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Società Oftalmologica Siciliana

Tavola Rotonda
L'Endofarmacologia in Oculistica

Neuro-Infiammazione retinica

Renato Nicoletti

NEUROINFIAMMAZIONE

patologie croniche oculari

Una risposta infiammatoria cronica su base immunologica associata ad un'alterazione dell'omeostasi tissutale caratterizza differenti patologie croniche oculari

- **Degenerazione Maculare Senile**
- **Retinopatia Diabetica**
- **Glaucoma**

Hesselink JK, J Ophthalmol, 2015

La Neuroinfiammazione caratterizza le principali patologie croniche oculari:

Immune mechanisms in inflammatory and degenerative eye disease

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It has recently been recognized that pathology of age-associated degenerative eye diseases such as adult macular degeneration (AMD), glaucoma and diabetic retinopathy, have strong immunological underpinnings. Attempts have been made to extrapolate to age-related degenerative disease insights from inflammatory processes associated with non-infectious uveitis, but these have not yet been sufficiently informative. Here we review recent findings on the immune processes underlying uveitis and those that have been shown to contribute to AMD, discussing in this context parallels and differences between overt inflammation and para-inflammation in the eye. We propose that mechanisms associated with ocular immune privilege, in combination with paucity of age-related antigen(s) within the target tissue, dampen what could otherwise be overt inflammation and result in the para-inflammation that characterizes age-associated neurodegenerative disease.

Trends in Immunology, June 2015, Vol. 36, No. 6

La Neuroinfiammazione caratterizza le principali patologie croniche oculari:

Review Article

Journal of Diabetes Research
Volume 2015, Article ID 582060, 16 pages

Diabetic Retinopathy: Vascular and Inflammatory Disease

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Academic Editor: Harald Sourij

Diabetic retinopathy (DR) is the leading cause of visual impairment in the working-age population of the Western world. The pathogenesis of DR is complex and several vascular, inflammatory, and neuronal mechanisms are involved. Inflammation mediates structural and molecular alterations associated with DR. However, the molecular mechanisms underlying the inflammatory pathways associated with DR are not completely characterized. Previous studies indicate that tissue hypoxia and dysregulation of immune responses associated with diabetes mellitus can induce increased expression of numerous vitreous mediators responsible for DR development. Thus, analysis of vitreous humor obtained from diabetic patients has made it possible to identify some of the mediators (cytokines, chemokines, and other factors) responsible for DR pathogenesis. Further studies are needed to better understand the relationship between inflammation and DR. Herein the main vitreous-related factors triggering the occurrence of retinal complication in diabetes are highlighted.

La Neuroinfiammazione caratterizza le principali patologie croniche oculari:

Curr Eye Res. 2014 February ; 39(2): 105–119.

From Mechanosensitivity to Inflammatory Responses: New Players in the Pathology of Glaucoma

David Križaj[1,2,3], **Daniel A. Ryskamp**[1,3], **Ning Tian**[1,3], **Gülgün Tezel**[4], **Claire H. Mitchell**[5,6], **Vladlen Z. Slepak**[7], and **Valery I. Shestopalov**[8,9,10]

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Abstract

Purpose of the study—Many blinding diseases of the inner retina are associated with degeneration and loss of retinal ganglion cells (RGCs). Recent evidence implicates several new signaling mechanisms as causal agents associated with RGC injury and remodeling of the optic nerve head. Ion channels such as Transient receptor potential vanilloid isoform 4 (TRPV4), pannexin-1 (Panx1) and P2X7 receptor are localized to RGCs and act as potential sensors and effectors of mechanical strain, ischemia and inflammatory responses. Under normal conditions, TRPV4 may function as an osmosensor and a polymodal molecular integrator of diverse mechanical and chemical stimuli, whereas P2X7R and Panx1 respond to stretch- and/or swelling-induced adenosine triphosphate release from neurons and glia. Ca^{2+} influx, induced by stimulation of mechanosensitive ion channels in glaucoma, is proposed to influence dendritic and axonal remodeling that may lead to RGC death while (at least initially) sparing other classes of retinal neuron. The secondary phase of the retinal glaucoma response is associated with microglial activation and an inflammatory response involving Toll-like receptors (TLRs), cluster of differentiation 3 (CD3) immune recognition molecules associated with the T-cell antigen receptor, complement molecules and cell type-specific release of neuroactive cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). The retinal response to mechanical stress thus involves a diversity of signaling pathways that sense and transduce mechanical strain and orchestrate both protective and destructive secondary responses.

Conclusions—Mechanistic understanding of the interaction between pressure-dependent and independent pathways is only beginning to emerge. This review focuses on the molecular basis of mechanical strain transduction as a primary mechanism that can damage RGCs. The damage occurs through Ca^{2+}-dependent cellular remodeling and is associated with parallel activation of secondary ischemic and inflammatory signaling pathways. Molecules that mediate these mechanosensory and immune responses represent plausible targets for protecting ganglion cells in glaucoma, optic neuritis and retinal ischemia.

La ridotta perfusione ematica della retina determina ipossia e ischemia che generano neuro-infiammazione

Cold Spring Harb Perspect Med. 2014 Jul 3.
The Complex Role of Neuroinflammation in Glaucoma.

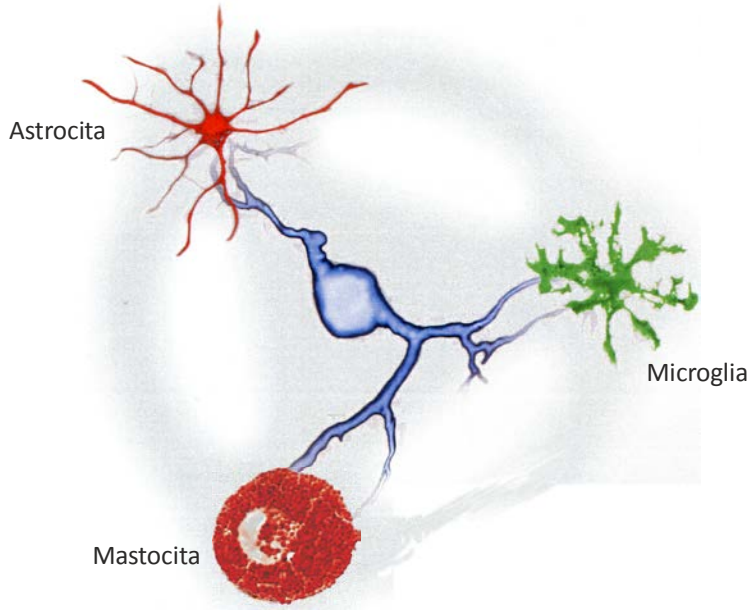
Soto I, Howell GR.

Glaucoma is a multifactorial neurodegenerative disorder affecting 80 million people worldwide. Loss of retinal ganglion cells and degeneration of their axons in the optic nerve are the major pathological hallmarks. Neuroinflammatory processes, inflammatory processes in the central nervous system, have been identified in human glaucoma and in experimental models of the disease. Furthermore, neuroinflammatory responses occur at early stages of experimental glaucoma, and inhibition of certain proinflammatory pathways appears neuroprotective. Here, we summarize the current understanding of neuroinflammation in the central nervous system, with emphasis on events at the optic nerve head during early stages of glaucoma.

RETINA e NEUROINFIAMMAZIONE

Mast cells, glia and neuroinflammation: partners in crime?

Stephen D. Skaper,* Laura Facci and Pietro Giusti
Dipartimento di Scienze del Farmaco, Largo "Egidio Meneghetti" 2, Università degli Studi di Padova, 35131 Padova, Italy



La massiva attivazione degli astrociti e quella della microglia favoriscono il rilascio di fattori tossici per le cellule ganglionari retiniche

- Neurodegenerazione -

RETINA e NEUROINFIAMMAZIONE

Tutte le patologie neuroinfiammatorie sono caratterizzate da:

ALTERAZIONI DEL MICROCIRCOLO:

- Aumentata permeabilità capillare
- Disfunzione vascolare
- Morte dei periciti
- Occlusione capillare
- Assottigliamento della membrana basale dei capillari retinici
- Microaneurismi

REAZIONI INFIAMMATORIE:
Incremento di numerosi biomarkers infiammatori (CRP, IL-6, TNF-α, IL–1β)

Yu Y, J Neuroinflamm, 2015

Lesioni retiniche (microaneurismi) evidenziati mediante retinal wholemount dopo 6 mesi di diabete

LA PALMITOILETANOLAMIDE:

Molecola Naturale «Sicura»

La PEA è una sostanza naturale lipidica sintetizzata «*on demand*»
a partire da un fosfolipide.

Le sue concentrazioni tissutali variano in funzione di differenti stress cellulari.
(Lo Verme et al, 2005).

La PEA agisce localmente e i suoi livelli tissutali sono fortemente regolati da un bilanciamento dell'attività degli enzimi coinvolti nei pathways anabolico e catabolico

Essa viene degradata dalla Idrolasi degli acidi grassi (FAAH), poiché i suoi cataboliti vengono riutilizzati, non è soggetta a fenomeni di accumulo *(Ueda et al. 2001).*

**L'efficacia e la sicurezza della PEA sono state documentate
in più di 40 studi clinici che hanno coinvolto più di 5000 pazienti**

Nessun effetto collaterale documentato

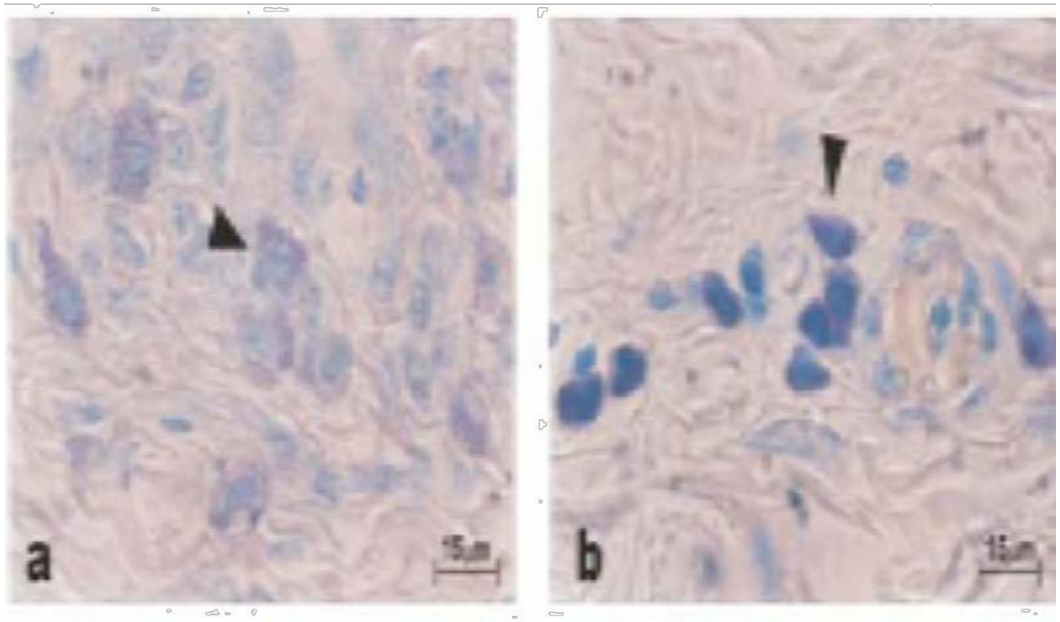
LA PALMITOILETANOLAMIDE:

Molecola Naturale «Sicura»

ALIA

Autacoid Local Inflammation Antagonism

Levi-Montalcini, R, J Neurosci, 1995



Lesioni cutanee in modello animale di dermopatia da ipersensibilità prima (**a**) e dopo (**b**) trattamento orale con PEA.

Frecce indicano il mastocita.

Il significativo aumento dell'intensità di colorazione conferma la riduzione della degranulazione mastocitaria in seguito a trattamento con PEA

La PEA agisce da antagonista locale del danno, inibendo ("down-regulation") la degranulazione dei mastociti attivati da uno stimolo infiammatorio.

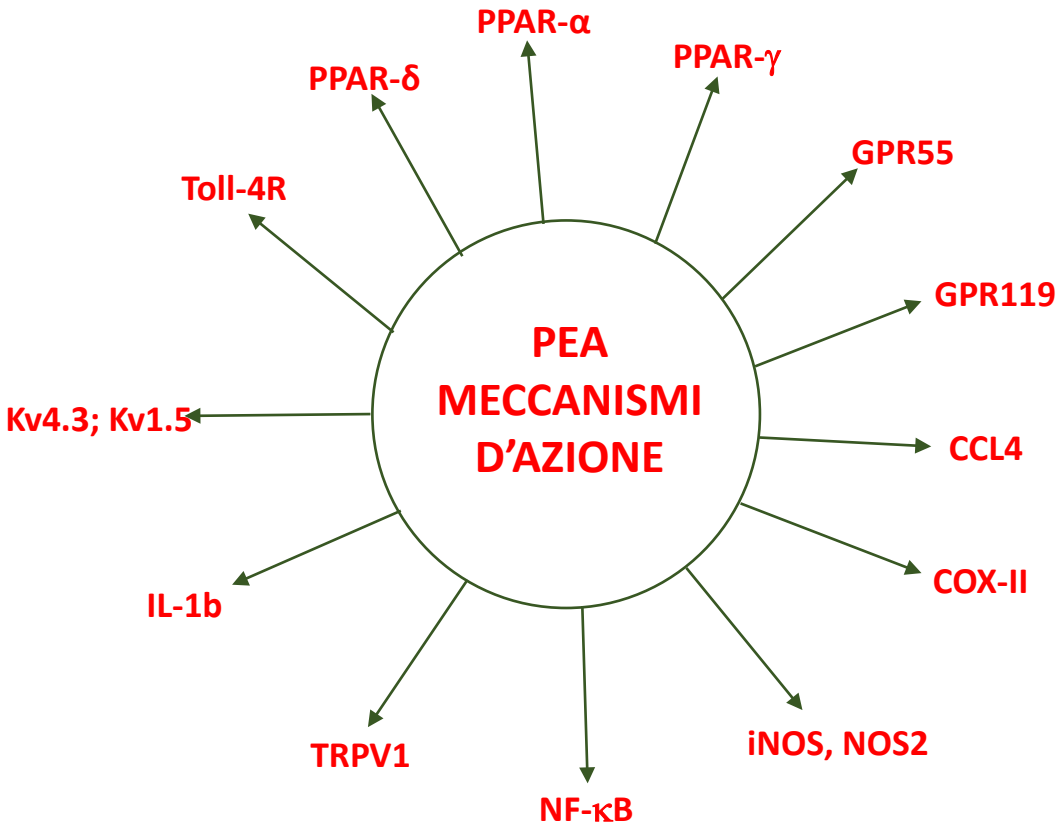
Viene infatti utilizzata «on demand» dal tessuto infiammato

LA PALMITOILETANOLAMIDE:

Meccanismo d'Azione

La PEA è una molecola endogena pleiotropica con attività prevalentemente antiflogistica capace di modulare e di ridurre l'iperattività dei mastociti, astrociti e microglia correlata a infiammazione, dolore, neuropatie.

Gli effetti della PEA sono dovuti all'interazione con numerosi recettori



Hesselink K, J Pain Relief, 2013; Skaper SD, Immunol, 2013

LA PALMITOILETANOLAMIDE: Neuroprotettore Retinico

I risultati sperimentali 2008-2015:

Table 1: Summary of preclinical studies related to PEA's cytoprotective effects.

Year	Dose PEA	Main results	Reference
2015	5 μM in vitro	Inhibition of the Ca^{2+}-dependent release of glutamate	[24]
2015	5 mg/kg	Diminished inflammation, demyelination, axonal damage, and inflammatory cytokine expression in a multiple sclerosis model	[25]
2015	0.1 μM in vitro	Protection cell viability in cultured cortical neurons and astrocytes against inflammation	[26]
2015	10^{-8}–10^{-6} M	Concentration-dependently reduced expression of proinflammatory and proangiogenic markers	[27]
2014	1 mg/kg	Prevention of induced afferent mechanical sensitization	[28]
2014	200, 400 and 800 μg/mL	Inhibition of inflammation markers and chymase expression in granulomatous tissue	[29]
2014	30 mg/kg sc	Increased AMP-activated protein kinase-α phosphorylation and carnitine palmitoyltransferase 1 transcription in adipose tissue; polarized adipose tissue macrophages to M2 lean phenotype	[30]
2014	10 mg/kg	Reduction of structural radiation injury, intestinal wall thickness, collagen deposition, intestinal inflammation, and increased anti-inflammatory IL-10 and IL-6	[31]
2013	10 mg/kg	Reduction of the clinical signs of type II collagen-induced arthritis as well as of paw edema compared to control	[32]
2013	2, 10 or 50 mg/kg	Improves all macroscopic signs of colitis and decreases the expression and release of all the proinflammatory markers	[33]
2013	30 mg/kg	Reduction of hypertension and protects kidney injury	[34]
2013	1 μM control in vivo	Protected SCI-associated neuroinflammation in vivo and in vitro	[35]
2013	0.1 μM, in vitro	Rescue of neuron damage by amyloid and reduction of neuroinflammation (decrease of astrocyte activation)	[36]
2013	5–10 mg/kg	Normalizing the activity of sensitized nociceptive neurons; significant reduction of mechanical allodynia and thermal hyperalgesia in a dose-dependent manner	[37]
2013	10 mg/kg	Strong reduction of microglia activation and PEA delayed mast cell recruitment, protection of mast cells against degranulation, and abolition of the nerve growth factor increase, reducing pain	[38]
2013	10 mg/kg	Protection of spinal cord damage; restoration of PPAR-δ and PPAR-γ expression in spinal cord after damage	[39]
2013	10, 20, 40, 60 mg/kg i.p.	Showing anti-epileptic properties in a rat model	[40]
2013	NR	Blunted Aβ1-42-induced neurotoxicity and controlled glial activation	[41]
2012	10 mg/kg	Significant attenuation of the degree of renal dysfunction, injury, and inflammation caused by ischemia-reperfusion injury	[42]
2012	10 mg/kg	Reduction of MPTP-induced microglial activation, the number of GFAP-positive astrocytes, and reduction of neutrophil infiltrations, reduction of TNF-α, IL-1β and iNOS in spinal cord and prevention of SCI-induced IκB-α degradation and Bax expression	[43]
2012	10 mg/kg	Reduction of apoptosis, brain infarctions, and various inflammatory parameters	[44]
2011	10 mg/kg	Significant reduction of mast cell infiltration, expression of mediators like NGF, the activation of microglia, and astrocytes expressing cannabinoid CB(2) receptor after spinal cord injury	[45]
2008	10 mg/kg	Significant reduction of spinal cord inflammation and tissue injury, neutrophil infiltration, and proinflammatory cytokine expression and significant amelioration of the recovery of motor limb function	[46]

NR: Nonreported.

LA PALMITOILETANOLAMIDE:

Neuroprotettore Retinico

I risultati sperimentali di numerosi studi ne danno conferma:

- **Inibisce l'induzione di geni codificanti molecole proinfiammatorie e proangiogenetiche (IL-1b, CCL-4, NOS2 ICAM-1, P-Selectin)**
- **Inibisce l'attivazione di NF-kB (fattore trascrizionale proinfiammatorio)**
- **Ha mostrato attività neuroprotettiva (antagonista del TNF-α)**
- **Riduce la perossidazione lipidica, la nitrazione proteica e l'induzione di pathway apoptotici**
- **Regola la risposta gliale a stimoli patologici; contrasta la morte delle cellule neuronali**
- **Favorisce l'aumento di fattori di crescita neuronale**

Riduzione della neuro-infiammazione

Riduzione dell'angiogenesi con miglioramento del microcircolo

Ocular Hypotensive Effect of Oral Palmitoyl-ethanolamide: A Clinical Trial

Caterina Gagliano,[1] Elina Ortisi,[1] Luigi Pulvirenti,[2] Michele Reibaldi,[1] Davide Scollo,[3] Roberta Amato,[3] Teresio Avitabile,[1] and Antonio Longo[1]

(*Invest Ophthalmol Vis Sci.* 2011;52:6096–6100)

Effect of Palmitoylethanolamide on Visual Field Damage Progression in Normal Tension Glaucoma Patients: Results of an Open-Label Six-Month Follow-Up

Ciro Costagliola,[1] Mario R. Romano,[1] Roberto dell'Omo,[1] Andrea Russo,[2] Rodolfo Mastropasqua,[3] and Francesco Semeraro[2]

JOURNAL OF MEDICINAL FOOD
J Med Food 00 (0) 2014, 1–6

Palmitoylethanolamide Effects on Intraocular Pressure After Nd:YAG Laser Iridotomy: An Experimental Clinical Study

Nicola Pescosolido,[1] Aloisa Librando,[2] Marta Puzzono,[2] and Marcella Nebbioso[2]

JOURNAL OF OCULAR PHARMACOLOGY AND THERAPEUTICS
Volume 27, Number 6, 2011

Effectiveness of Palmitoylethanolamide on Endothelial Dysfunction in Ocular Hypertensive Patients: A Randomized, Placebo-Controlled Cross-Over Study

Ernesto Strobbe, Mauro Cellini, and Emilio C. Campos

(*Invest Ophthalmol Vis Sci.* 2013;54:968–973)

La Palmitoiletanolamide e Neuroinfiammazione Retinica

European Journal of Pharmacology ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Immunopharmacology and inflammation

Palmitoylethanolamide treatment reduces retinal inflammation in streptozotocin-induced diabetic rats

Irene Paterniti [a,1], Rosanna Di Paola [a], Michela Campolo [a], Rosalba Siracusa [a], Marika Cordaro [a], Giuseppe Bruschetta [a], Gemma Tremolada [c], Anna Maestroni [d], Francesco Bandello [c], Emanuela Esposito [a,1], Gianpaolo Zerbini [d], Salvatore Cuzzocrea [a,b,1,*]

[a] Department of Biological and Environmental Sciences, University of Messina, Italy
[b] Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, USA
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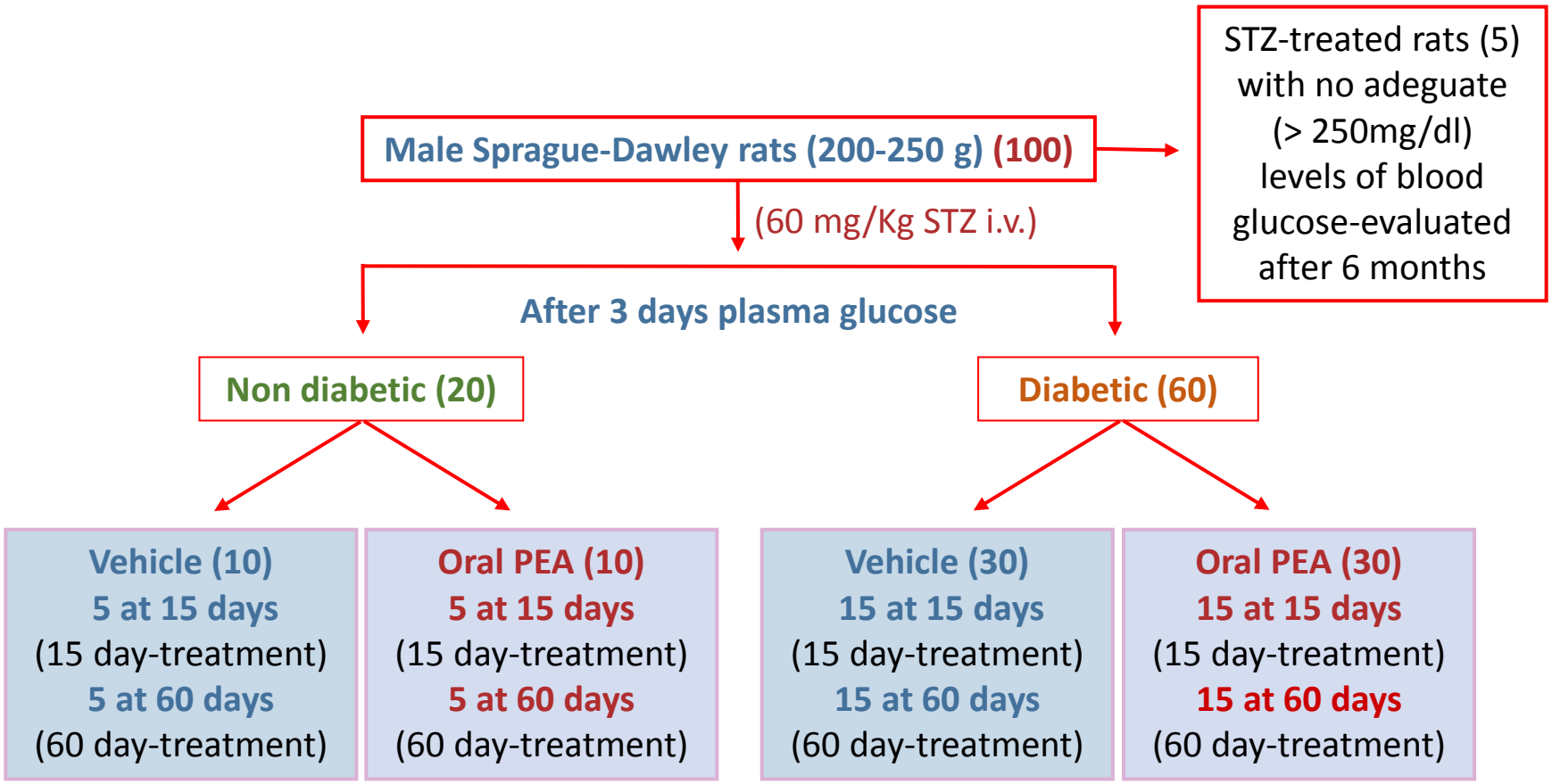
A B S T R A C T

Although the pathogenesis of diabetic retinopathy (DR) is still insufficiently understood, new evidences indicate 'retinal inflammation' as an important player in the pathogenesis of the complication. Accordingly, common sets of upregulated inflammatory cytokines are found in serum, vitreous and aqueous samples obtained from subjects with DR, and these cytokines can have multiple interactions to impact the pathogenesis of the disease. Thus, based on previously published data, we investigated the effects of Palmitoylethanolamide (PEA), an endogenous lipid amide that belongs to the N-acyl-ethanolamines family, on DR in streptozotocin (STZ)-induced diabetic rats. PEA (10 mg/kg) was administered orally daily starting 3 days after the iv administration of STZ. The rats were killed 15 and 60 day later and eyes were enucleated to evaluate, through immunohistochemical analysis, the key inflammatory events involved in the breakdown of blood retinal barrier (BRB). Immunohistochemical analysis confirmed the presence of VEGF, ICAM-1, nitrotyrosine (a marker of peroxynitrite), and tight junctions in the retina of STZ-treated rats. Of interest, the extent of injury was significantly reduced after treatment with PEA.

Altogether, this study provides the first evidence that PEA attenuates the degree of inflammation while preserving the blood–retinal barrier in rats with experimental DR.

La Palmitoiletanolamide e Retinopatia Diabetica

Experimental Design



Moderate hyperglycemia will be stabilized by subcutaneous injection of 2 units Ultralente Insulin every 2 days

La Palmitoiletanolamide e Retinopatia Diabetica

Endpoints for DR

To assess the retinal expression of biomarkers linked to retinal dysfunction
VEGF, TNF-α, ICAM-1 evaluated by mean ELISA method
n 28 eyes at 15 days **+ 20 eyes** at 60 days

To assess the retinal oxidative stress
NF-kB p65, IkB-α, PPAR-α evaluated by mean western blot analysis
n 26 eyes at 15 days **+ 18 eyes** at 60 days

To assess retinal nytrosative stress as well as endothelial alterations
Nitrotyrosine, ICAM-1, ZO-1, Claudin-5, VEGF evaluated by mean immunohistochemistry
n 26 eyes at 15 days **+ 22 eyes** at 60 days

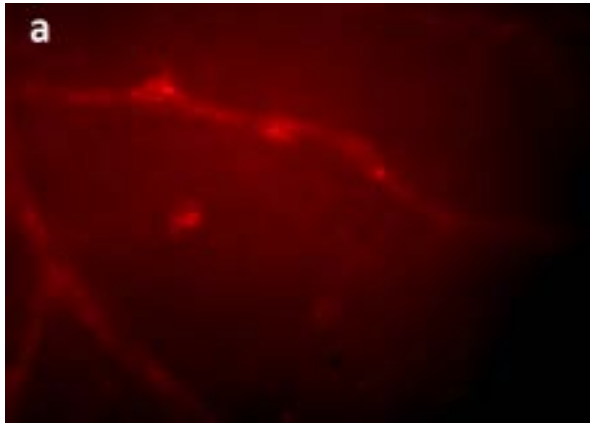
To assess retinal abnormalities
Retinal and veins capillaries evaluated by mean Retinal Whole Mount methods
n 20 eyes at 6 months

La Palmitoiletanolamide e Retinopatia Diabetica

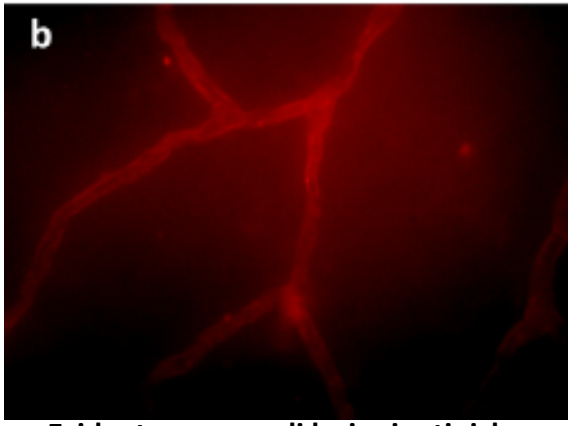
- La PEA RIDUCE SIGNIFICATIVAMENTE la PRODUZIONE di:
  - TNF-α (infiammazione)
  - VEGF (angiogenesi)
  - ICAM-1 (alterazione endoteliale)
  - Nitrotirosina (stress nitrosativo retinico)

- La PEA PROTEGGE LA STRUTTURA MICROVASCOLARE RETINICA indispensabile per il mantenimento della funzione barriera della retina.
  - Regolare distribuzione delle proteine ZO-1 (Zonula Occludents-1) e Claudina

- La PEA PRESERVA DA LESIONI RETINICHE (microaneurismi)

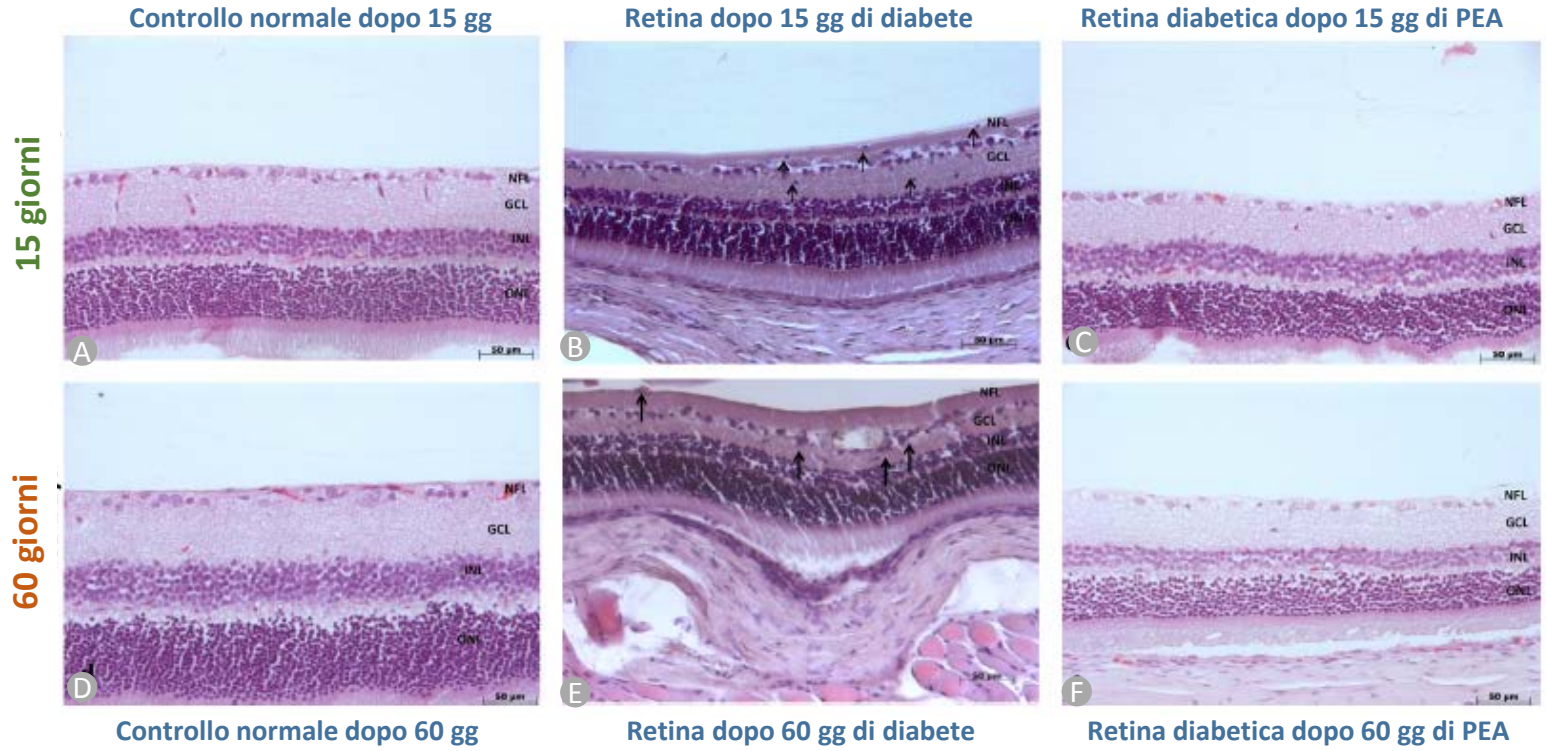


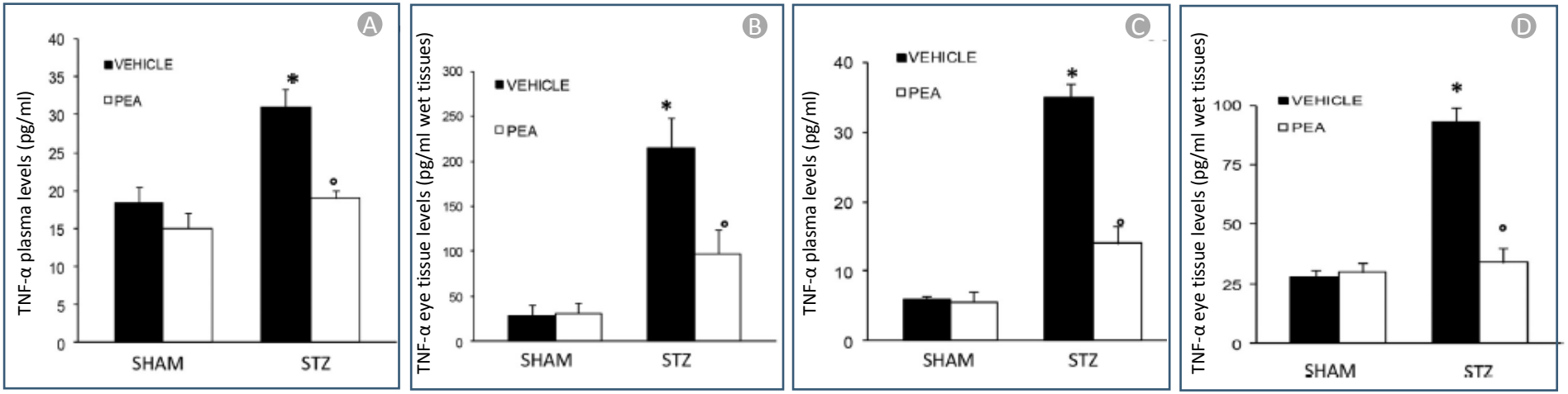
Evidenti microaneurismi nella retina di ratti dopo 6 mesi di diabete



Evidente assenza di lesioni retiniche nella retina di ratti diabetici dopo 6 mesi di trattamento con PEA

La PEA riduce significativamente l'aumento della citochina infiammatoria TNF-α

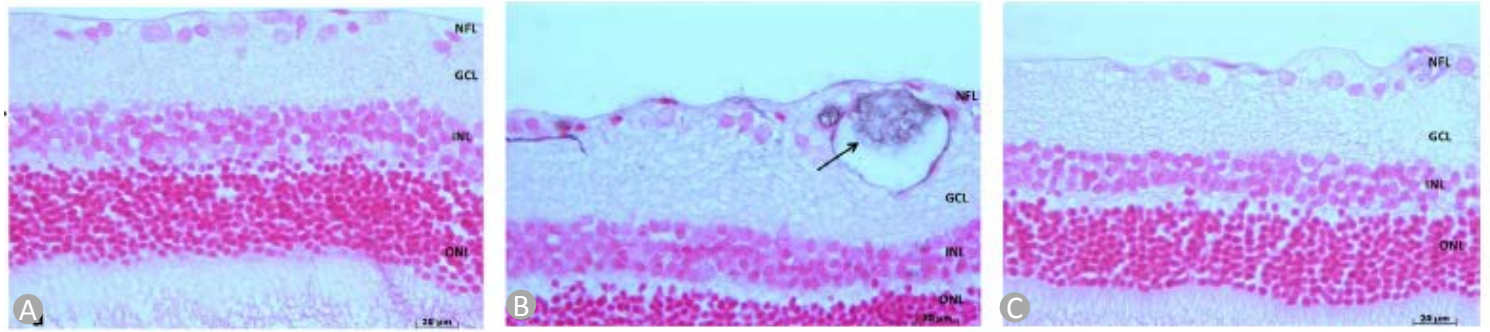




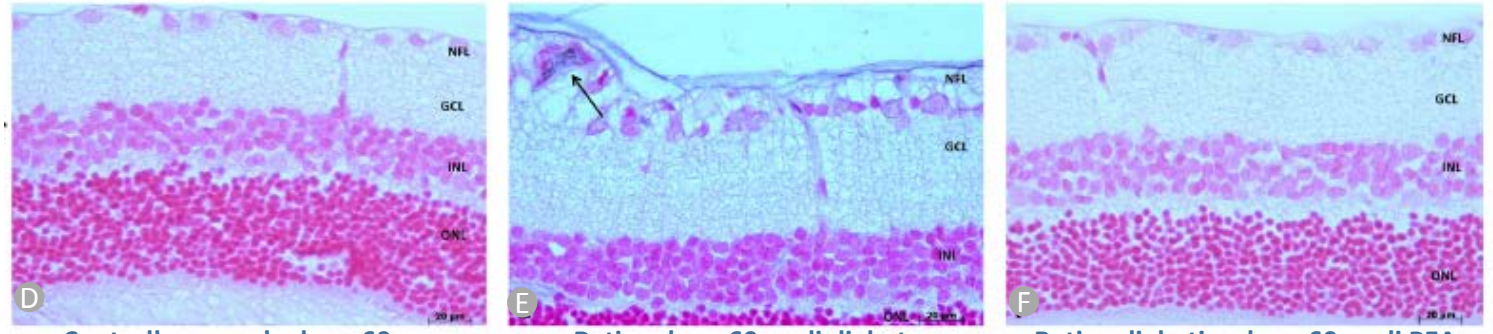
La PEA riduce significativamente la produzione di VEGF nei tessuti retinici

Controllo | Retina dopo 15 gg di diabete | Retina diabetica dopo 15 gg di PEA

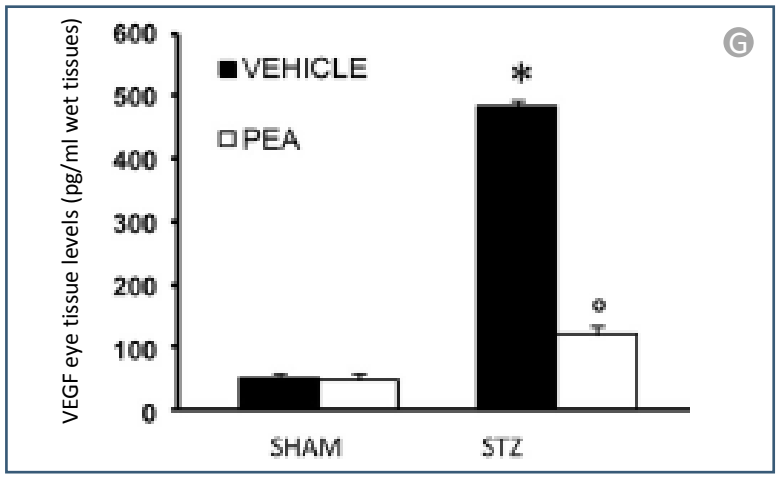
15 giorni



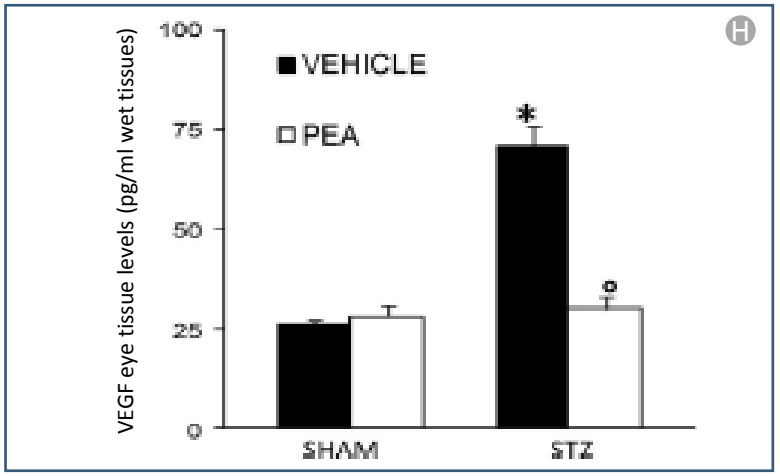
60 giorni



Controllo normale dopo 60 gg | Retina dopo 60 gg di diabete | Retina diabetica dopo 60 gg di PEA

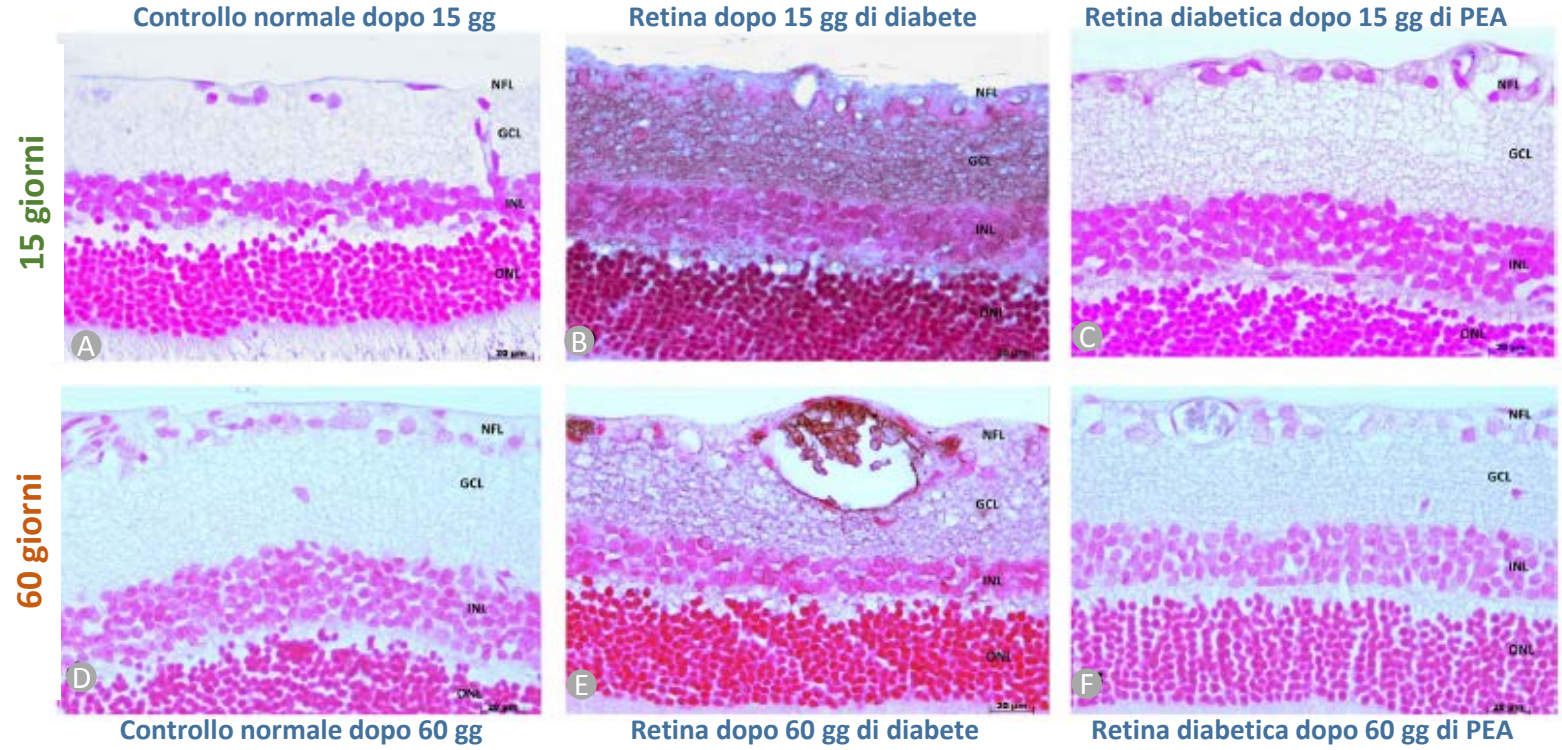


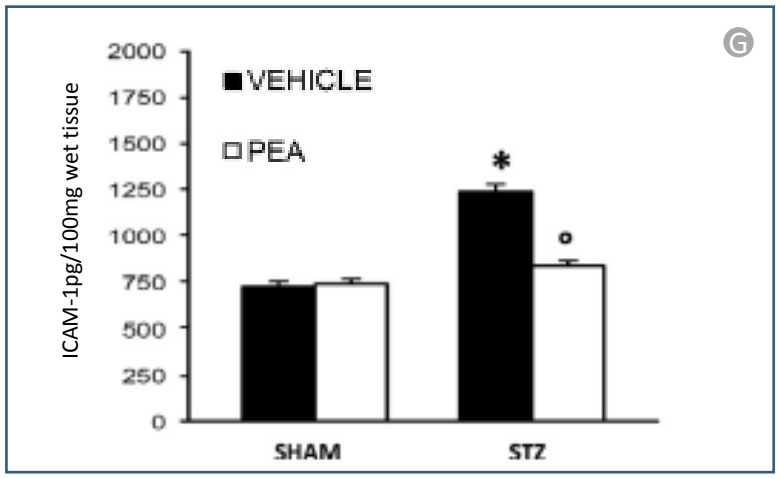
15 giorni



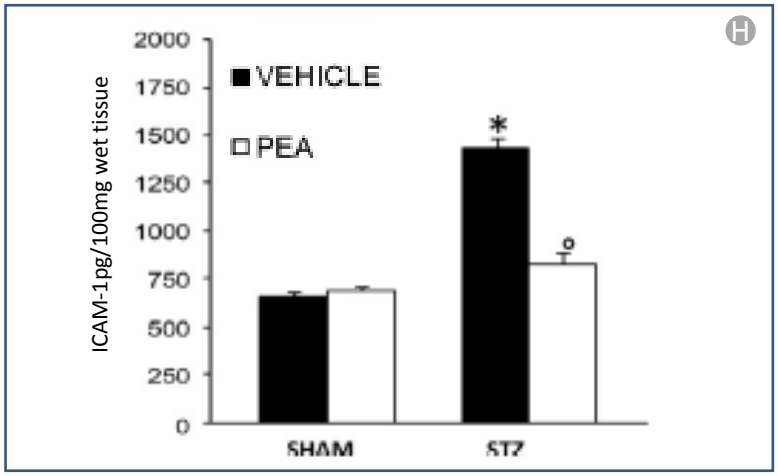
60 giorni

La PEA riduce significativamente la produzione di ICAM-1





15 giorni

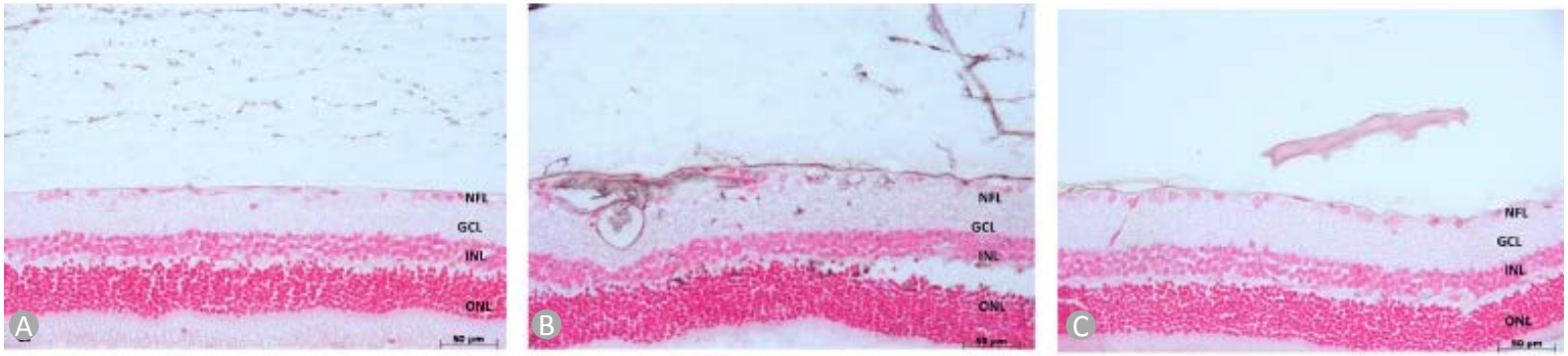


60 giorni

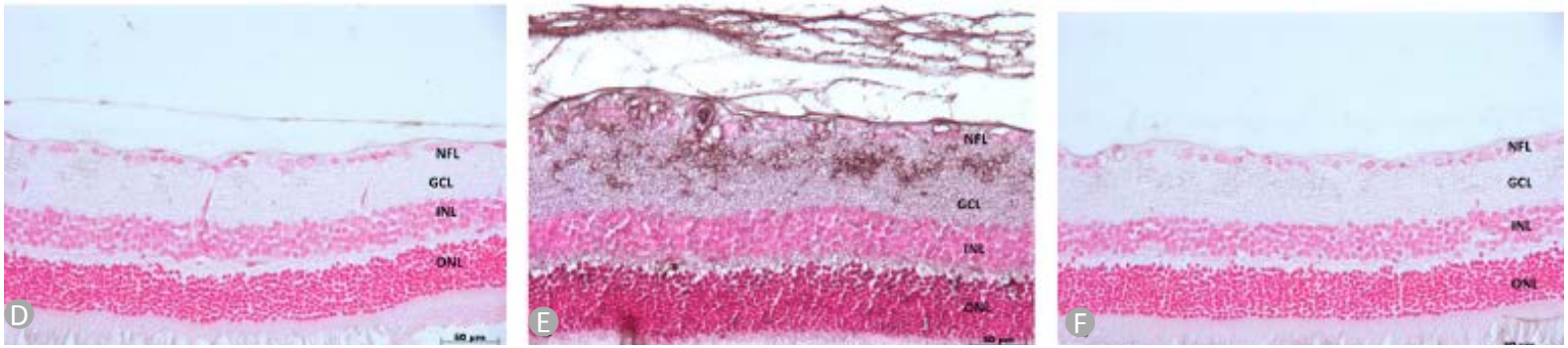
La PEA riduce lo stress nitrosativo retinico

Controllo normale dopo 15 gg | Retina dopo 15 gg di diabete | Retina diabetica dopo 15 gg di PEA

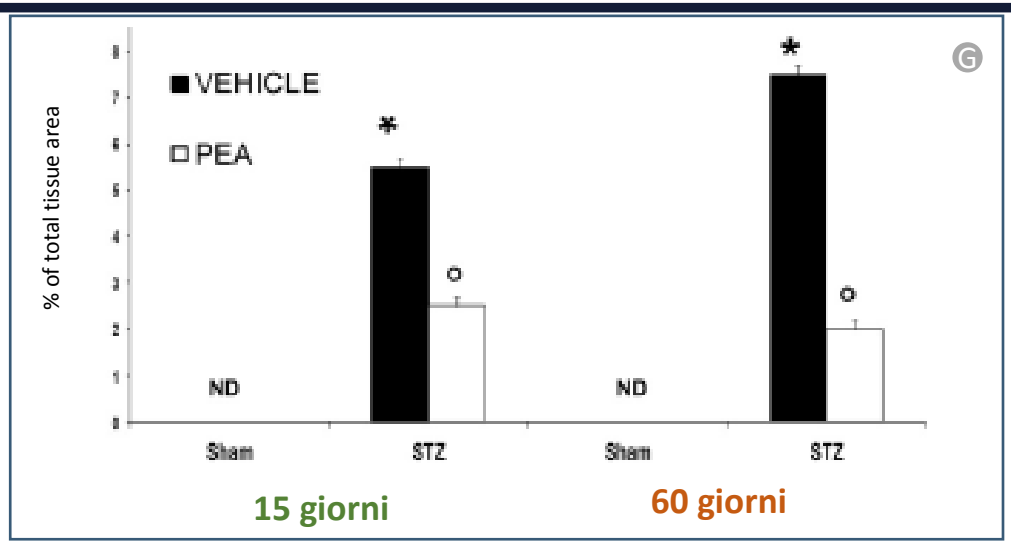
15 giorni



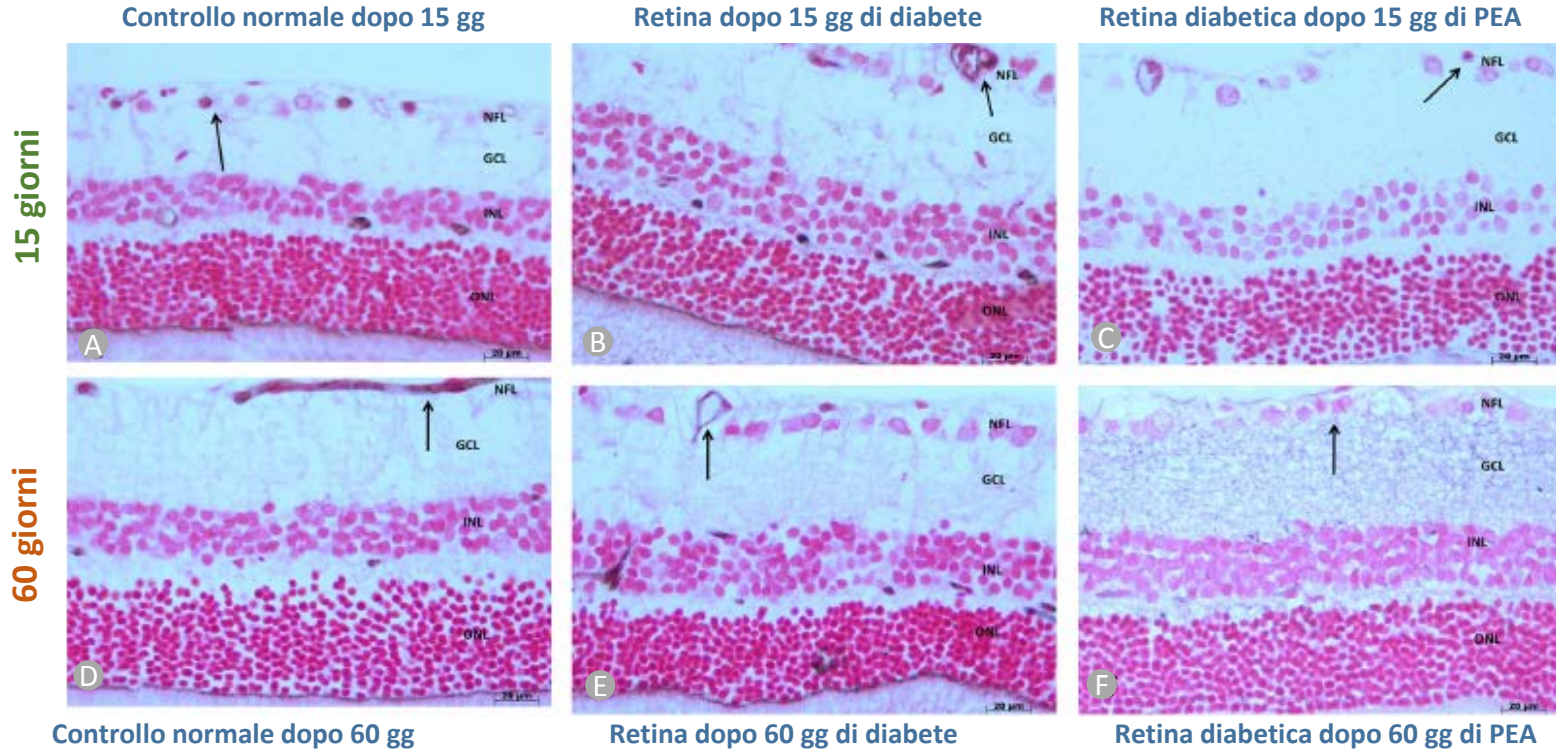
60 giorni

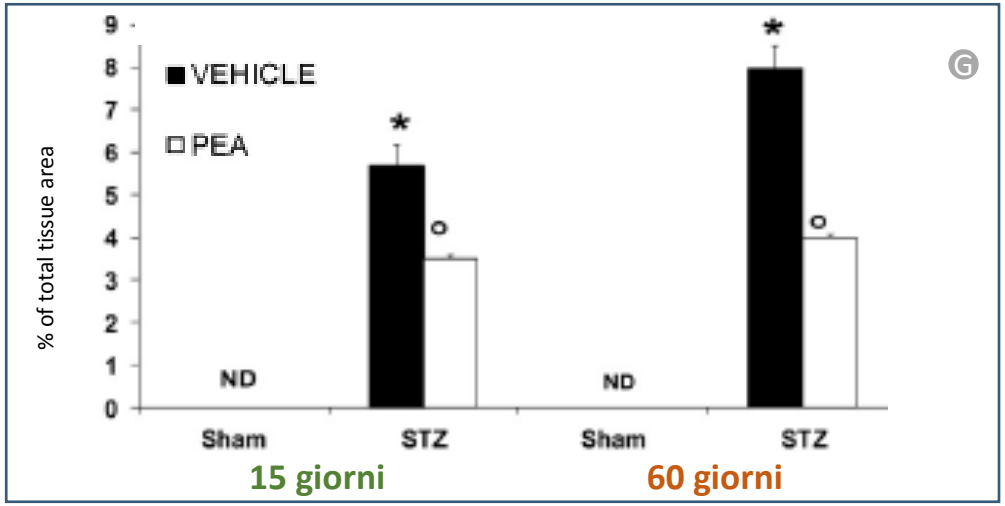


Controllo normale dopo 60 gg | Retina dopo 60 gg di diabete | Retina diabetica dopo 60 gg di PEA



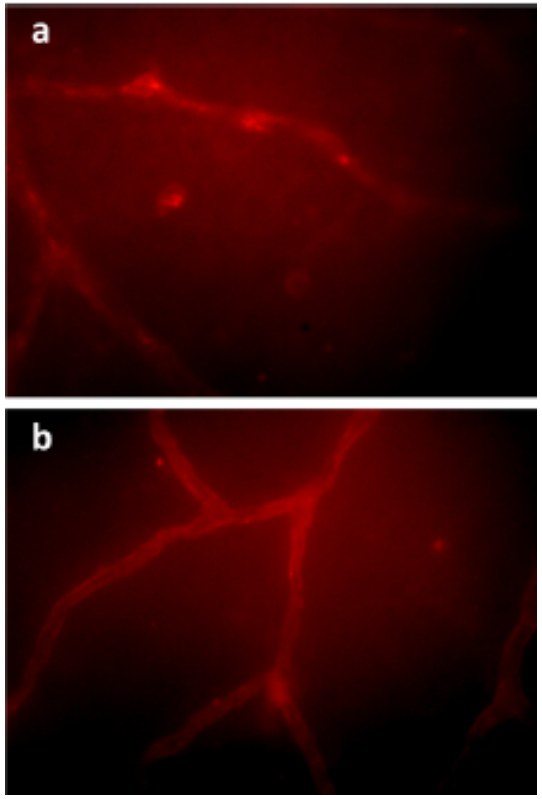
La PEA protegge la struttura microvascolare retinica indispensabile per il mantenimento della funzione barriera della retina

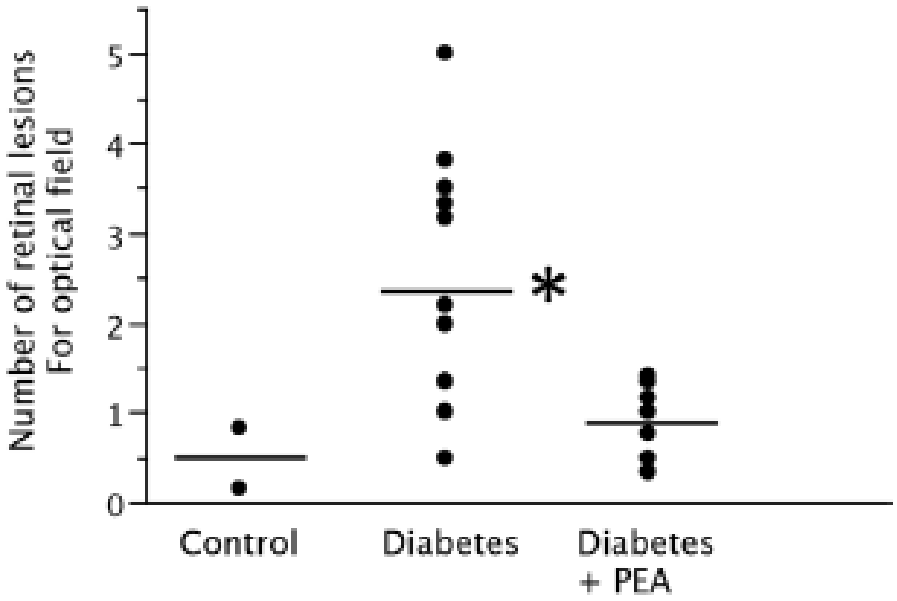




regolare distribuzione delle proteine ZO-1 e Claudina

La PEA preserva da lesioni retiniche (microaneurismi) analizzate nei preparati istologici di retina intera





Questo risultato appare molto significativo da un punto di vista clinico poiché evidenzia lesioni retiniche a livello vascolare confrontabili con quelle rilevabili nei soggetti con retinopatia diabetica confermando l'effetto protettivo che la PEA può avere nei confronti di questa grave e invalidante malattia oculare.

La Palmitoiletanolamide

Neuroinfiammazione Retinica

I risultati dei numerosi studi sperimentali confermano l'effetto "farmacologico" della

PEA

Modula/Controlla tutti i processi neuroinfiammatori oculari

- **Glaucoma**
- **Maculopatie**
- **Neuropatia Diabetica**
- **Dolore Neurogenico**

Grazie per l’attenzione