Peer Review File

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Reviewer A

Comment:

This manuscript entitled "Nitric oxide: an old drug but 1 with new horizons in ophthalmology- a narrative review" submitted by Dr. Choul Yong Park and coworkers reported a systematic review on the biosynthesis and biology of NO, reactive nitrogen species, NO-regulated downstream signaling pathways. From a clinical perspective, multiple potentials of NO as a novel antiglaucoma treatment, in retinal diseases, as a wound healing modulator, as an ocular inflammatory mediator, as antimicrobial agent in cornea infection, and as a stem cell modulator were also discussed. Moreover, perspective on the future of NO in ophthalmology, from a clinical point of view, is another positive feature of this review article. Consequently, this reviewer highly suggests the publication of this manuscript as is.

Reply: Thank you very much for your positive feedback

Changes in the text: No change was applied.

Reviewer B

This is a well-written and comprehensive review regarding the dual role of nitric oxide in physiological and pathological ocular functions.

Comment 1: One main concern is the fact that authors overlooked the important role of NO and iNOS in diabetic retinopathy.

Reply: As suggested, the role of NO and iNOS in diabetic retinopathy was added.

Changes in the text

NO plays an important role in diabetic retinopathy (DR). There are some experimental evidences that excessive synthesis of NO can contribute to the development and aggravation of DR.(59-61) Especially, high concentrations of NO produced by retinal iNOS are known as a major mediator in DR. In a immunohistochemical study using human retinas, iNOS was observed only in retina with DR.(61) Several studies reported that intraocular NO concentration significantly increased in diabetic patients.(62,63) As well known, increased leukostasis and vascular permeablity is typical pathologic finding in DR. Interestingly, previous study has revealed that NOS inhibition by L-NAME or iNOS knockout in mice can reverse these pathologies.(64) The role of eNOS in DR also appears to be important. One study revealed that eNOS knock out mice developed an accelerated DR in streptozotocin induced diabetes.(65) In this study, although total retinal NO levels in non-diabetic eNOS knock out mice were similar to those in wild type mice, , induction of diabetic in eNOS knockout mice resulted in highly increased total retinal NO level with increased iNOS mRNA

expression. (65) Furthermore, some human studies have reported interaction between eNOS gene polymorphisms and the development and progression of DR.(66,67)

A few minor grammatical and typographic errors can be corrected:

Comment 2: Line 249: Latanoprostene bunod is hydrolyzed (is and not in)

Reply: Thank you very much to find this important typo.

Changes in the text: Latanoprostene bunod is hydrolyzed to latanoprost acid

Comment 3: Line 343: Hypoxia and inflammation are frequently coexist (delete are)

Reply: Thank you very much to find this important typo.

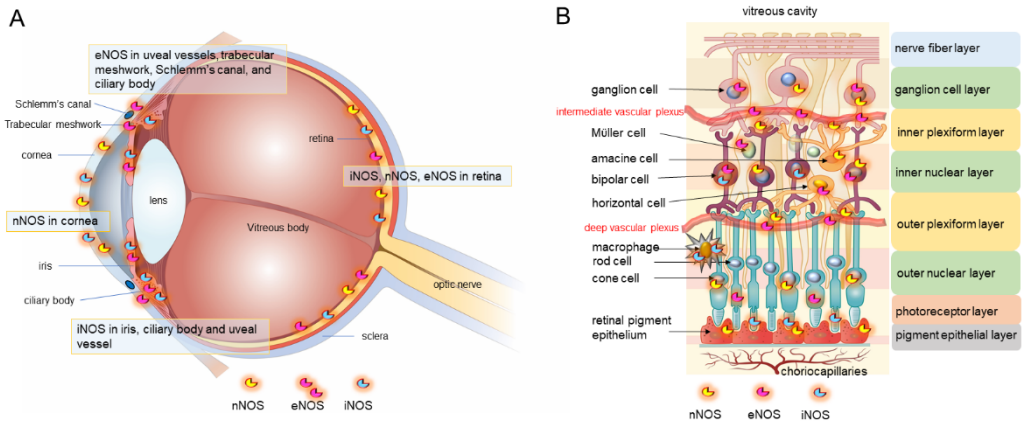
Changes in the text : Hypoxia and inflammation frequently coexist

Comment 4: Figure 3B: read amacrine cell

Reply: Thank you very much to find this important typo.

Changes in the text : Figure 3B was corrected. “amarcrine” was corrected as “amacrine”.

Revised figure 3

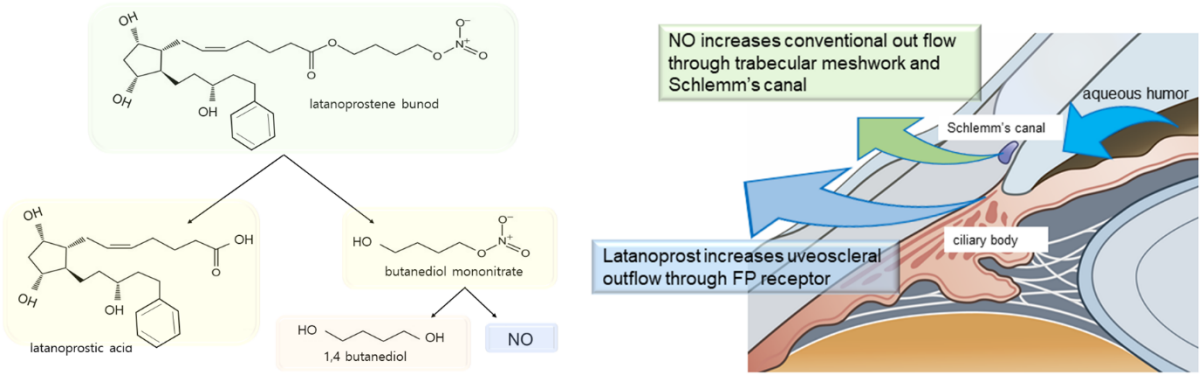


Comment 5: Figure 4 legend (lines 738 and 740): increases (with s). Latanoprost increases (in the figure itself)

Reply: Thank you very much to find this important typo.

Changes in the text : “Latanoprost increase” was changed as “Latanoprost increases”

Revised figure 4 was attached.



Comment 6: Figure 6: the reference from which data were published seems wrong (67 or 69). Revise accordingly in the text also because this figure contains two models (human keratocytes and murine corneal wound healing)

Reply: Thank you very much to find this important typo. Panel A in figure 6 is an unpublished data by the authors. And Panels B and C are from the previous published data.

Changes in the text: The following correction was made.

Figure 6. Nitric oxide (NO) effect on corneal wound healing. NaNO2 was used as an NO donor. A: Cultured human keratocytes were stimulated by transforming growth factor β1 and myofibroblast differentiation was verified by increased alpha smooth muscle actin (αSMA) expression in protein blots. Addition of NaNO2 in the culture media inhibited myofibroblast differentiation of keratocytes, as verified by decreased αSMA expression. Approximately 20% decreased αSMA expression was observed when 1000 μM of NaNO2 was added to the medium. (unpublished data by the authors) B: In vivo effect of NO in corneal chemical burn in Balb/c mice. Corneal opacity grade after healing from chemical burns significantly decreased with the topical treatment of NaNO2 compared with PBS treatment. C: Representative ocular surface pictures of healing process of chemical burn shows more transparent cornea in NaNO2-treated mouse compared with PBS control. Panels B and C are from Figure 6 in "Effect of Nitric Oxide on Human Corneal Epithelial Cell Viability and Corneal Wound Healing" Park et al. Sci Rep. 2017;7(1):8093 under Creative Commons Attribution 4.0 International License.