

**Peroxynitrite Mediates Retinal Neurodegeneration by
Inhibiting NGF Survival Signal in Experimental and Human
Diabetes**

Tayyeba K. Ali, M.D.^{1,4}, Suraporn Matragoon, M.S.^{1,4}, Bindu A. Pillai, M.S.^{1,4};
Gregory I. Liou, Ph.D.³, and Azza B. El-Remessy, Ph.D., R.Ph.^{1,2,3,4}

¹Program in Clinical and Experimental Therapeutics, University of Georgia;
²Department of Pharmacology and Toxicology; ³Department of Ophthalmology,
Medical College of Georgia;
⁴VA Medical Center, Augusta, GA 30912

Running Title: Peroxynitrite and NGF signal

Corresponding Author: A.B. El-Remessy, Ph.D., R.Ph.
Program in Clinical and Experimental Therapeutics
College of Pharmacy
University of Georgia
Augusta, Georgia 30912
aelremessy@mcg.edu

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ABSTRACT

Objective: Recently we have shown diabetes-induced retinal neurodegeneration positively correlates with oxidative stress and peroxynitrite. Studies also show peroxynitrite impairs nerve growth factor (NGF) survival signal in sensory neurons. However, the causal role of peroxynitrite and the impact of tyrosine nitration on diabetes-induced retinal neurodegeneration and NGF survival signal have not been elucidated.

Research Design and Methods: Expression of NGF and its receptors was examined in retinas from human and STZ-diabetic rats and retinal ganglion cells (RGC). Diabetic animals were treated with FeTPPs (IP, 15mg/kg/day), which catalytically decomposes peroxynitrite to nitrate. After 4 weeks of diabetes, retinal cell death was determined by TUNEL assay. Lipid peroxidation and nitrotyrosine were determined using MDA assay, immunofluorescence and Slot-Blot analysis. Expression of NGF and its receptors was determined by ELISA, real-time PCR, immunoprecipitation and Western Blot analyses.

Results: Analyses of retinal neuronal death and NGF showed 9-fold and 2-fold increases, respectively, in diabetic retinas compared to controls. Diabetes also induced increases in lipid peroxidation, nitrotyrosine, and the pro-apoptotic p75^{NTR} receptor in human and rat retinas. These effects were associated with tyrosine nitration of the pro-survival TrkA receptor, resulting in diminished phosphorylation of TrkA and its down-stream target, Akt. Furthermore, peroxynitrite induced neuronal death, TrkA nitration, and activation of p38 MAPK in RGC even in the presence of exogenous NGF. FeTPPs prevented tyrosine nitration, restored NGF survival signal and prevented neuronal death *in vitro* and *in vivo*.

Conclusion: Together, these data suggest that diabetes-induced peroxynitrite impairs NGF neuronal survival by nitrating TrkA receptor and enhancing p75^{NTR} expression.

KEYWORDS. Peroxynitrite, apoptosis, tyrosine nitration, diabetic retinopathy, neuroprotection, NGF, TrkA, p75^{NTR}

ABBREVIATIONS. Nerve growth factor (NGF), retinal ganglion cells (RGC), relative optical density (ROD), peroxynitrite (PN), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, enzyme-linked immunosorbent assay (ELISA).

