Retinal ischemia & its relevance: Steroid, anti-VEGF, Protectant, Stem cells, Gene Therapy, Retinal Chip

Hsiao-Ming Chao, PhD (U. of Oxford)

趙效明

振興醫院眼科部科主任（Retinal Surgeon）

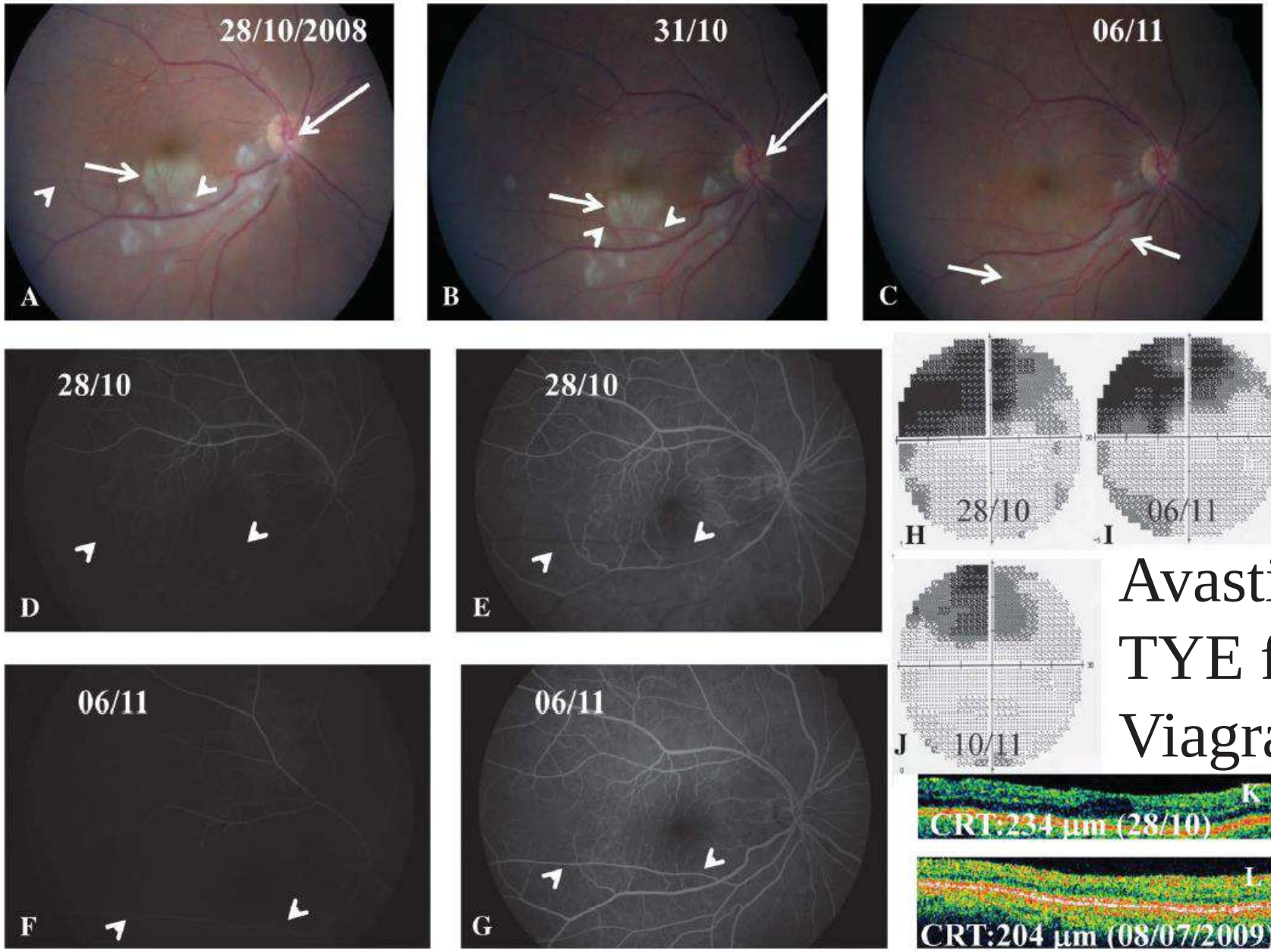
陽明大學部定副教授（Associate Professor）

Radcliff Camera, University of Oxford

Intra-arterial thrombolysis for retinal artery occlusion: the Calgary experience: 10-30 mg rtPA (none CRAO>20/300)
Can J Nrol Sci. 2005 Nov;32(4):507-11.

Vitreous surgery with direct CRA massage for CRAO
(2 M postop BCVA: ↑ ≥3L; n=6/10) *Eye (Lond)*. 2009 Apr;23(4):867-72.

Embolus-induced BRAO with initial BCVA < 0.5:
TYE restores a perfusion & vision. **Chao** *et al.*（**趙效明**）
Retina Cases Brief Report. 2013 Summer;7(3):210-6.
栓塞引發網膜分支動脈栓塞；視力<0.5 (n=6):
激光栓子切除術復灌流（16 M postTYE BCVA:↑≥4L ）



Avastin + Kenacort;
TYE for single embolus;
Viagra for many emboli

Retinal Cases & Brief Reports. 7(3):210-216, 2013

CLINICAL AND EXPERIMENTAL
OPTOMETRY

CLINICAL COMMUNICATION

Retinal angiomatous proliferation responds safely to a double dose (1.0 mg) of ranibizumab

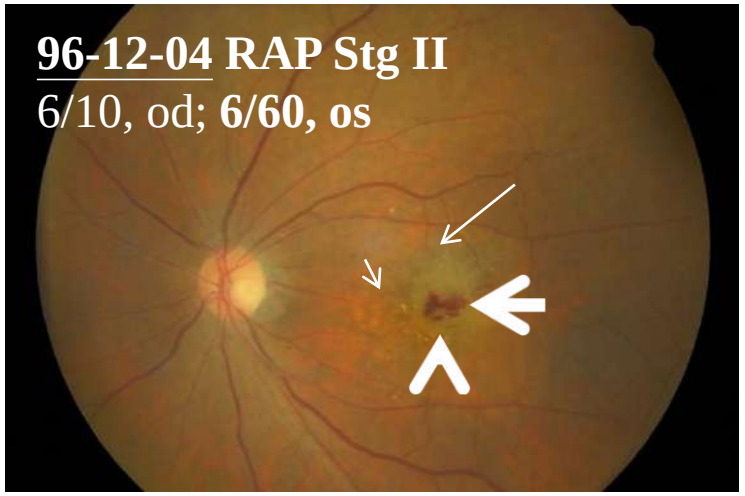
Clin Exp Optom 2012

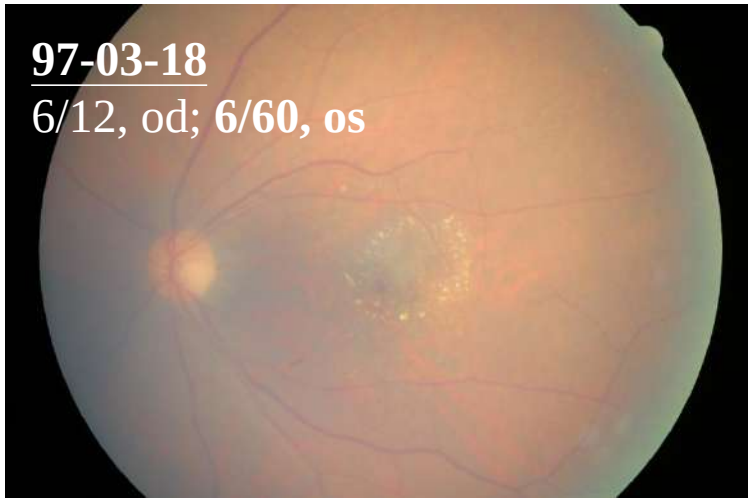
DOI:10.1111/j.1444-0938.2012.00766.x

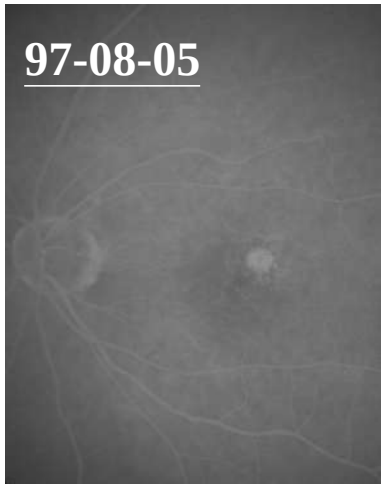
Tsui-Kang Hsu* MD
Jou-Horn Liu*† MD
Jianqin Lei§ MD PhD
Hsiao-Ming Chao*†‖ MD PhD

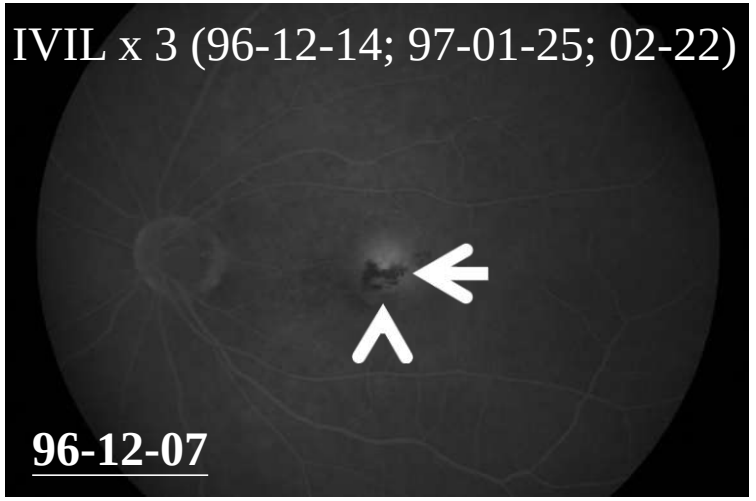
Correspondent author

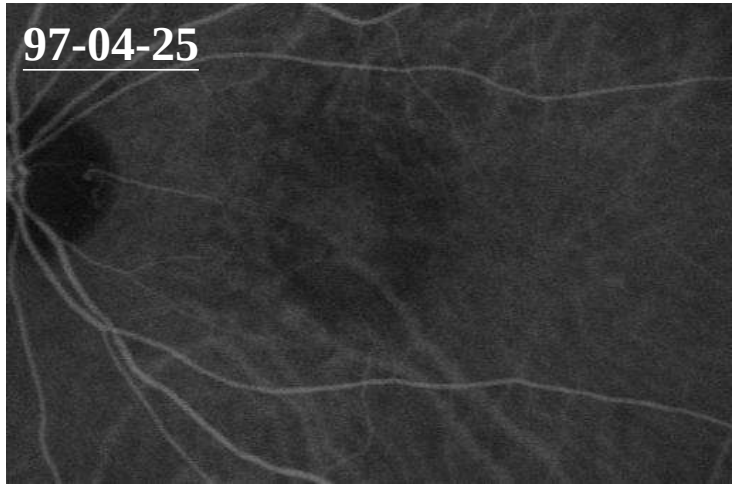
A 77-year-old man presented with sudden foggy central vision in the right eye. The visual acuity (VA) was 6/60 (R) and 6/6 (L). Funduscopy revealed superficial macular haemorrhage in the right eye. Using fluorescein angiography and indocyanine green angiography, retinal angiomatous proliferation was confirmed. Two intra-vitreal injections

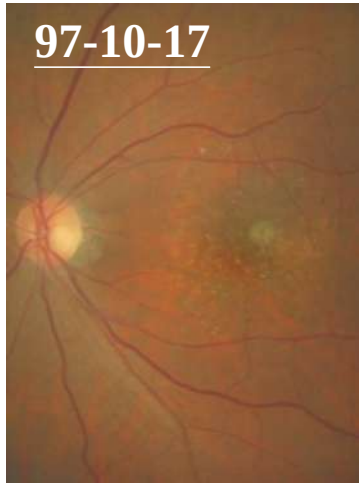












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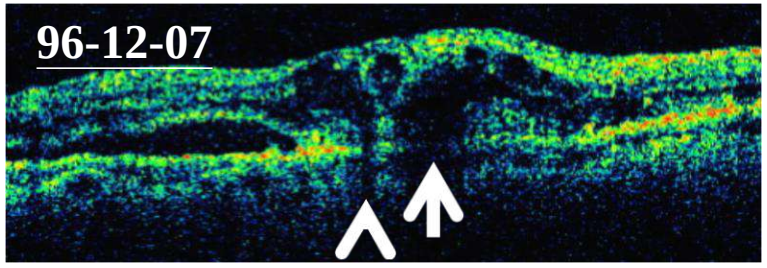
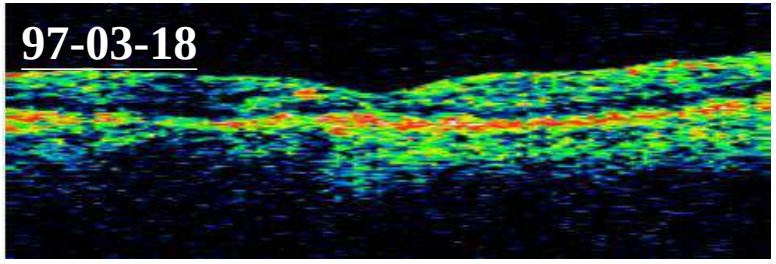
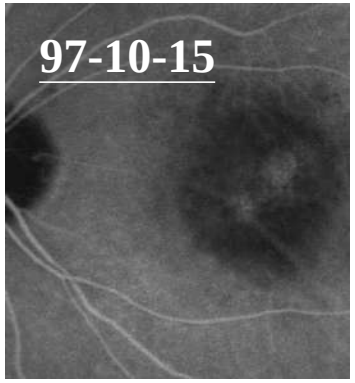
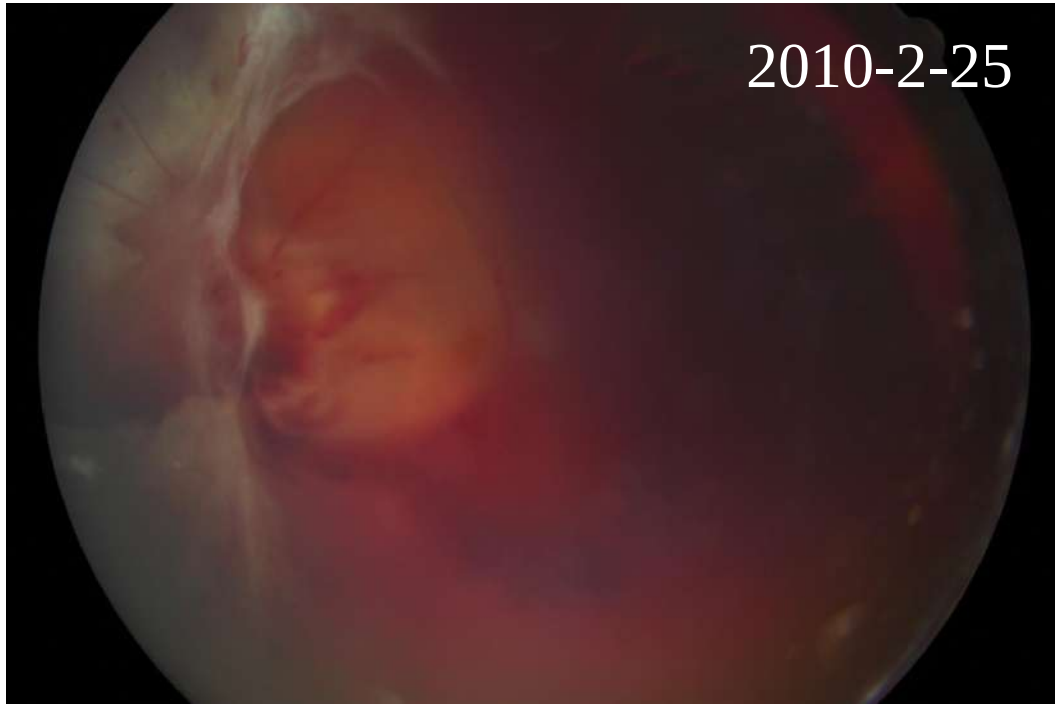






Chao *et al.* 2012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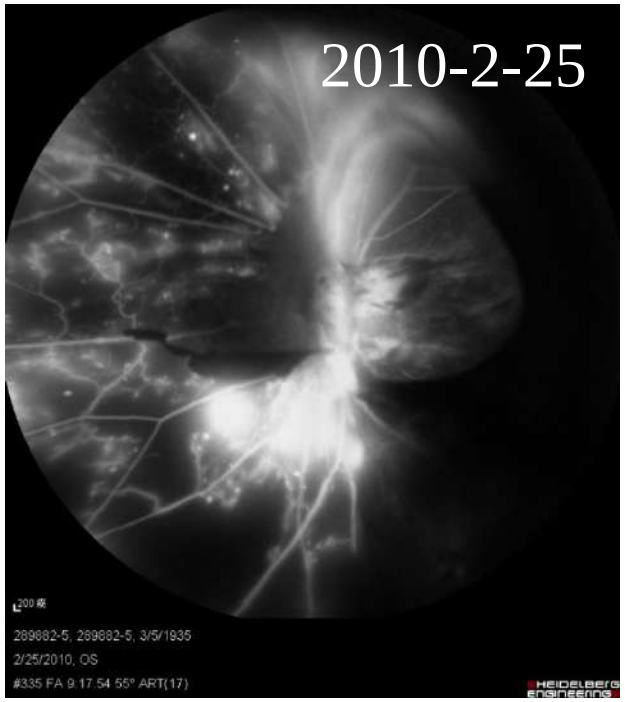
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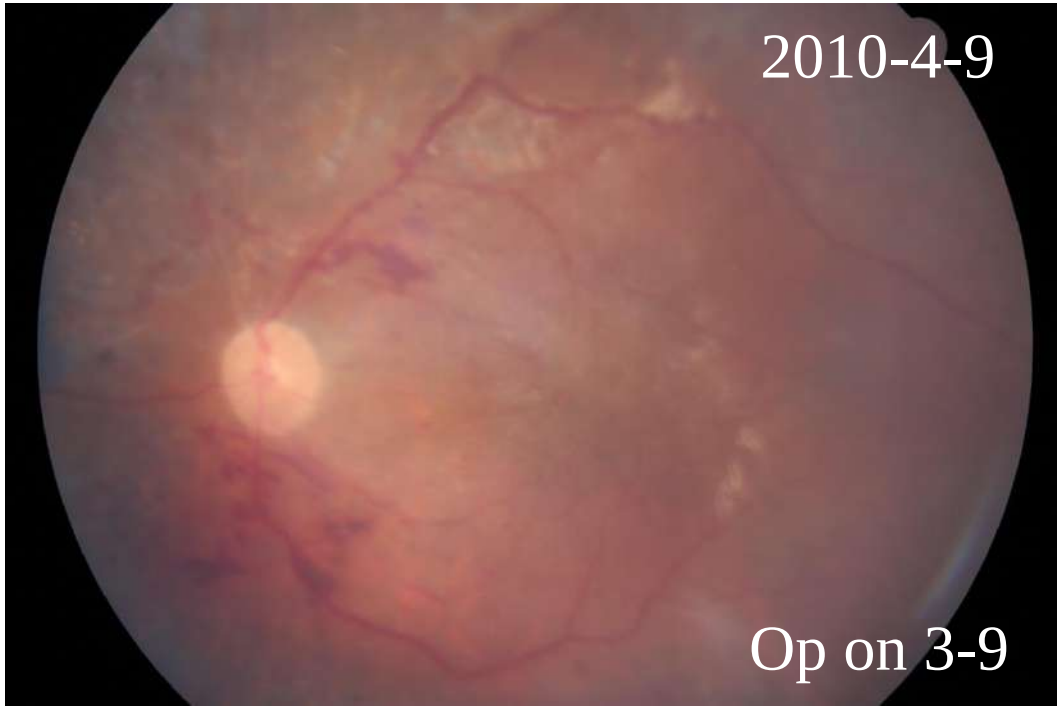
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Brain Research 877 (2000) 47–57

BRAIN RESEARCH

www.elsevier.com/locate/bres

Research report

An investigation into the potential mechanisms underlying the neuroprotective effect of clonidine in the retina

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Accepted 9 May 2000

Abstract

α_2-Adrenoceptor agonists, such as clonidine, attenuate hypoxia-induced damage to brain and retinal neurones by a mechanism of action which likely involves stimulation of α_2-adrenoceptors. In addition, the neuroprotective effect of α_2-adrenoceptor agonists in the retina may involve stimulation of bFGF production. The purpose of this study was to examine more thoroughly the neuroprotective properties of clonidine. In particular, studies were designed to ascertain whether clonidine acts as a free radical scavenger. It is thought that betaxolol, a β_1-adrenoceptor antagonist, acts as a neuroprotective agent by interacting with sodium and L-type calcium channels to reduce the influx of these ions into stressed neurones. Studies were therefore undertaken to determine whether clonidine has similar properties. In addition, studies were undertaken to determine whether i.p. injections of clonidine or betaxolol affect retinal bFGF mRNA levels. In vitro data were generally in agreement that clonidine and bFGF counteracted the effect of NMDA as would occur in hypoxia. No evidence could be found that clonidine interacts with sodium or L-type calcium channels, reduces calcium influx into neurones or acts as a free radical scavenger at concentrations below 100 μM. Moreover, i.p. injection of clonidine, but not betaxolol, elevated bFGF mRNA levels in the retina. The conclusion from this study is that the neuroprotective properties of α_2-adrenoceptor agonists, like clonidine, are very different from betaxolol. The fact that both betaxolol and clonidine blunt hypoxia-induced death to retinal ganglion cells suggests that combining the two drugs may be a way forward to producing more effective neuroprotection.

ELSEVIER

Brain Research 904 (2001) 126–136

BRAIN RESEARCH

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Research report

Topically applied clonidine protects the rat retina from ischaemia/reperfusion by stimulating α_2-adrenoceptors and not by an action on imidazoline receptors

Hsiao-Ming Chao, Neville N. Osborne*

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Accepted 3 April 2001

Abstract

Ischaemia was induced to the rat retina by raising the intraocular pressure above the systolic blood pressure for 45 min. After a reperfusion period of 5 days, alterations in the localisation of choline acetyltransferase (ChAT) and calretinin immunoreactivities, a reduction in the thickness of the inner retinal layers and a decline in the b-wave amplitude of the electroretinogram were recorded. These changes were blunted when clonidine was injected intraperitoneally before or after ischaemia or when applied topically by a specific regime. Other α_2-adrenoceptor agonists, brimonidine and apraclonidine, acted in a similar way to clonidine when applied topically but because of the number of experiments carried out a comparison between the effectiveness of the different α_2-adrenoceptor agonists was not possible. The protective effect of clonidine was attenuated when the α_2-adrenoceptor antagonists yohimbine or rauwolscine were co-administered, suggesting that the mechanism of action of the drug is to stimulate α_2-adrenoceptors. In addition, the imidazoline receptor ligands, BU-226 and AGN-192403 did not blunt the effect of ischaemia/reperfusion, supporting the notion that the protective action of the α_2-adrenoceptor agonists does not involve imidazoline sites but rather the activation of α_2-adrenoceptors. The protective effect of 0.5% clonidine appeared to be greater when topically applied to the eye that received ischaemia than when applied by the same regime to the contralateral eye. These studies suggest that while most of topically applied clonidine reaches the retina by a systemic route one cannot rule out additional pathways.

Cutaneous and Ocular Toxicology, 25: 1–10, 2006

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DOI: 10.1080/15569520600695702

Taylor & Francis
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Case Reports

SIDEROSIS OCULI: VISUAL DYSFUNCTIONS EVEN AFTER IRON REMOVAL: A ROLE OF OCT

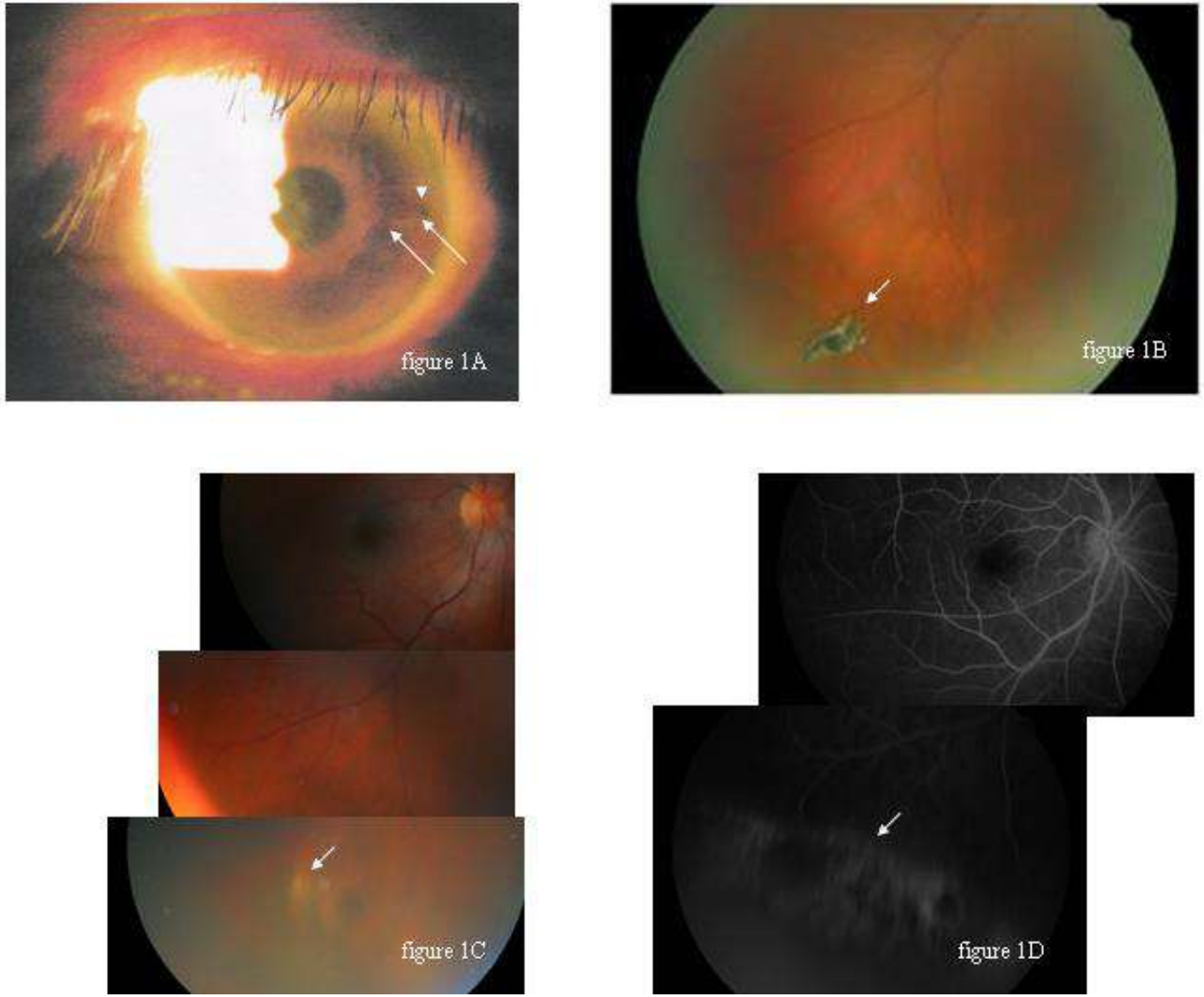
Hsiao-Ming Chao

Department of Ophthalmology, Taipei Veterans General Hospital, Taipei, Taiwan, Department of Ophthalmology, Faculty of Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan, and Institute of Pharmacology, School of Medicine, National Yang-Ming University, Taipei, Taiwan

Shih-Jen Chen, Weng-Ming Hsu, Fenq-Lih Lee, and Ko-Hua Chen

Department of Ophthalmology, Taipei Veterans General Hospital, Taipei, Taiwan and Department of Ophthalmology, Faculty of Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan

Four males with sidersosis oculi were reviewed. Vitreouslanterior chamber angle irons (cases 1 and 3) were misdiagnosed initially and discovered later. In case 2, the retina-incarcerated iron was long ignored. Exceptionally in case 4, the iron was encapsulated by using optical coherence tomography (OCT). Preoperatively, in cases 1 and 4, the injured eye's vision, electro-oculogram, and electroretinogram were reduced compared with the other eye. In three cases, field defects were relevant to their iron locations. Postremoval, iron-impaired retinal functions didn't obviously improve. Early iron removal seems vital. OCT identified iron encapsulation, ameliorating iron toxicity. Consistently, field defect in case 4 was nonprogressive.



Chao *et al.*
Cutaneous and Ocular Toxicology, 25: 1–10, 2006
(also accepted by Human and Experimental Toxicology)

Chao *et al.*
Cutaneous and Ocular Toxicology, 25: 1–10, 2006
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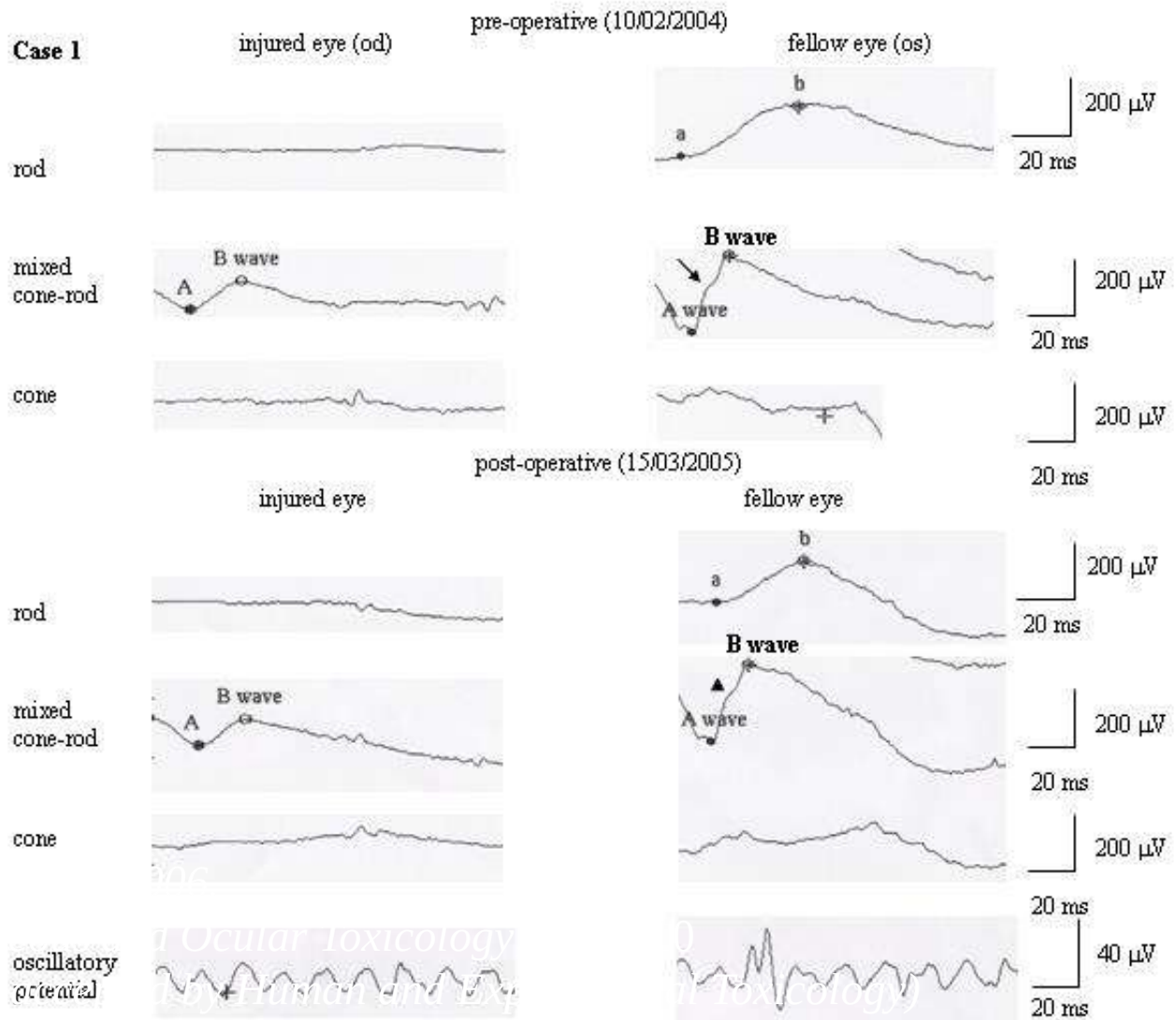


Human & Experimental Toxicology (2008) 27: 327–339
www.het.sagepub.com

Article

Iron-generated hydroxyl radicals kill retinal cells *in vivo*: effect of ferulic acid

HM Chao[1,2,3,5,10], YH Chen[1,3], JH Liu[2,4], SM Lee[1,2], FL Lee[1,2], Y Chang[5,6], PH Yeh[3], WHT Pan[3], CW Chi[3,7], TY Liu[3,7], WY Lui[8,9], LT Ho[7], CD Kuo[7], DE Lin[1,5], CC Chan[1,6], DM Yang[6,7], AMY Lin[7] and FP Chao[1,7]

[1]Department of Ophthalmology, Veterans General Hospital, Taipei, Taiwan, Republic of China; [2]Department of Ophthalmology, Faculty of Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan, Republic of China; [3]Institute of Pharmacology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, Republic of China; [4]Cheng Hsin Rehabilitation Medical Center, Taipei, Taiwan, Republic of China; [5]Institute of Biomedical Engineering, National Yang-Ming University, Taipei, Taiwan, Republic of China; [6]Institute of Biophotonics, National Yang-Ming University, Taipei, Taiwan, Republic of China; [7]Department of Medical Research and Education, Veterans General Hospital, Taipei, Taiwan, Republic of China; [8]Department of Surgery, Veterans General Hospital, Taipei, Taiwan, Republic of China; [9]Department of Surgery, Faculty of Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan, Republic of China; and [10]Department of Ophthalmology, China Medical University Hospital, Taiwan, Republic of China

Siderosis bulbi is vision threatening. An investigation into its mechanisms and management is crucial. Experimental siderosis was established by intravitreous administration of an iron particle (chronic) or $FeSO_4$ (acute). After siderosis, there was a significant dose-responsive reduction in eletroretinogram (a/b-wave) amplitude, and an increase in $^{\bullet}OH$ level, greater when caused by 24 mM $FeSO_4$ than that by 8 mM $FeSO_4$. Furthermore, the $FeSO_4$-induced oxidative stress was significantly blunted by 100 μM ferulic acid (FA). Siderosis also resulted in an excessive glutamate release, increased $[Ca^{++}]_i$, and enhanced superoxide dismutase immunoreactivity. The latter finding was consistent with the Western blot result. Obvious disorganization including loss of photoreceptor outer segments and cholinergic amacrines together with a wide-spreading ferric distribution across the retina was present, which were related to the eletro-retinographic and pathologic dysfunctions. Furthermore, b-wave reduction and amacrine damage were respectively, significantly, dose-dependently, and clearly ameliorated by FA. Thus, siderosis stimulates oxidative stress, and possibly, subsequent excitotoxicity, and calcium influx, which explains why the retina is impaired electrophysiologically and pathologically. Importantly, FA protects iron toxicity perhaps by acting as a free radical scavenger. This provides an approach to the study and treatment of the iron-related disorders such as retained intraocular iron and Alzheimer disease.

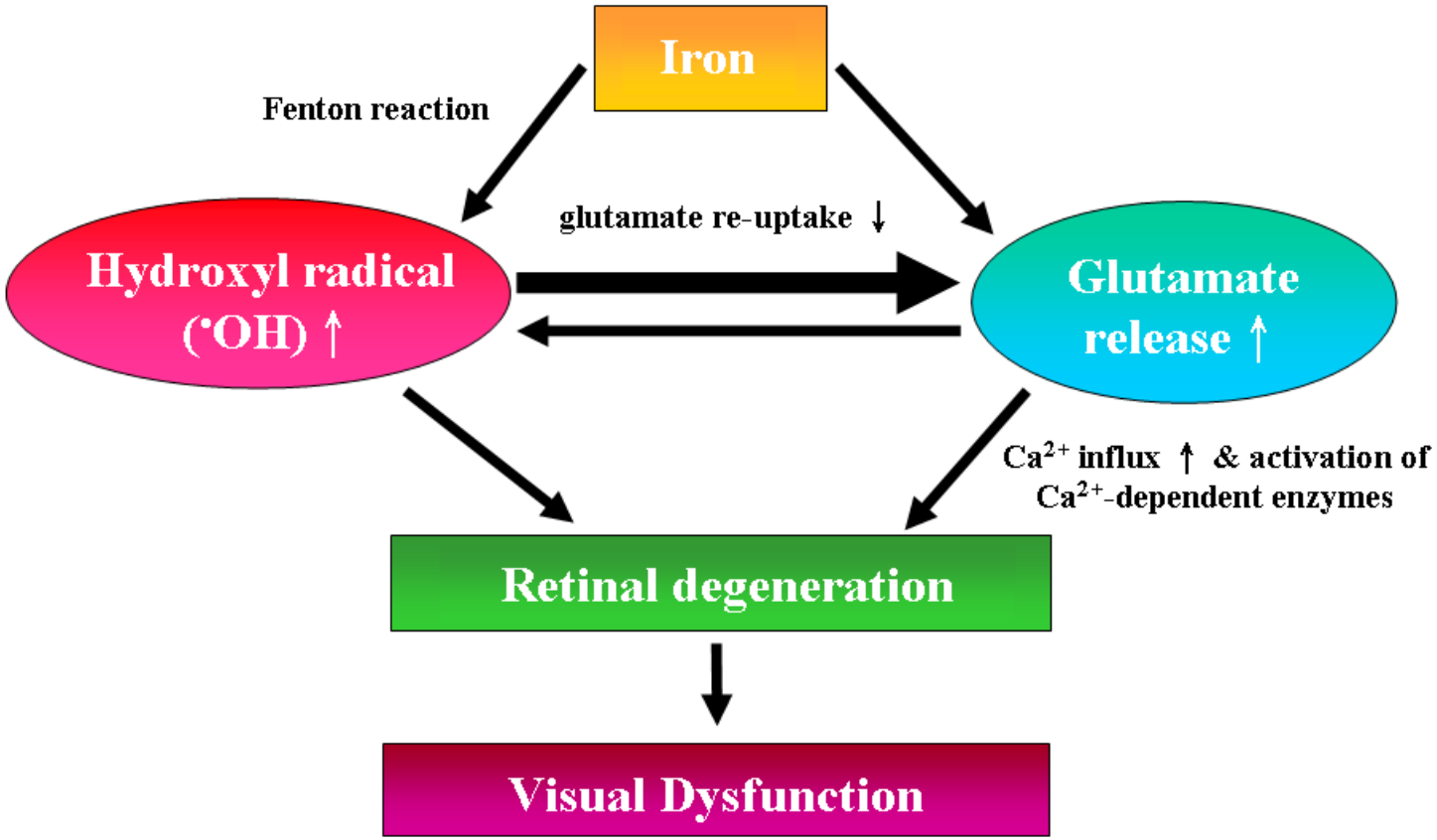
Key words: Ca^{++}; ferulic acid; glutamate; iron; $^{\bullet}OH$

Fe-induced ·OHs Kill Retinal Cells In Vivo: Ferulic Acid Effect

Hum Exp Toxicol. 27(4):327-39, 2008 **Chao** *et al.*

鐵誘導·OH損傷網膜細胞：阿魏酸療效

Chao *et al.*
Cutaneous and Ocular Toxicology, 25: 1–10, 2006
(also accepted by Human and Experimental Toxicology)



Chao *et al.*
Cutaneous and Ocular Toxicology, 25: 1–10, 2006
(also accepted by Human and Experimental Toxicology)

JOURNAL OF OCULAR PHARMACOLOGY AND THERAPEUTICS
Volume 24, Number 5, 2008

DOI: 10.1089/jop.2008.0005

Ferulic Acid, but not Tetramethylpyrazine, Significantly Attenuates Retinal Ischemia/Reperfusion-Induced Alterations by Acting as a Hydroxyl Radical Scavenger

Hsiao-Ming Chao,[1–3] De-Ean Lin,[1,4] Ying Chang,[4,5] Weng-Ming Hsu,[6] Shui-Mei Lee,[1,2] Feng-Lih Lee,[1,2] Chin-Wen Chi,[3,7] Wynn H.T. Pan,[3] Tsung-Yun Liu,[3,7] Wing-Yiu Lui,[8,9] Low-Tone Ho,[7] Cheng-Deng Kuo,[7] Chia-Chin Chan,[1,5] and Fang-Ping Chao[1,7]

Abstract

Purpose: Ischemia plays an important role in glaucomatous optic neuropathy and retinal vascular occlusive disorders, which renders investigation vital.
Methods: Retinal ischemia was induced by raising intraocular pressure to 120 mmHg. Its mechanism and management was evaluated by measuring ˙OH levels, electroretinogram (ERG) b-wave amplitudes, immunohistochemistry, and reverse transcriptase polymerase chain reaction.
Results: Ischemia for 45, 60, and 75 min caused significant and time-dependent increased ˙OH levels, which might contribute to retinal ischemic injures. Specifically, 60 min of ischemia plus reperfusion, causing moderate oxidative stress, resulted in retinal changes that were characterized by decreased ERG b-wave amplitudes, loss of choline acetyltransferase immunolabeled amacrine cell bodies/neuronal processes, downregulated Thy-1 m-RNA levels (indexing retinal ganglion cells; RGCs), and reduced thickness of the Thy-1 immunolabeled RGC and inner plexiform layers. Of clinical importance, this is the first study to show that ischemic detrimental effects are significantly blunted when 0.5 nmol of ferulic acid, one active ingredient of *Ligusticum wallichi* (Chuanxiong), was applied 24 h before retinal ischemia. Further, but not to a significant level, 0.5 nmole of tetramethylpyazine, another Chuanxiong-active component, showed such an ameliorating trend. Moreover, the 60-min ischemia-induced significant increase in ˙OH production was significantly attenuated by FA.
Conclusions: FA is able to protect against retinal ischemia and possibly glaucoma by, at least in part, acting as a ˙OH scavenger.

Ferulic acid * attenuates retinal I/R by scavenging ·OH

J. Ocul. Pharmacol. Ther., 24(5):461-72, 2008

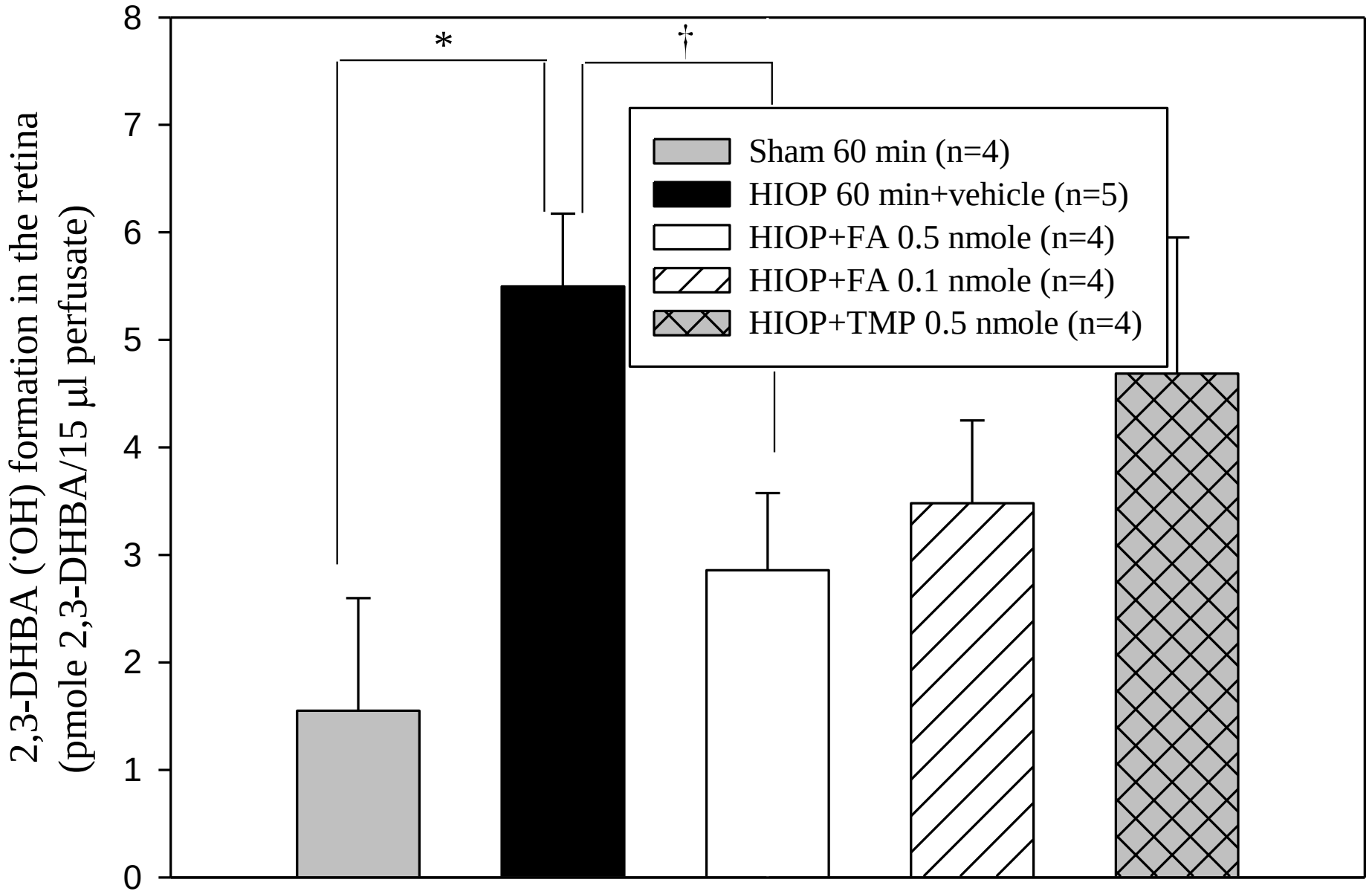
阿魏酸吞噬氫氧根游離基，明顯地減弱網膜缺血損傷

2008年3月通過“ferulic acid”療效之中華民國發明專利

（適應:**網膜缺血**、**青光眼**及**老年黃斑病**；

證號:I353248；效期:**2028年3月**；發明人:**趙效明**；專利權人:**台北榮總**）

預防或治療“網膜缺血”或“wAMD”**上**，具**學**術、臨床貢獻


2,3-DHBA (˙OH) formation in the retina
(pmole 2,3-DHBA/15 µl perfusate)
0
1
2
3
4
5
6
7
8
*
†
Sham 60 min (n=4)
HIOP 60 min+vehicle (n=5)
HIOP+FA 0.5 nmole (n=4)
HIOP+FA 0.1 nmole (n=4)
HIOP+TMP 0.5 nmole (n=4)

中華民國專利證書

發明第 I 353248 號

發明名稱：一種治療視網膜缺血及青光眼之中草藥組成物

專利權人：行政院國軍退除役官兵輔導委員會臺北榮民總醫院

發明人：趙效明

專利權期間：自2011年12月1日至2028年3月30日止

上開發明業經專利權人依專利法之規定取得專利權

經濟部智慧財產局

局長 王美花

中華民國 101 年 11 月 15 日（換發）

注意：專利權人未依法繳納年費者，其專利權自原繳費期限屆滿之次日消滅。

Therapeutic Effects and Mechanisms of Action of Mannitol During H_2O_2-Induced Oxidative Stress in Human Retinal Pigment Epithelium Cells

Jorn-Hon Liu,[1,2] Mi-Mi Chen,[1] Jhao-Wei Huang,[3] Hsiung Wann,[1,3] Li-Kang Ho,[3] Wynn H.T. Pan,[3] Yei-Ching Chen,[1] Chi-Ming Liu,[4] Ming-Yang Yeh,[1] Shen-Kou Tsai,[1] Mason Shing Young,[1] Low-Tone Ho,[5] Cheng-Deng Kuo,[5] Hui-Yen Chuang,[1] Fang-Ping Chao,[1] and Hsiao-Ming Chao[1–3]

Correspondent Author

J Ocul Pharmacol Ther. **2010** Jun;26(3):249-57

Abstract

Background: Age-related macular degeneration (AMD) is a leading cause of blindness in the elderly. At a later stage, neovascular or exudative AMD can lead to severe central vision loss that is related to aging-associated cumulative oxidative stress of the human retinal pigment epithelium (hRPE) and choroid capillary. Early prevention with antioxidants is mandatory. The aim of this study was to determine whether and how mannitol can act as an antioxidant.

Methods: The methods used included measurements of cell viability, oxygen free radical (OFR) levels, lipid peroxide (LP) levels, and OFR-related enzyme protein levels.

Results: H_2O_2 dose-dependently reduced the cell viability of hRPE cells. This negative effect was significantly counteracted by pretreatment with mannitol (1 mM). H_2O_2 significantly stimulated the formation of OFR and LP. These increases were dose-dependently and significantly blunted by mannitol. Furthermore, treatment with H_2O_2 was associated with a reduction in the level of catalase, but not of manganese superoxide dismutase (MnSOD). In contrast, it was shown that mannitol protected hRPE cells against the H_2O_2-induced oxidative stress by increasing the level of catalase, but not the level of MnSOD.

Conclusion: This study supports an antioxidative role for mannitol that acts through up-regulating the level of catalase, which is decreased by H_2O_2.

Introduction

AGE-RELATED MACULAR DEGENERATION (AMD) is a leading cause of blindness in the elderly.[1–3] The early stages of the disease are characterized by drusen and retinal pigmentary changes. Fifteen percent of affected individuals experience profound loss of central vision owing to the development of choroidal neovascularization (CNV), namely exudative AMD.[4] This has a profound effect on the lives of the affected patients.[5] These include depression, and loss of the ability to drive, read, and recognize faces, as well as reduced mobility and impaired orientation. Vision loss also puts patients at an increased risk of falls and increases the likelihood of needing residential nursing care.

Edema is observable swelling due to fluid accumulation in a body tissue. The body's organs such as the retina have interstitial spaces where fluid can accumulate. In diabetic retinopathy, and exudative AMD, there is neovascularization or a change in retinal vessel permeability, whereby fluid leaks and accumulates. The retinal edema may be located in the macula, which can result in vision loss or blindness. Mannitol has been used to treat or prevent medical conditions that are caused by an increase in interstitial fluid and/or water.[6] It is reasonable, therefore, to suggest that this might also be the case when treating the macular edema found with diabetes and in patients with neovascular AMD.

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[2]Department of Ophthalmology, Faculty of Medicine and [3]Institute of Pharmacology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, Republic of China.
[5]Department of Medical Research and Education, Veterans General Hospital, Taipei, Taiwan, Republic of China.

H_2O_2 oxidative stress in hRPEs: Effects/mechanisms of mannitol
J Ocul Pharmacol Ther.,26(3):249-57, 2010

過氧化氫對人類網膜色素上皮細胞之氧化壓力：甘露醇作用及機制

Mannitol: ↓ ROS, ↓ lipid peroxides, ↑ catalase, ↔ SOD

甘露醇治Brain edema

Wet AMD: 預防或治療上（IRF, SRF or RPED），具學術、臨床貢獻

Baicalein Significantly Protects Human Retinal Pigment Epithelium Cells Against H_2O_2-Induced Oxidative Stress by Scavenging Reactive Oxygen Species and Downregulating the Expression of Matrix Metalloproteinase-9 and Vascular Endothelial Growth Factor

Jorn-Hon Liu,[1–3] Hsiung Wann,[1,4] Mi-Mi Chen,[1] Wynn H.T. Pan,[4] Yei-Ching Chen,[1] Chi-Ming Liu,[5] Ming-Yang Yeh,[3] Shen-Kou Tsai,[3] Mason Shing Young,[3] Hui-Yen Chuang,[1] Fang-Ping Chao,[1] and Hsiao-Ming Chao [1,2,4]

Correspondent Author

J Ocul Pharmacol Ther. **2010** Oct;26(5):421-9

Abstract

Purpose: Age-related macular degeneration is a leading cause of blindness in the elderly. At a later stage, neovascular or exudative age-related macular degeneration can lead to severe central vision loss that is related to aging-associated cumulative oxidative stress of the human retinal pigment epithelium (hRPE) cells. Early prevention with antioxidants is mandatory. The aim of this study was to determine whether and how baicalein can act as an antioxidant.

Methods: The methods used included lactate dehydragenase, 2′,7′-dichloro-fluorescein diacetate, or enzyme-linked immunosorbent assay to measure cell viability, oxygen-free radical levels, or the levels of vascular endothelial growth factor (VEGF)/matrix metalloproteinase-9 (MMP-9), respectively.

Results: H_2O_2 dose-dependently reduced the cell viability of hRPE cells. This negative effect was dose-dependently (with a lower effect at 20 μM) and significantly counteracted by pretreatment with baicalein (50 μM). Treatment with H_2O_2 significantly stimulated the formation of oxygen-free radicals. This increase was dose-dependently and significantly blunted by baicalein. Further, treatment with a sublethal dose of H_2O_2 was associated with an upregulation in the levels of VEGF and MMP-9. The increases in these proteins were also dose-dependently (with a lower effect at 20 μM) and significantly (50 μM) blunted by pretreatment with baicalein.

Conclusion: This study supports an antioxidative role for baicalein whereby it protects hRPE cells against H_2O_2-induced oxidative stress by downregulating the levels of VEGF and MMP-9, which are increased by H_2O_2.

Introduction

AGE-RELATED MACULAR DEGENERATION (AMD) is a leading cause of blindness in the elderly.[1] The prevalence of AMD increases with age. The early stages of the disease are characterized by drusen and retinal pigmentary changes. Fifteen percent of affected individuals experience profound loss of central vision owing to the development of choroidal neovascularization (CNV), namely exudative AMD.[2] This has a profound effect on the lives of the affected patients.[3] These include depression and loss of ability to drive, read, and recognize faces, as well as reduced mobility and impaired orientation. Vision loss also puts patients at an increased risk of falls and increases the likelihood of them needing residential nursing care.

The retinal pigment epithelium (RPE) is in the outmost layer of the retina. RPE synthesizes the extracellular matrix. The RPE cells absorb redundant light and process via phagocytosis of the photoreceptor outer segments, and therefore, it is usually under high oxidative stress. It has been shown that the RPE phagocytosis of the photoreceptor outer segments increases extracellular H_2O_2 by 9-fold.[4] Oxidative

[1]Department of Ophthalmology, Cheng Hsin General Hospital, Taipei, Taiwan, R.O.C.
[2]Department of Ophthalmology, Faculty of Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan, R.O.C.
[3]Cheng Hsin General Hospital, Taipei, Taiwan, R.O.C.
[4]Institute of Pharmacology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, R.O.C.
[5]Department of Medical Research and Education, Cheng Hsin General Hospital, Taipei, Taiwan, R.O.C.

H_2O_2-induced oxidative stress in hRPE cells:
baicalein scavenged ROS & ↓MMP-9 & **VEGF** expression
J Ocul Pharmacol Ther. **2010** Oct;26(5):421-9

人類網膜色素上皮細胞之過氧化氫的氧化壓力：
黃芩素吞噬氫氧根游離基、↓基質金屬蛋白酶-9/血管內皮生長因子的表達

Anti-VEGF: avastin/lucentis/eyelea

預防或治療“wAMD”**上**，具**學**術、臨床貢獻

JOURNAL OF OCULAR PHARMACOLOGY AND THERAPEUTICS
Volume 29, Number 6, 2013

DOI: 10.1089/jop.2012.0179

Baicalein Protects Against Retinal Ischemia by Antioxidation, Antiapoptosis, Downregulation of *HIF-1α*, *VEGF*, and *MMP-9* and Upregulation of *HO-1*

Hsiao-Ming Chao,[1,2,3*] Min-Jay Chuang,[3*] Jorn-Hon Liu,[1,2] Xiao-Qian Liu,[4] Li-Kang Ho,[3] Wynn H.T. Pan,[3] Xiu-Mei Zhang,[4] Chi-Ming Liu,[5,6] Shen-Kou Tsai,[5] Chi-Woon Kong,[5] Shou-Dong Lee,[5] Mi-Mi Chen,[1] and Fang-Ping Chao[1]

Current Eye Research, 36(3), 238–246, 2011

ISSN: 0271-3683 print/ 1460-2202 online
DOI: 10.3109/02713683.2010.539760

informa
healthcare

ORIGINAL ARTICLE

Pharmacological Preconditioning by Low Dose Cobalt Protoporphyrin Induces Heme Oxygenase-1 Overexpression and Alleviates Retinal Ischemia-Reperfusion Injury in Rats

Pai-Huei Peng[1,2], Hsiao-Ming Chao[3,7], Shu-Hui Juan[4], Chau-Fong Chen[5], Jorn-Hon Liu[3], and Mei-Lan Ko[6]

[1]*Department of Ophthalmology, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan*
[2]*School of Medicine, Catholic Fu-Jen University, Taipei, Taiwan*
[3]*Department of Ophthalmology, Cheng Hsin General Hospital, Taipei, Taiwan*
[4]*Department of Physiology, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[5]*Department of Physiology, College of Medicine, National Taiwan University, Taipei, Taiwan*
[6]*Department of Ophthalmology, General Hsin-Chu Hospital, Hsin-Chu, Taiwan*
[7]Institute of Pharmacology, School of Medicine, National Yang-Ming University, Taipei, Taiwan

JOURNAL OF OCULAR PHARMACOLOGY AND THERAPEUTICS
Volume 00, Number 00, 2011

DOI: 10.1089/jop.2011.0099

ORIGINAL ARTICLE

The Effects and Underlying Mechanisms of *S*-Allyl L-Cysteine Treatment of the Retina After Ischemia/Reperfusion

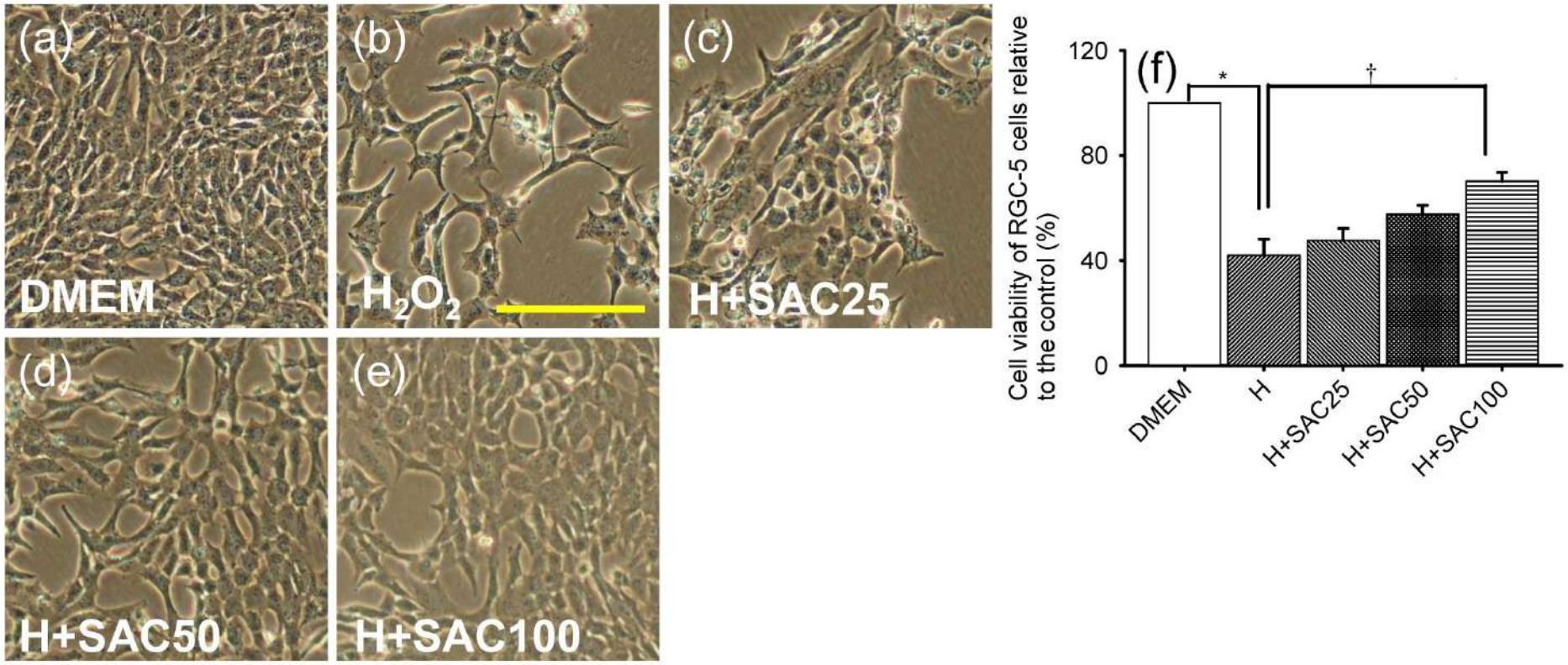
Yan-Qing Chen,[1–3] Wynn H.T. Pan,[3] Jorn-Hon Liu,[1,4] Mi-Mi Chen,[1] Chi-Ming Liu,[4,5] Ming-Yang Yeh,[4] Shen-Kou Tsai,[4] Mason Shing Young,[4] Xiu-Mei Zhang,[2] and Hsiao-Ming Chao[1,3]

1. Retina I/R: Effects & Mechanisms of S-allyl L-cysteine
J. Ocul. Pharmacol. Ther., 28(2):110-7, 2012 Chao *et al.*

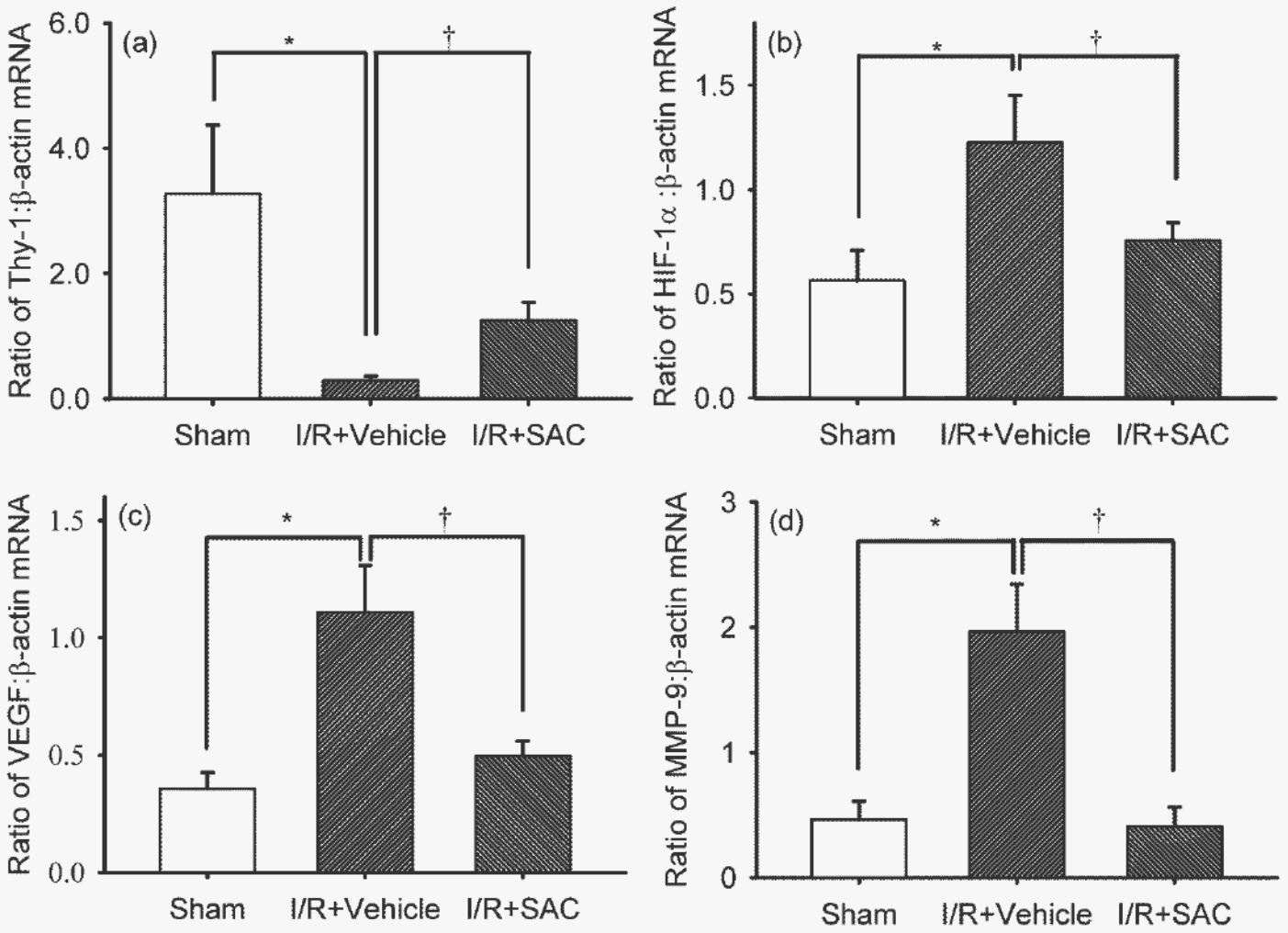
S-烯丙基-半胱氨酸（SAC）對網膜缺血損傷的作用及機制

產（睛明舫）、官（潘）、學（陽明藥理所）、醫（振興醫院）
: 兩岸學術交流 & Food Science貢獻

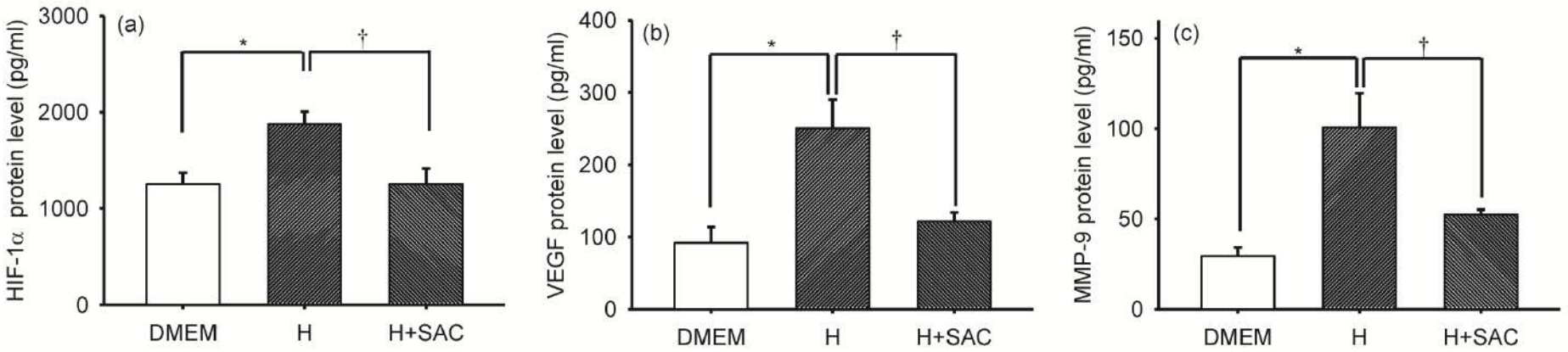
SAC: ↓**HIF-1α**, **VEGF**, **MMP-9**→**CNV** induction factor（**元凶**）
預防或治療“網膜缺血”或“wetAMD”**上**，具**學**術、臨床貢獻



J. Ocul. Pharmacol. Ther., 28(2):110-7, 2012 Chao *et al.*



J. Ocul. Pharmacol. Ther., 28(2):110-7, 2012 Chao *et al.*



J. Ocul. Pharmacol. Ther., 28(2):110-7, 2012 Chao *et al.*

The United States of America

The Director of the United States Patent and Trademark Office

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person(s) having title to this patent the right to exclude others from making, using, offering for sale, or selling the invention throughout the United States of America or importing the invention into the United States of America, and if the invention is a process, of the right to exclude others from using, offering for sale or selling throughout the United States of America, or importing into the United States of America, products made by that process, for the term set forth in 35 U.S.C. 154(a)(2) or (c)(1), subject to the payment of maintenance fees as provided by 35 U.S.C. 41(b). See the Maintenance Fee Notice on the inside of the cover.

Deputy Director of the United States Patent and Trademark Office

US008569372B2

(12) **United States Patent**
Chao

(10) **Patent No.: US 8,569,372 B2**
(45) **Date of Patent: Oct. 29, 2013**

(54) **METHOD FOR PREVENTING OR TREATING A DISEASE, DISORDER OR CONDITION INDUCED BY RETINA ISCHEMIA**

(75) Inventor: **Hsiao-Ming Chao**, Taipei (TW)

(73) Assignees: **Hsiao-Ming Chao**, Taipei (TW); **Chieh-Cheng Ke**, Taipei (TW)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 99 days.

(21) Appl. No.: **13/246,057**

(22) Filed: **Sep. 27, 2011**

(65) **Prior Publication Data**

US 2013/0079411 A1 Mar. 28, 2013

(51) **Int. Cl.**
A61K 31/19 (2006.01)

(52) **U.S. Cl.**
USPC **514/557**

(58) **Field of Classification Search**
USPC 514/557
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

7,851,501 B2 * 12/2010 Aydt et al. 514/438

OTHER PUBLICATIONS

Saravanan et al., Phytomedicine, 2010, 17(14): 1086-1089.*
Tsujikawa et al., Am. J. Physiology, 2000, 279(3,Pt. 2):R980-R989.*

* cited by examiner

Primary Examiner — Rei-tsang Shiao
(74) *Attorney, Agent, or Firm* — WPAT, P.C.; Anthony King

(57) **ABSTRACT**

The present invention provides a method for preventing or treating a disease, disorder or condition induced by retina ischemia, comprising administering to a subject in need thereof a S-allyl-L-cysteine in a therapeutically effective amount.

8 Claims, 5 Drawing Sheets

The American Journal of Chinese Medicine, Vol. 42, No. 3, 1–16

DOI: 10.1142/S0192415X14500451

S-Allyl L-Cysteine Protects the Retina Against Kainate Excitotoxicity in the Rat

Hsiao-Ming Chao,*,†,‡ Ing-Ling Chen*,†,‡ and Jom-Hon Liu*

Contents lists available at ScienceDirect

Experimental Eye Research

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

Effects of epigallocatechin-3-gallate on rat retinal ganglion cells after optic nerve axotomy

Exp Eye Res. **2010** Apr;90(4):528-34

Pai-Huei Peng [a], Lan-Fen Chiou [b], Hsiao-Ming Chao [c], Shan Lin [d], Chau-Fong Chen [b], Jorn-Hon Liu [c], Mei-Lan Ko [e,*]

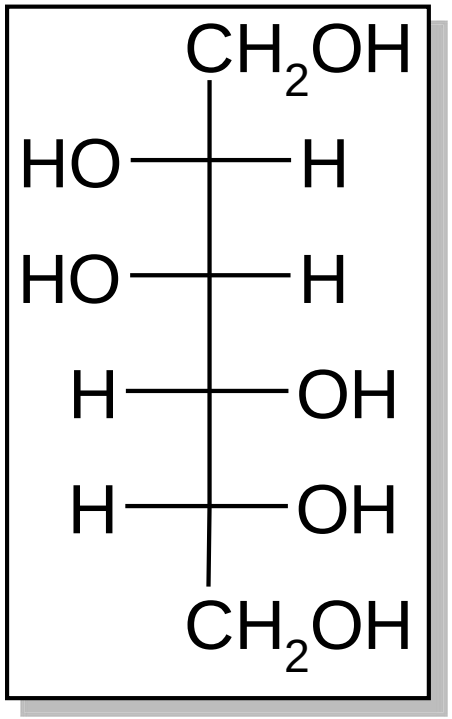
JOURNAL OF OCULAR PHARMACOLOGY AND THERAPEUTICS
Volume 29, Number 1, 2013

DOI: 10.1089/jop.2012.0141

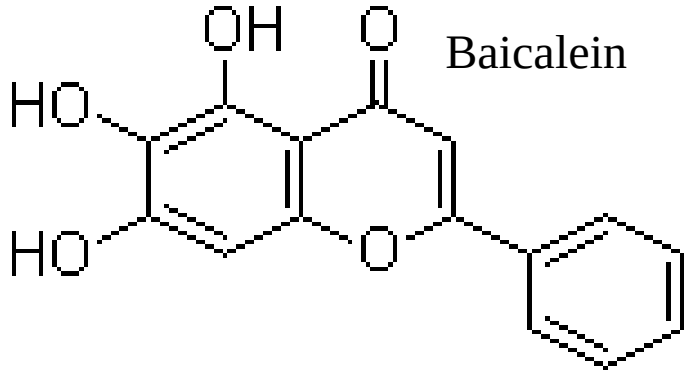
Resveratrol Mitigates Rat Retinal Ischemic Injury: The Roles of Matrix Metalloproteinase-9, Inducible Nitric Oxide, and Heme Oxygenase-1

Xiao-Qian Liu,[1] Bing-Jhih Wu,[2,3] Wynn H.T. Pan,[2,3] Xiu-Mei Zhang,[1] Jorn-Hon Liu,[4] Mi-Mi Chen,[4] Fang-Ping Chao,[4] and Hsiao-Ming Chao[2–4]

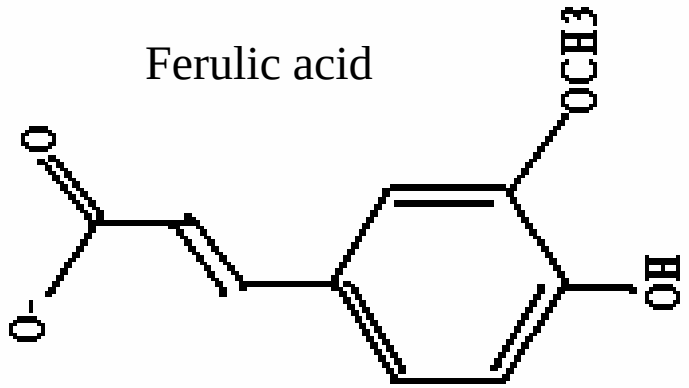
Mannitol



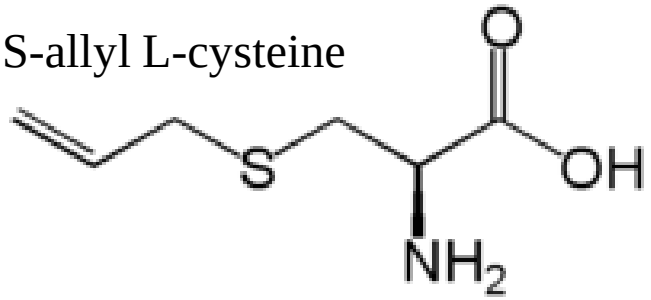
Baicalein



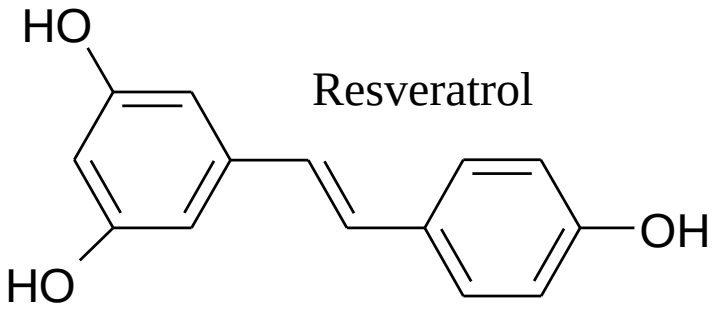
Ferulic acid



S-allyl L-cysteine



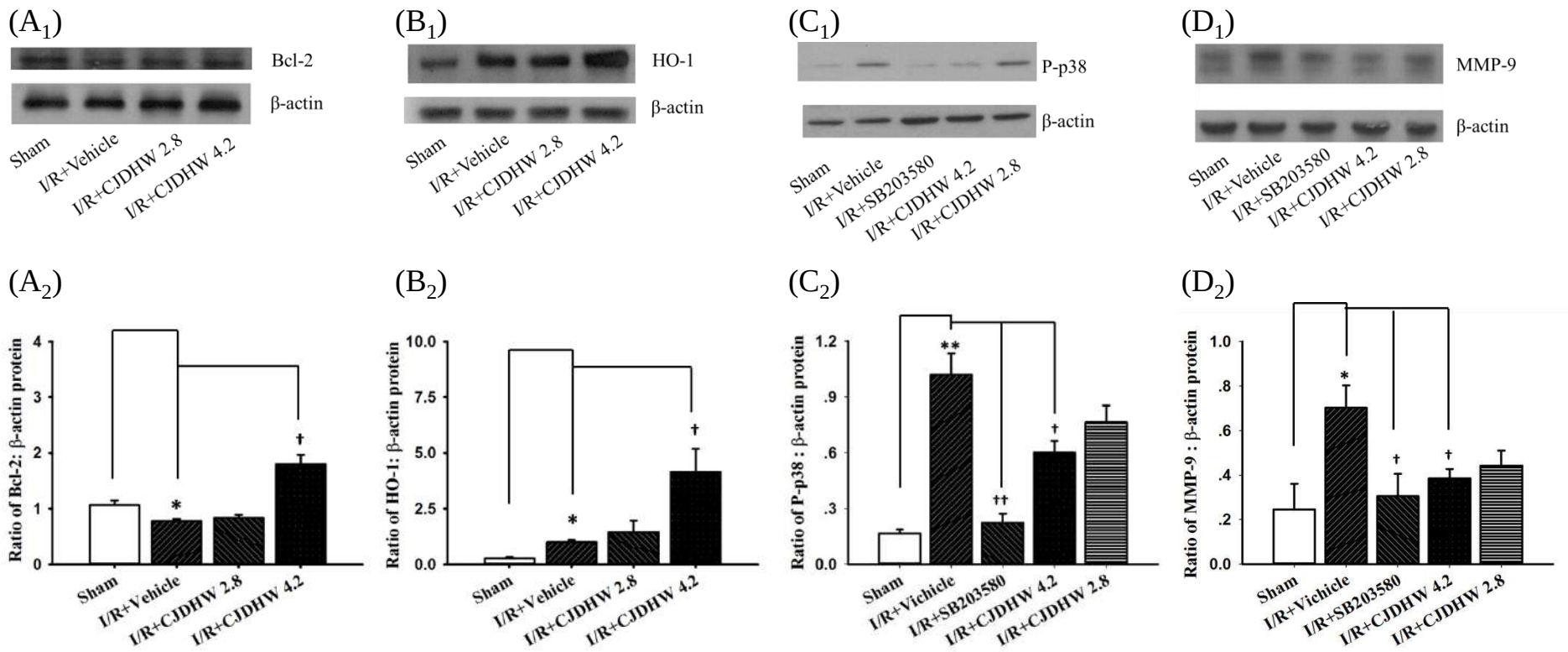
Resveratrol



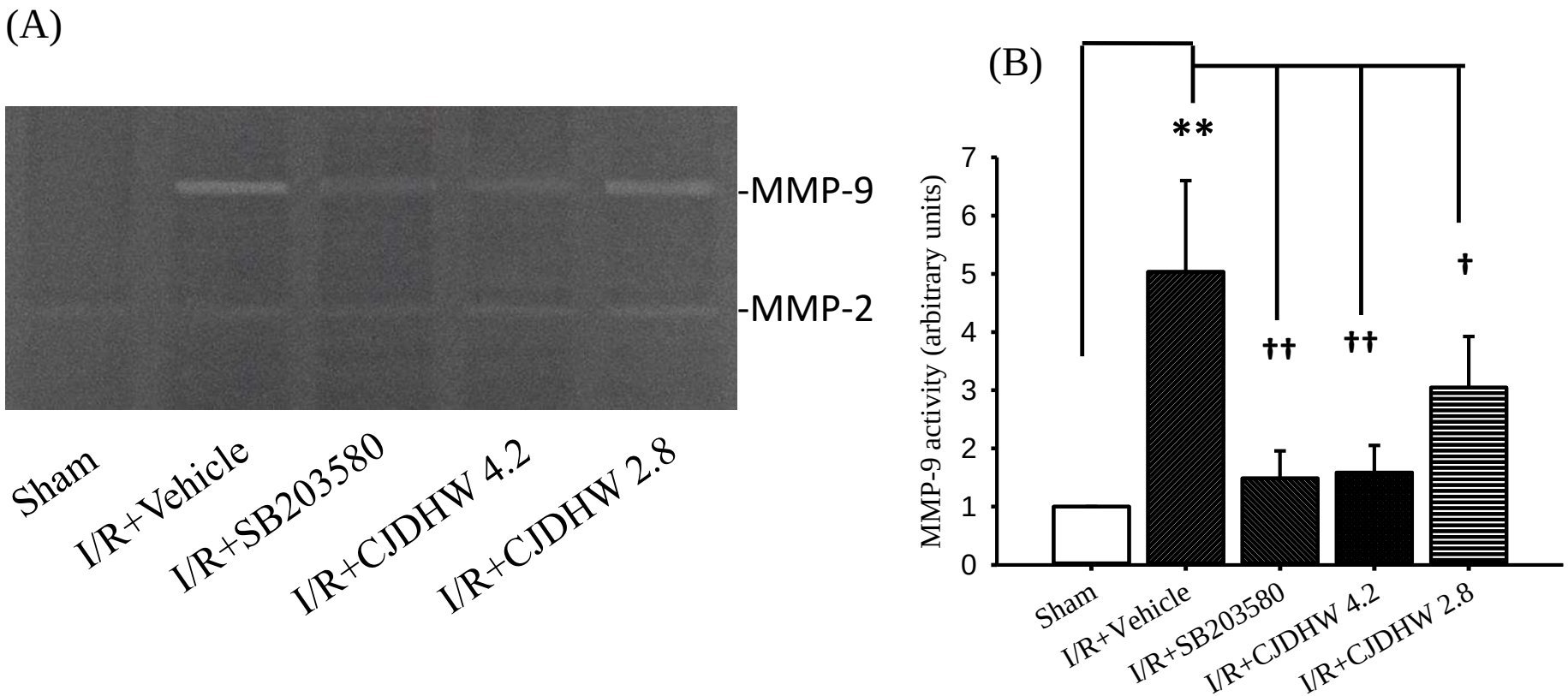
Chi-Ju-Di-Huang-Wan protects against retinal ischemia in the rat, ↓ MMP-9 & ↓ p38 MAPK

Ji-Min Cheng[1], Xiao-Qian Liu[2], Jorn-Hon Liu[3], Wynn Hwai-Tzong Pan[4], Xiu-Mei Zhang[2], Lei Hu[1*§], **Hsiao-Ming Chao**[3,4,5*§]

Accepted by *Chi Med* & Published in Early 2016



Chao et al., Accepted by *Chi Med* & Published in Early 2016



Chao et al., Accepted by *Chi Med* & Published in Early 2016

Chao et al (2008). Iron-generated Hydroxyl Radicals Kill Retinal Cells *In Vivo*: Effect of **Ferulic Acid**

Chao et al (2008). **Ferulic acid**, but not tetramethylpyrazine, significantly attenuates retinal ischaemia/reperfusion-induced alterations by acting as a hydrodyl **radical scavenger**

Liu et al. (2010). Therapeutic effects and mechanisms of action of **mannitol** during H_2O_2-induced oxidative stress in human retinal pigment epithelium cells

Liu et al. (2010). **Baicalein** significantly protects human retinal pigment epithelium cells against H_2O_2-induced oxidative stress by scavenging ROS, and down-regulating **MMP-9**/**VEGF** expression.

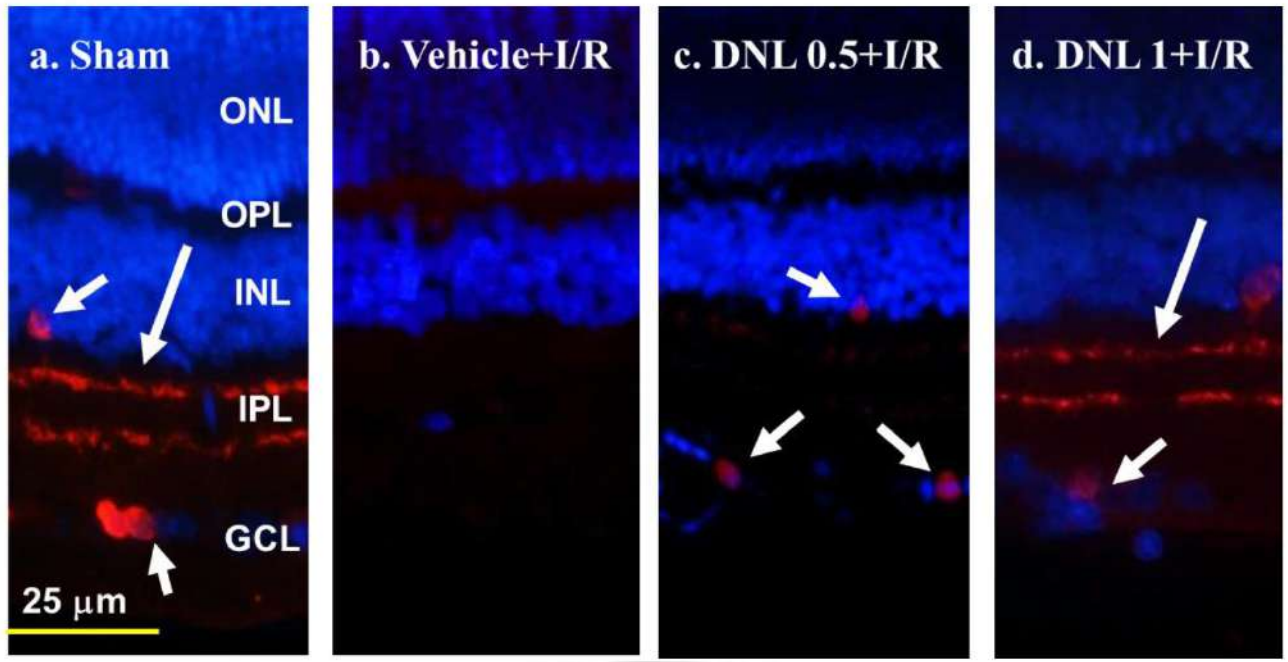
Liu et al. **Baicalein** protects against retinal ischemia by **anti-oxidation**, **anti-apoptosis**, downregulation of **HIF-1α**, **VEGF** and **MMP-9**, and upregulation of **HO-1**

Chen et al (2011). The effects and underlying mechanisms of **S-allyl L-cysteine** treatment of retina after ischaemia/reperfusion . In Revision (**HIF-1α**)

Resveratrol mitigates rat retinal ischemic injury: roles of **MMP-9**, **iNOS**, and **HO-1**

Protection/mechanisms of *Dendrobium nobile Lindl* in retinal ischemia

金釵石斛對於視網膜缺血的保護效果及機轉

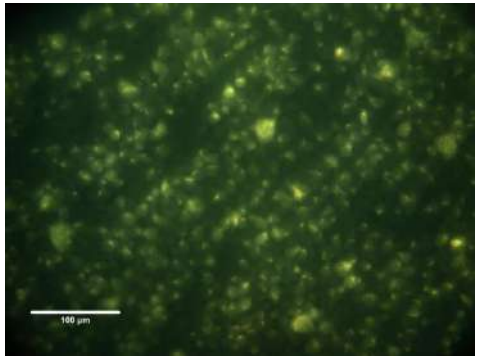

a. Sham
ONL
OPL
INL
IPL
GCL
25 μm
b. Vehicle+I/R
c. DNL 0.5+I/R
d. DNL 1+I/R

Retinal ischemia: Xuefu Zhuyu Decoction’s effect & mechanisms
對於視網膜缺血的保護效果及機轉

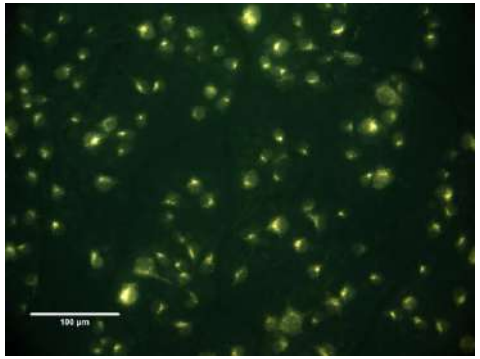
CHGH104：

Retinal ischemia: Xuefu Zhuyu Decoction's effect & mechanisms

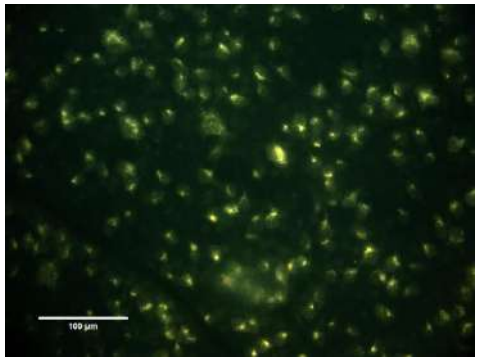
Sham



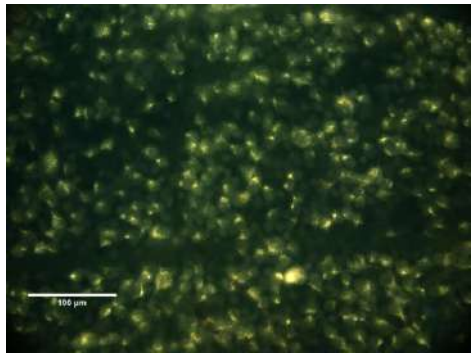
Vehicle + I/R



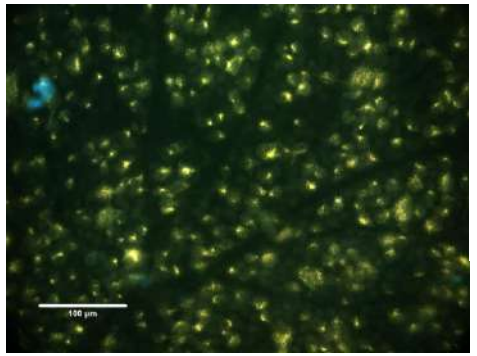
$XFZYD_{1.35}$ + I/R

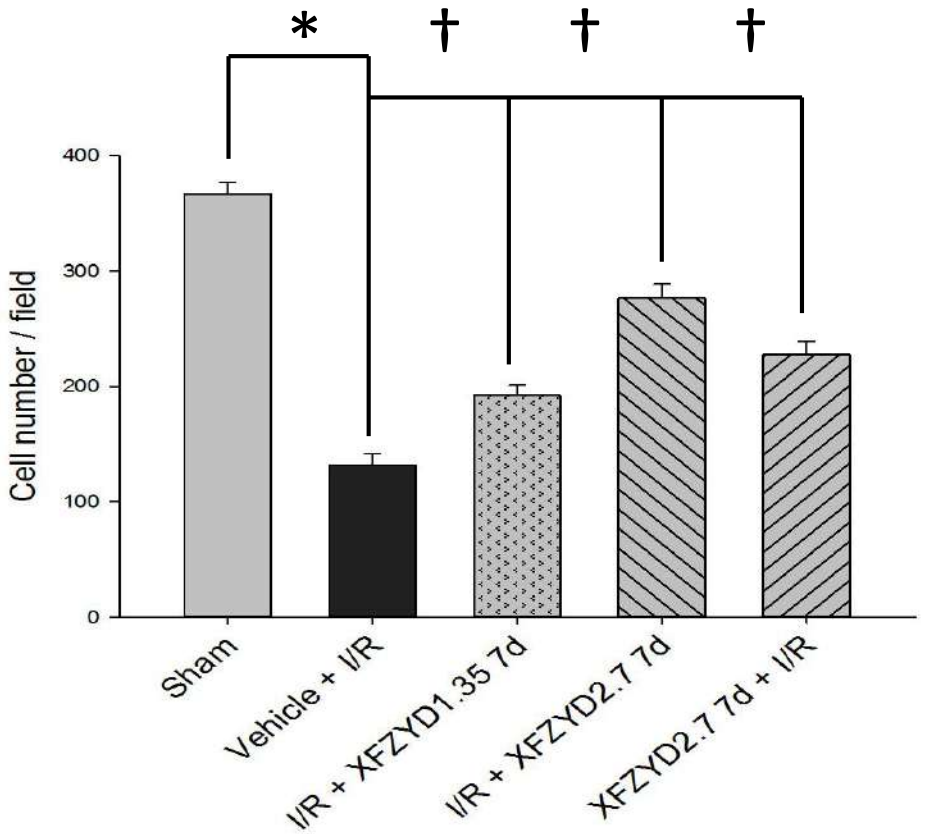


$XFZYD_{2.7}$ + I/R



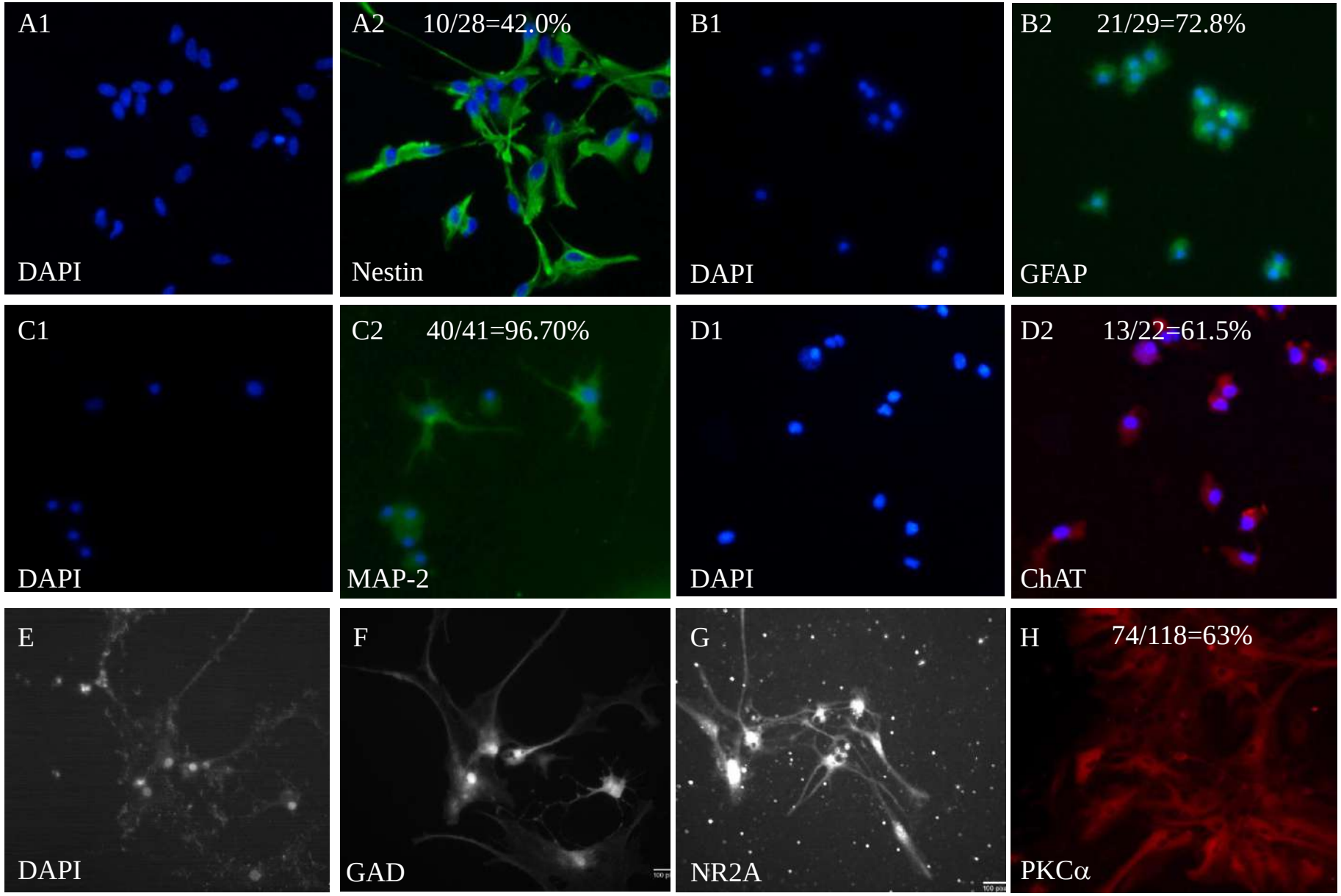
I/R + $XFZYD_{2.7}$



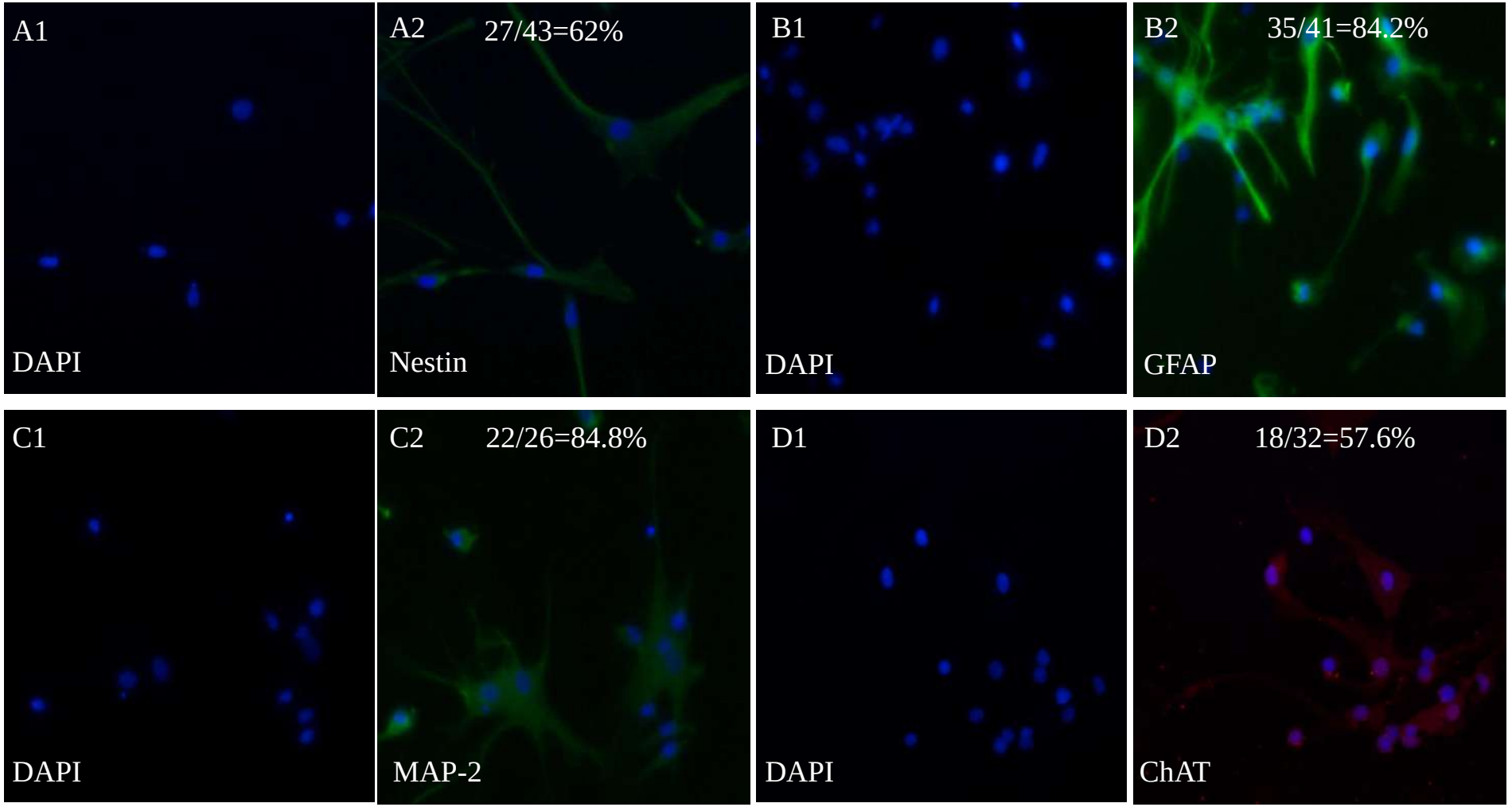


A comparison of cortical & retinal stem cells in self-renewal & differentiation:

Retina


A1
DAPI
A2
10/28=42.0%
Nestin
B1
DAPI
B2
21/29=72.8%
GFAP
C1
DAPI
C2
40/41=96.70%
MAP-2
D1
DAPI
D2
13/22=61.5%
ChAT
E
DAPI
F
GAD
G
NR2A
H
74/118=63%
PKCα

Cortex


A1
DAPI
A2
27/43=62%
Nestin
B1
DAPI
B2
35/41=84.2%
GFAP
C1
DAPI
C2
22/26=84.8%
MAP-2
D1
DAPI
D2
18/32=57.6%
ChAT

Early “Prevention”—Nutrient 上功治未病

Beverage

Tea (EGCA) Chao et al., *Exp. Eye Res.* 90(4):528-34, 2010.

Red Wine (Resveratrol) Chao et al., *J. Ocul. Pharmacol. Ther.* 29(1): 33-40, 2013.

Food

Asian:

S-allyl L-cysteine (garlic大蒜) Chao et al., *Am. J. Chin. Med.* 28(2):110-7, 2014

Ferulic acid (當歸川芎) Chao et al., *Hum Exp Toxicol.* 27(4):327-39, 2008

CJDHW (枸杞), *MS submitted* *Leung et al (2001), IOVS;42(2):466-71.*

Western:

Olive

Gingko biloba (dementia)

Lutein (macula), Vitamin E (ML Ko et al), Vitamin A (RP)

Early "Treatment"--Neuroprotectant in Eurocondor Project中工治欲病

Whether topical brimonidine & somatostatin can prevent/arrest develop./progress. of early stages of DR & neurodeg.

Alphagan-P (**Brimonidine**)
Saylor *et al*. Experimental & Clinical Evidence for **Brimonidine** as an Optic Nerve & Retinal Neuroprotectant. *Arch Ophthalmol*. 2009;127(4):402-406.

Chao HM & Osborne NN. Topical **clonidine** protects the rat retina from I/R by stimulating **α_2 adrenoceptors** & not by imidazoline receptors. *Brain Res*. 2001;904(1):126-36.

Somatostatin生長抑素
Neuropeptides, trophic factors, & others providing morphofunctional & metabolic protection in experimental diabetic retinopathy. *Int Rev Cell Mol Biol*. 2014;311:1-121.

研究主題及發現

Neuroprotection: 西、傳醫、食、胞；基因

學術貢獻與創新

預防或治療、兩岸四地、產官學醫；專利

研究關聯性與整體性

“網缺”：胞、動、人（IRB、科技產業）

未來研究方向與展望

臨床：VO, NTG, DR, wet AMD (Retina ischemia vs Stroke)

學術：研究關聯性與整體性（Retina vs Brain）

共同解決人類醫學難題（Global）

Thanks for your attention

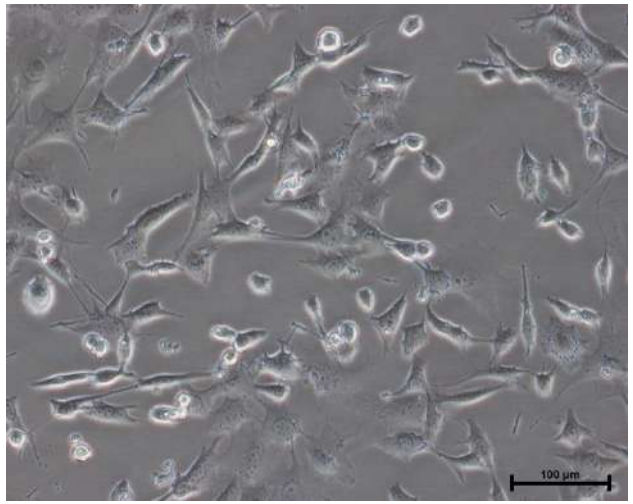
Primary cell culture：Pig/human/rat RPE or RGC-5 cells

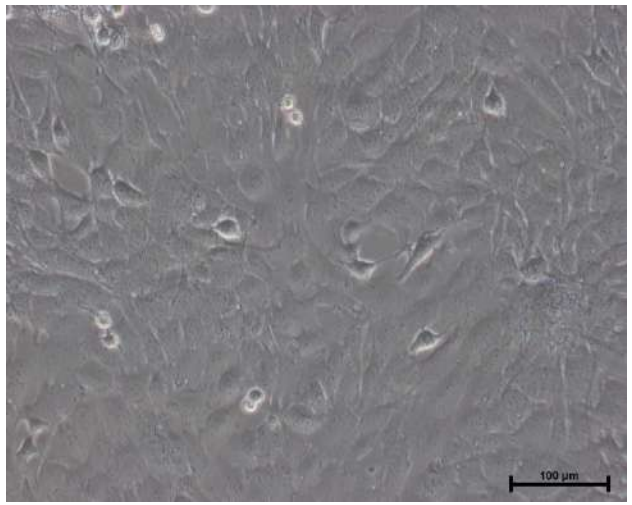
- Pre-administration (15-min) of SAC/Baicalein or vehicle (PBS).
- H_2O_2 induced oxidative stress or
  oxygen glucose depriviation
- **Analysis**

1) MTT assay
2) ROS assay
3) Western blotting & ELISA
4) Real-time PCR

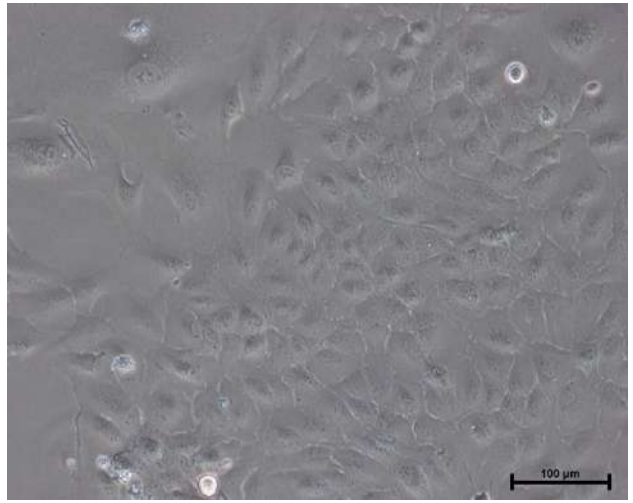
2D culture

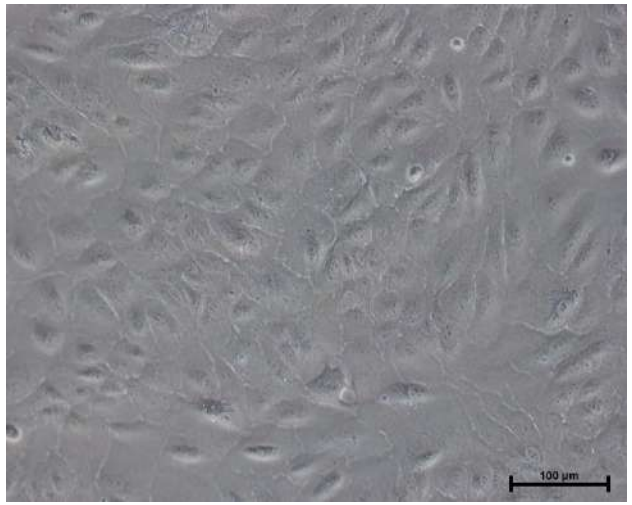
- **RGC-5 cell**





- **RPE cell**





3D culture

- **RGC-5 cell**