The amount and duration of VEGF exposure required for blood-retina barrier breakdown is less than that required for neovascularization [5]. Elevated levels of VEGF come into play even before the signs of retinopathy set in [6].

Advances in spectral-domain optical coherence tomography (SD-OCT) technology have enhanced the understanding of morphological alterations in individual layers of retina and their association with various molecular mechanisms [7]. The external limiting membrane (ELM) and ellipsoid zone (EZ) can be observed by SD-OCT. Status of ELM and EZ has been studied in brown Norwegian rats on OCT. It was found that the EZ and ELM disappeared after euthanasia. The origin of the EZ and ELM was found to be related to the biological activities of the photoreceptor cells [8].

The ELM separates the layers of rods and cones from the overlying outer nuclear layer and is a linear confluence of junctional complexes between Muller cells and photoreceptors [9, 10]. It serves as a barrier against macromolecules [11]. The subcellular compartment of the photoreceptors includes an outer segment that absorbs light and converts it into electrical signals and an inner segment that has the metabolic functions of generating energy and proteins [12].

The EZ clinically defines the photoreceptor integrity. The biological EZ consists mainly of mitochondria. This enables higher levels of energy consumption within the photoreceptors. Focal or global absence of the EZ on SD-OCT corresponds to the reduced reflectivity or anatomic absence of the EZ. Dysfunction of mitochondria in the foveal photoreceptors results in reduced VA in DME [7].

The ELM and EZ integrity is essential for the maintenance of normal vision [13]. DME is known to be associated with ELM and EZ disruption, which in turn affects visual acuity [14–18]. Jain et al. discovered the mechanism of ELM and EZ disruption [6]. An increase in VEGF correlated with increased severity of DR, increased central subfield thickness (CST) and sequential disruption of ELM and EZ [6]. An intact ELM was highlighted as a prerequisite for an intact EZ [6, 19, 20]. Accordingly, disruption of ELM and EZ has been

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graded as follows: grade 0—no disruption of ELM and EZ; grade 1—ELM disrupted, EZ intact; grade 2—both ELM and EZ disrupted. The disruption scale correlates significantly with logMAR visual acuity [6]. Integrity of ELM and EZ has been found to be a positive predictor for visual outcome [20–24].

Omri et al. [25] demonstrated in rat and monkey retina that ELM comprised of attachment of outer process of glial Muller cells to one another and also to inner photoreceptor segments. They revealed that tight junctions (TJ) existed in the ELM. On ultrastructural analysis it was suggested that TJ existed between glial Muller cells and photoreceptors. Occludin, a protein, was found as a key component of TJ. In ELM, occludin was found to be organized between the glial Muller cells and the photoreceptors. It was suggested that the ELM should be considered as part of a retinal barrier. In DME, at the ELM level, glial Muller cells are swollen and lose their occludin content. Therefore, ELM junctions could be considered as unique regulatory targets in treatment [25].

VEGF alters TJs and promotes vascular permeability in many retinal as well as brain diseases. The molecular mechanisms of this barrier regulation is however, not very well understood. Murukami et al. [26] highlighted that VEGF induced phosphorylation-dependent occludin ubiquitination. This is necessary for increased permeability to both macromolecules and ions. Role for occludin in regulation of endothelial barrier properties was highlighted. They suggested occludin as important potential therapeutic targets for the control of vascular permeability in diseases of the blood–brain and blood-retinal barrier. They demonstrated that occludin had a significant role in regulation of barrier properties and might serve as a possible therapeutic target.

Anti-VEGFs are considered as the first-line treatment for DME. Administration of intravitreal anti-VEGF agents has been found to be associated with reduction in CST and improvement in visual acuity (VA) [27–29]. Restoration of the foveal photoreceptors occurs following administration of intravitreal ranibizumab in DME [7]. Improvement in photoreceptor integrity takes place after second and third dose of ranibizumab with improvement in VA and colour vision [30]. A larger foveal photoreceptor microstructure defect is associated with lower VA. Patients with larger foveal photoreceptor microstructure defects at baseline had lesser VA improvements [31]. The improvement in EZ defect size is dependent on the pattern of DME on SD-OCT [32].

De et al. discovered the mechanism of ELM and EZ restoration after anti-VEGF therapy in DME. Anti-VEGF therapy led to restoration of barrier effect of ELM. The ELM was established as a retinal structural barrier and was found to restore first followed by EZ restoration. Decrease in logMAR VA was more pronounced in patients associated with restoration of ELM and EZ [33].

An increase in VEGF results in sequential ELM and EZ disruption on SD-OCT. An intact ELM is a prerequisite for an intact EZ in DME. Anti-VEGF therapy leads to restoration of barrier effect of ELM. The ELM restores first followed by EZ restoration.

Authors' contribution
SS, LA and CHM were equally involved in the preparation of manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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Received: 9 October 2020 Accepted: 13 February 2021
Published online: 04 March 2021

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