Acta Ophthalmologica

Acta Ophthalmologica 2019

Elevation of serum oxidative stress in patients with retina vein occlusions

Kuan-Hsuan Chen,[1,2,3] En-Lin Hsiang,[2,4] Min-Yen Hsu,[5,6] Yueh-Ching Chou,[2,3,7] Tai-Chi Lin,[1,8] Yuh-Lih Chang,[2,3] Ching-Yao Tsai,[4,9] Tzu-Hsuan Li,[4,5] Lin-Chung Woung,[4,9] Shih-Jen Chen,[4,8] Chi-Hsien Peng[10] and De-Kuang Hwang[4,8]

[1]Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan
[2]Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan
[3]Department of Pharmacy, Taipei Veterans General Hospital, Taipei, Taiwan
[4]School of Medicine, National Yang-Ming University, Taipei, Taiwan
[5]Department of Ophthalmology, Chung Shan Medical University Hospital, Taichung, Taiwan
[6]School of Medicine, Chung Shan Medical University, Taichung, Taiwan
[7]School of Pharmacy, Taipei Medical University, Taipei, Taiwan
[8]Department of Ophthalmology, Taipei Veterans General Hospital, Taipei, Taiwan
[9]Department of Ophthalmology, Taipei City Hospital, Taipei,Taiwan
[10]Department of Ophthalmology, Shin Kong Wu Ho-Su Memorial Hospital and Fu-Jen Catholic University, Taipei, Taiwan

ABSTRACT.

Purpose: **Retina vein occlusion (RVO) is a visual-threatening retinal disease that causes irreversible impaired quality of life. The contribution of oxidative stress behind clinical course of RVO was rarely investigated. The study aimed to measure the serum oxidative biomarker in patients with RVO to investigate further physical response.**

Methods: **We measured the serum levels of malondialdehyde (MDA), 8-hydroxy-2-deoxyguanosine (8OHdG), Sirutin 1 (SIRT1), peroxisome proliferator- activated receptor gamma (PPAR-r), Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), orkhead box protein O1 (FOXO1), orkhead box protein O3 (FOXO3), catalase, (SOD) and hydrogen peroxide (H_2O_2) among 19 patients with cataract as control group and 36 patients with RVO, respectively.**

Results: **The mean MDA, 8OHdG and hydrogen peroxide in the serum were significantly higher in patients with RVO compared with the results in control group subjects. Whereas SIRT1, PPAR-r, PGC-1, FOXO1, FOXO3, catalase and SOD levels in serum were significantly decreased in patients with RVO compared with control group.**

Conclusion: **We demonstrated that the serum level of MDA, 8OHdG and hydrogen peroxide is increased in patients with RVO. Among these, the elevation of MDA, 8OHdG and hydrogen peroxide suggests the increasing of serum oxidative stress in RVO patients. All enzymes related reactive oxygen species scavenge were decreased. Thus, focal RVO may increase systemic oxidative stress within serum.**

Key words: oxidative stress – retinal vein occlusions

Acta Ophthalmol. 2019: 97: e290–e295

doi: 10.1111/aos.13892

Introduction

Retinal vein occlusion (RVO) is one of the most common retinal diseases which may lead to visual loss. Clinical presentations of RVO include retinal haemorrhage, tortuous retinal veins, optic nerve swelling and macular oedema. The incidence was 2.3% in the United States. Although the occlusion of retinal vein was undoubted, the initial pathogenesis and following pathophysiology of RVO remain controversial (Kirkegaard et al. 2017). Various treatments including laser, thrombolytic therapy, surgery and intravitreal administration of drugs have been used. Among these, intravitreal anti-vascular endothelium growth factors (anti-VEGFs) are widely used for treating macular oedema and other angiogenic complications in RVO.

Studies have demonstrated that patients with older age, diabetes, hypertension, hyperlipidemia, cardiovascular disease and glaucoma were at the higher risks for developing RVO. Previous research also revealed that the intraocular angiogenic and inflammatory cytokines play a crucial role in the progression and incidence of ocular complications in RVO patients. Besides, elevation of serum heparanase and lipoprotein was also found in patients

with RVO (Hu et al. 2017; Kuhli-Hattenbach et al. 2017).

Among various types of oxidative damages in cellular structures or biomolecules induced by reactive oxidative stress (ROS), 8-hydroxy-2-deoxyguanosine (8-OHdG) is one of the most abundant oxidative products of DNA oxidation and has been reported as a noninvasive and sensitive biomarker for oxidative stress (Simic 1992; Eisinger et al. 2012). Sabri et al. (2003) previously reported that ROS stimulates cell growth, matrix remodelling and cellular dysfunction in cardiomyocytes through the activation of a broad variety of hypertrophic and apoptotic signalling pathways and transcription factors. In cultured cardiomyocytes, Murdoch et al. (2006) showed that intracellular ROS production and antioxidants have been stimulated by angiotensin-II, endothelin-1, norepinephrine, tumour necrosis factor-α (TNF-α) or pulsatile mechanical stretch. In a previous study, urinary 8-OHdG can reflect the oxidative status in hypertension and be used as an effective marker for monitoring the changes of oxidative stress. In addition, 8-OHdG is also commonly used to evaluate oxidative DNA damage in several disorders including cancer, atherosclerosis, diabetes and cardiovascular diseases (Wu et al. 2004; Mendes et al. 2013).

Toll-like receptors (TLRs) are a class of proteins that play a crucial role in the innate immune system. Activation of TLRs recruits a lot of adaptors to initiate the downstream signalling and leads to the production of proinflammatory cytokines (O'Neill & Bowie 2007). TLR4 is one of the TLRs family, plays an important role in the autoimmune diseases and therapeutic targets (Eisinger et al. 2012). SIRT1, which resides in the nucleus, binds and deacetylates p53, NF-κB and transcription factors (Orimo et al. 2009). SIRT1 plays a vital role in metabolism and can affect complex biological phenomena including ageing and disease (Li 2013). It has many biological functions, including anti-apoptosis, cell cycle regulation and transcription regulation. Oxidative stress can inhibit SIRT1 activity and decreased activity of SIRT1 which induces inflammatory responses (Salminen et al. 2013).

Methods

This study was conducted by the Declaration of Helsinki; the protocols were approved by the institutional review board under No. 2013-06-025B. It is necessary for all patients to provide the written consent forms before enrolling the study.

We measured the serum levels of malondialdehyde (MDA), 8-hydroxy-2-deoxyguanosine (8OHdG), Sirutin 1 (SIRT1), peroxisome proliferator-activated receptor gamma (PPAR-r),

Table 1. Clinical characteristics of study population.

	Cataract $n = 19$	BRVO $n = 18$	CRVO $n = 18$	p-Value*
Age (years) (mean ± SD)	76.1 ± 4.2	73.7 ± 4.4	78.4 ± 2.3	0.07
Women (*n*, (%))	9 (47.4%)	10 (55.6%)	8 (44.4%)	0.84
Body mass index (kg/m^2) (mean ± SD)	24.2 ± 2.0	25.4 ± 1.7	25.1 ± 1.9	0.15
eGFR (ml/min/1.73 m^2) (mean ± SD)	67.9 ± 4.8	65.2 ± 6.6	66.4 ± 8.9	0.92
Hypertension (*n*, (%))	15 (78.9%)	12 (66.7%)	14 (77.8%)	0.74
Usage of ACE inhibitor or ARB (*n*, (%))	13 (68.4%)	11 (61.1%)	10 (55.6%)	0.74
Diabetes mellitus (*n*, (%))	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.00
Alzheimer's disease (*n*, (%))	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.00

ACE = angiotensin-converting enzyme; ARB = angiotensin-II receptor blocker; BRVO = branch retina vein occlusion; CRVO = central retina vein occlusion; eGFR = estimated glomerular filtration rate.
* Comparison among groups was calculated using Kruskal–Wallis test and chi-square exact test.

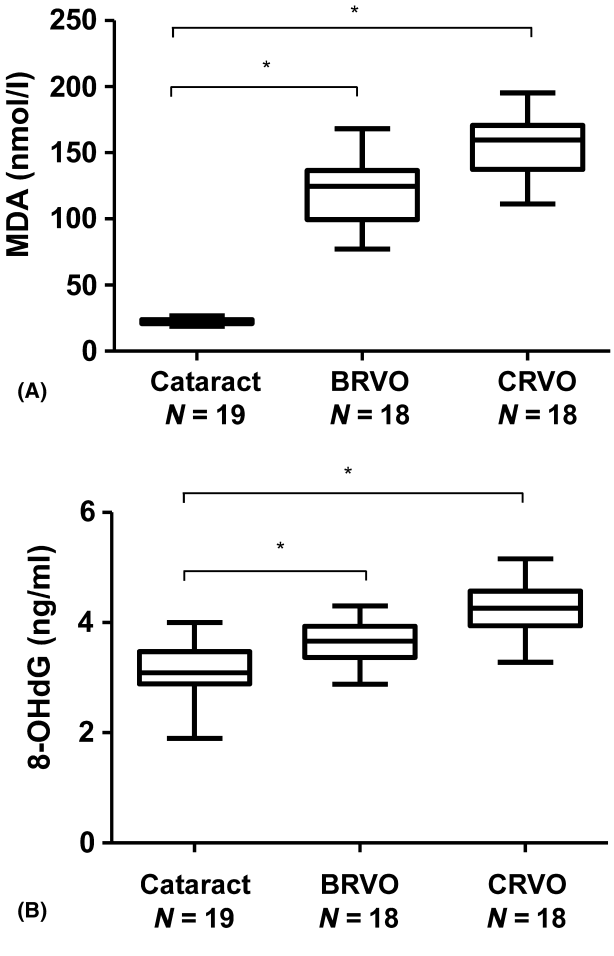

Fig. 1. Expression levels of malondialdehyde (MDA) and 8-OHdG in patients with retina vein occlusion (RVO). The serum MDA and 8-OHdG concentration was also higher in patients with RVO than in the control subjects (MDA, Cataract versus BRVO: 22.5 ± 2 nmol/l versus 121.6 ± 26.2 nmol/l, $p < 0.05$. Cataract versus CRVO: 22.5 ± 2 nmol/l versus 156.8 ± 22.3 nmol/l, $p < 0.05$.; 8-OHdG, Cataract versus BRVO: 3.2 ± 0.5 ng/ml versus 3.6 ± 0.4 ng/ml, $p < 0.05$. Cataract versus CRVO: 3.2 ± 0.5 ng/ml versus 4.3 ± 0.5 ng/ml). *$p < 0.05$.

peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), orkhead box protein O1 (FOXO1), forkhead box protein O3 (FOXO3), catalase, (SOD) and hydrogen peroxide (H_2O_2) among 19 patients with cataract as control group and 36 patients with RVO, respectively.

Patients

Eighteen patients diagnosed as branched RVO (BRVO) (n = 18; median age = 73.7 years; range = 69.3–78.1 years) and 18 patients diagnosed as central RVO (CRVO; n = 18; median age = 78.4 years; range = 76.1–80.7 years) were included. Further 19 age- and sex-matched cataract patients were included as controls (patients with cataract, n = 19) Blood serum sample was collected once the condition of RVO was diagnosed.

Isolation of mRNA and quantitative real-time polymerase chain reaction

Human blood was collected in BD K2-EDTA tubes (5 ml) from patients with BRVO before intravitreal injection. Total blood RNA was isolated with the RNeasy Plus mini kit (Qiagen, Hilden, Germany). The quality of RNA was confirmed via an Experion Automated Electrophoresis Station (Bio-Rad, Hercules, CA, USA). Oligonucleotide specificity was determined by a homology search within the human genome (BLAST, National Center for Biotechnology Information, Bethesda, MD) and confirmed by dissociation curve analysis. Oligonucleotides for MDA, 8-ohdg, SIRT1, peroxisome proliferator-activated receptor gamma (PPAR-r), PPAR-r coactivator-1 alpha (PGC-1α), p53, FOXO1, FOXO3 and β-actin were designed using the computer software package Primer Express 2.0 (Applied Biosystems, Foster City, CA, USA). All the oligonucleotides were synthesized by Invitrogen (Breda, the Netherlands). PCR was performed with SYBR Green in an ABI 7000 sequence detection system (Applied Biosystems) according to the manufacturer's guidelines.

Antioxidant enzyme activity measurement

To determine the antioxidant enzyme activity, plasma was collected from whole blood after centrifugation at 2500 g at 4°C for 10 min. Superoxide dismutase (SOD; Cell Biolabs, San Diego, CA, USA, STA-340) and catalase activity (Cell Biolabs, STA-341) in the plasma were determined by using an enzymatic assay from a commercial kit according to the manufacturer's instructions. Enzyme activity was converted to units per milligram of protein.

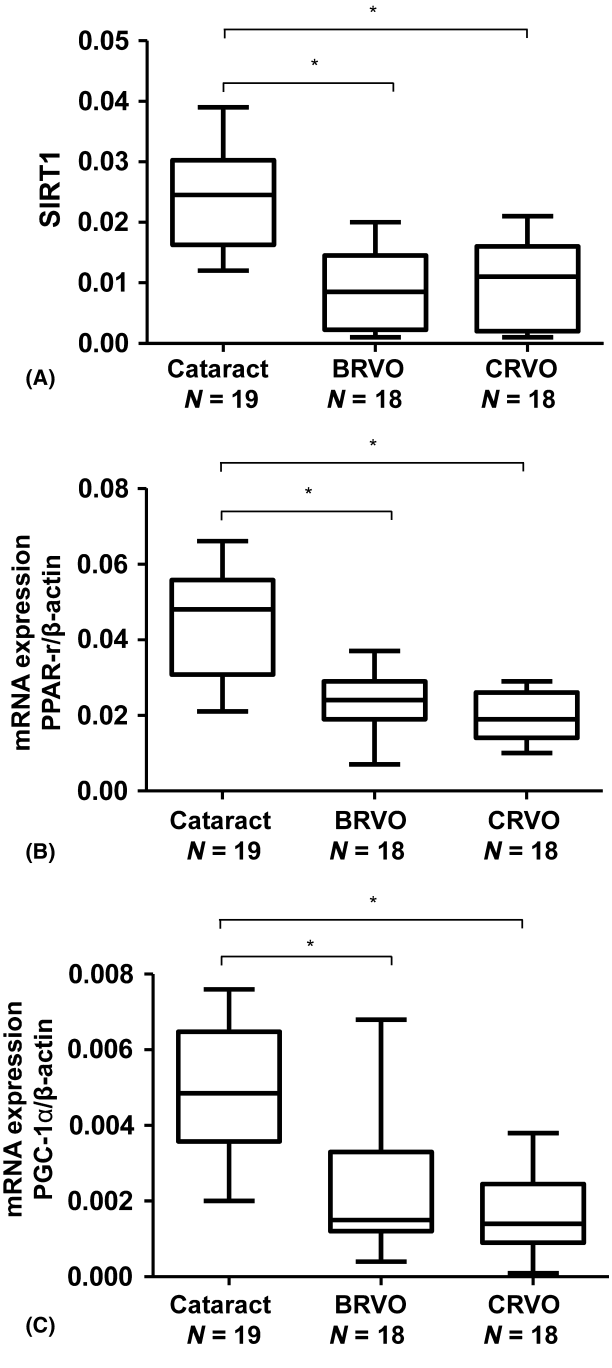

Fig. 2. Decreasing levels of SIRT1 in patients with RVO. The data showed that the expression levels of SIRT1 (Cataract versus BRVO: 0.02 ± 0.008 versus 0.008 ± 0.006, $p < 0.05$; Cataract versus CRVO: 0.02 ± 0.008 versus 0.009 ± 0.007, $p < 0.05$, respectively) (A), PPAR-r (Cataract versus BRVO: 0.05 ± 0.01 versus 0.02 ± 0.007, $p < 0.05$; Cataract versus CRVO: 0.05 ± 0.01 versus 0.02 ± 0.006, $p < 0.05$, respectively) (B) and PGC-1α (Cataract versus BRVO: 0.005 ± 0.002 versus 0.002 ± 0.002, $p < 0.05$; Cataract versus CRVO: 0.005 ± 0.002 versus 0.002 ± 0.001, $p < 0.05$, respectively) (C) were both decreased in patients with RVO. *$p < 0.05$.

Hydrogen peroxide measurement

To determine the concentration of hydrogen peroxide, plasma has to be collected from whole blood after centrifugation at 2500 g at 4°C for 10 min. According to the manufacturer's instructions, hydrogen peroxide (Cell

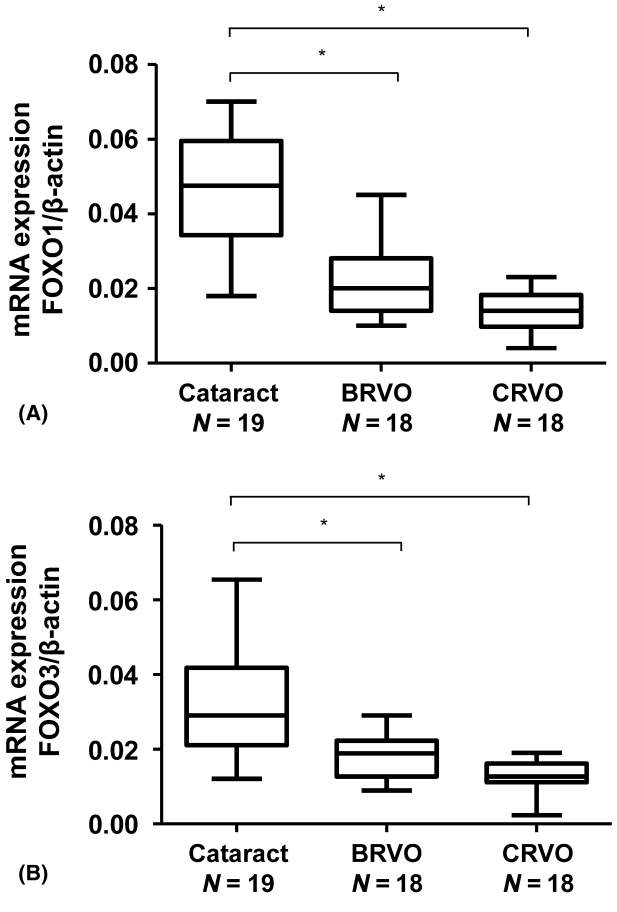

Fig. 3. Decreased levels of FOXO family in patients with RVO. Our data showed that FOXO1 (Cataract versus BRVO: 0.05 ± 0.02 versus 0.02 ± 0.01, $p < 0.05$; Cataract versus CRVO: 0.05 ± 0.02 versus 0.01 ± 0.01, $p < 0.05$, respectively) (A) and FOXO3 (Cataract versus BRVO: 0.03 ± 0.01 versus 0.01 ± 0.005, $p < 0.05$; Cataract versus CRVO: 0.03 ± 0.01 versus 0.01 ± 0.004, $p < 0.05$, respectively) (B) expression levels were strongly decreased in patients with RVO. *$p < 0.05$.

Biolabs, STA-344) in the plasma was determined using an enzymatic assay with a commercial kit. The concentration of hydrogen peroxide was converted to μM.

Statistical analysis

All results are reported as the mean ± standard deviation or number (%). Variables were compared with Kruskal–Wallis test and one way ANOVA (with Bonferroni correction) or Pearson's chi-squared test when it's appropriate. Statistical analysis was performed with SPSS, version 13.0 (SPSS, Inc., Chicago, IL, USA). Statistical significance was set at $p < 0.05$.

Results

Patients with RVO increased serum oxidative stress

Table 1 shows the characteristics of the study participants. We enrolled patients with BRVO ($n = 18$; median age = 73.7 years; range = 69.3–78.1 years), CRVO ($n = 18$; median age = 78.4 years; range = 76.1–80.7 years) and age- and sex-matched controls (patients with cataract, $n = 19$; median age = 72.5 years; range = 66.7–78.3 years). None of the patients has diabetes or Alzheimer's disease in our study. The proportion of patients with obesity, hypertension, under medications of angiotensin-converting enzyme inhibitor or Angiotensin-II receptor blocker were similar among three study groups.

The serum MDA and 8-OHdG concentration was also higher in RVO patients compared with control subjects (MDA, Cataract versus BRVO: 22.5 ± 2 nmol/l versus 121.6 ± 26.2 nmol/l, $p < 0.05$. Cataract versus CRVO: 22.5 ± 2 nmol/l versus 156.8 ± 22.3 nmol/l, $p < 0.05$.; 8-OHdG, Cataract versus BRVO: 3.2 ± 0.5 ng/ml versus 3.6 ± 0.4 ng/ml, $p < 0.05$. Cataract versus CRVO: 3.2 ± 0.5 ng/ml versus 4.3 ± 0.5 ng/ml; Fig. 1).

The downstream genes of SIRT1 are decreased by oxidative stress

The activation of SIRT1 (Cataract versus BRVO: 0.02 ± 0.008 versus 0.008 ± 0.006, $p < 0.05$; Cataract versus CRVO: 0.02 ± 0.008 versus 0.009 ± 0.007, $p < 0.05$, respectively) (Fig. 2A) modulates downstream genes that also exert anti-inflammatory functions, such as the PPAR-r and PGC-1α. We used real-time PCR to investigate these genes compared to control. The data showed that the expression levels of PPAR-r (Cataract versus BRVO: 0.05 ± 0.01 versus 0.02 ± 0.007, $p < 0.05$; Cataract versus CRVO: 0.05 ± 0.01 versus 0.02 ± 0.006, $p < 0.05$, respectively; Fig. 2B) and PGC-1α (Cataract versus BRVO: 0.005 ± 0.002 versus 0.002 ± 0.002, $p < 0.05$; Cataract versus CRVO: 0.005 ± 0.002 versus 0.002 ± 0.001, $p < 0.05$, respectively; Fig. 2C) were both decreased in patients with RVO.

Expression levels of FOXO family were decreased in patients with RVO

We further tested the mRNA expression levels of FOXO family in patients with RVO. Our data showed that FOXO1 (Cataract versus BRVO: 0.05 ± 0.02 versus 0.02 ± 0.01, $p < 0.05$; Cataract versus CRVO: 0.05 ± 0.02 versus 0.01 ± 0.01, $p < 0.05$, respectively; Fig. 3A) and FOXO3 (Cataract versus BRVO: 0.03 ± 0.01 versus 0.01 ± 0.005, $p < 0.05$; Cataract versus CRVO: 0.03 ± 0.01 versus 0.01 ± 0.004, $p < 0.05$, respectively; Fig. 3B) expression levels were strongly decreased in patients with RVO.

Antioxidant enzyme activities are decreased in patients with RVO

We isolated the plasma from the whole blood and tested the antioxidant enzyme activities in all patients. Our data showed that the activity of catalase (Cataract versus BRVO: 3.5 ± 0.9 versus 2.3 ± 0.5, $p < 0.05$; Cataract versus CRVO: 3.5 ± 0.9 versus 1.6 ± 0.5, $p < 0.05$, respectively; Fig. 4A) and SOD (Cataract versus BRVO: 0.8 ± 0.2 versus 0.5 ± 0.1, $p < 0.05$; Cataract versus CRVO: 0.8 ± 0.2 versus 0.4 ± 0.1, $p < 0.05$, respectively; Fig. 4B) were declined in patients with RVO. Otherwise, we found that hydrogen peroxide concentrations,

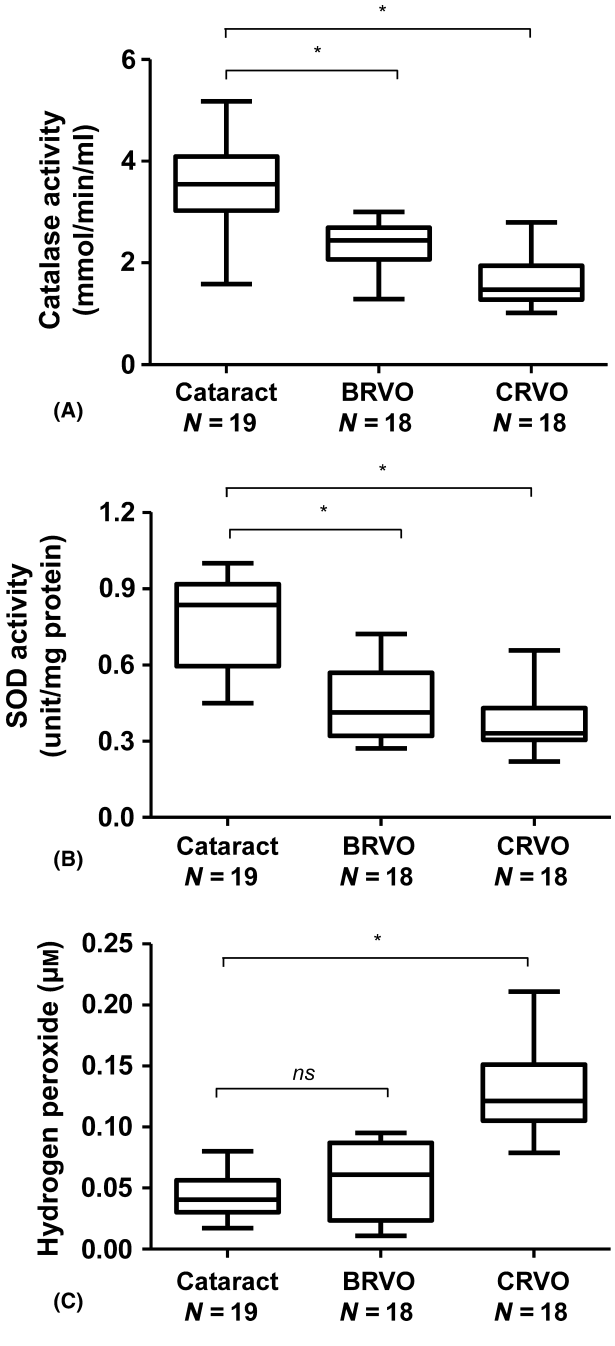

Fig. 4. Antioxidant enzyme activity and reactive oxidative stress concentrations in patients with retina vein occlusion. Blood plasma was isolated from patients. The activity of (A) catalase (Cataract versus BRVO: 3.5 ± 0.9 versus 2. 3 ± 0.5, $p < 0.05$; Cataract versus CRVO: 3.5 ± 0.9 versus 1.6 ± 0.5, $p < 0.05$, respectively) and (B) Superoxide dismutase (SOD) (Cataract versus BRVO: 0.8 ± 0.2 versus 0.5 ± 0.1, $p < 0.05$; Cataract versus CRVO: 0.8 ± 0.2 versus 0.4 ± 0.1, $p < 0.05$, respectively) were investigated using enzymatic assay kits. Both of catalase and SOD showed (C) H_2O_2 concentrations were investigated using enzymatic assay kits (Cataract versus BRVO: 0.04 ± 0.02 versus 0.06 ± 0.03, $p = 0.08$; Cataract versus CRVO: 0.04 ± 0.02 versus 0.13 ± 0.03, $p < 0.05$, respectively). *$p < 0.05$.

represented as serum oxidative stress, were elevated in patients with RVO (Cataract versus BRVO: 0.04 ± 0.02 versus 0.06 ± 0.03, $p = 0.08$; Cataract versus CRVO: 0.04 ± 0.02 versus 0.13 ± 0.03, $p < 0.05$, respectively; Fig. 4C).

Discussion

The cause of RVO is multifactorial. We had shown increased oxidative stress in RVO cases as seen by elevated 8-OHdG, MDA and hydrogen perioxide (H_2O_2) levels, markers of peroxidation, as well as decreased antioxidant activity of enzyme like catalase and SOD. Decreased of antioxidant related pathway such as SIRT1, PPAR-r, PGC-1 FOXO1 and FOXO3 also reflects reduced antioxidant defence (Zheng et al. 2010; Rodrigues et al. 2011; Saint-Geniez et al. 2013). In addition to decrease in the antioxidant enzyme such as catalase and SOD, the lower non-enzymatic antioxidant (SIRT1, PPAR-r, PGC-1 FOXO1 and FOXO3) is also responsible for the elevated oxidative stress within serum (Zheng et al. 2010; Golestaneh et al. 2016). Our study thus indicated that RVO can cause increased oxidative stress within serum.

Myocytes contain large amount of mitochondria and ROS produced in mitochondria can damage mitochondrial DNA within myocytes. Mitochondria dysfunction might lead to a vicious cycle that further generates ROS and eventually decreases contractility of myocardium, myocyte loss and increased cell death (Giacco & Brownlee 2010).

In one article, Malondialdehyde (MDA), a marker of lipid peroxidation was lowered by the intraperitoneal administration of edaravone at a dose of 3 mg/kg and superoxide dismutase (SOD) was enhanced in rodent retinal tissue (Song et al. 2008). MDA which exhibits cytotoxicity is a product of lipid peroxidation and SOD which neutralizes superoxide anions is an antioxidant enzyme. Moreover, visual dysfunction and apoptosis of retinal neurons within the inner nuclear, ganglion cell and outer nuclear layers were induced by edaravone inhibited the retinal ischaemia/reperfusion. In our study, ROS is scavenged by antioxidant enzyme and the activity of serum antioxidant enzyme was decreased when RVO happened (Fig. 5).

Our study also showed some limitations. First, we assumed that the increasing of these target markers was mainly contributed by RVO itself rather than by other systemic condition because that not only the distribution of age and sex but also general health conditions were similar between patients with RVO and controls in our study. However, there is a possibility that the elevation of these markers mainly came from other systemic condition which associated with the development of RVO. Second, our sample

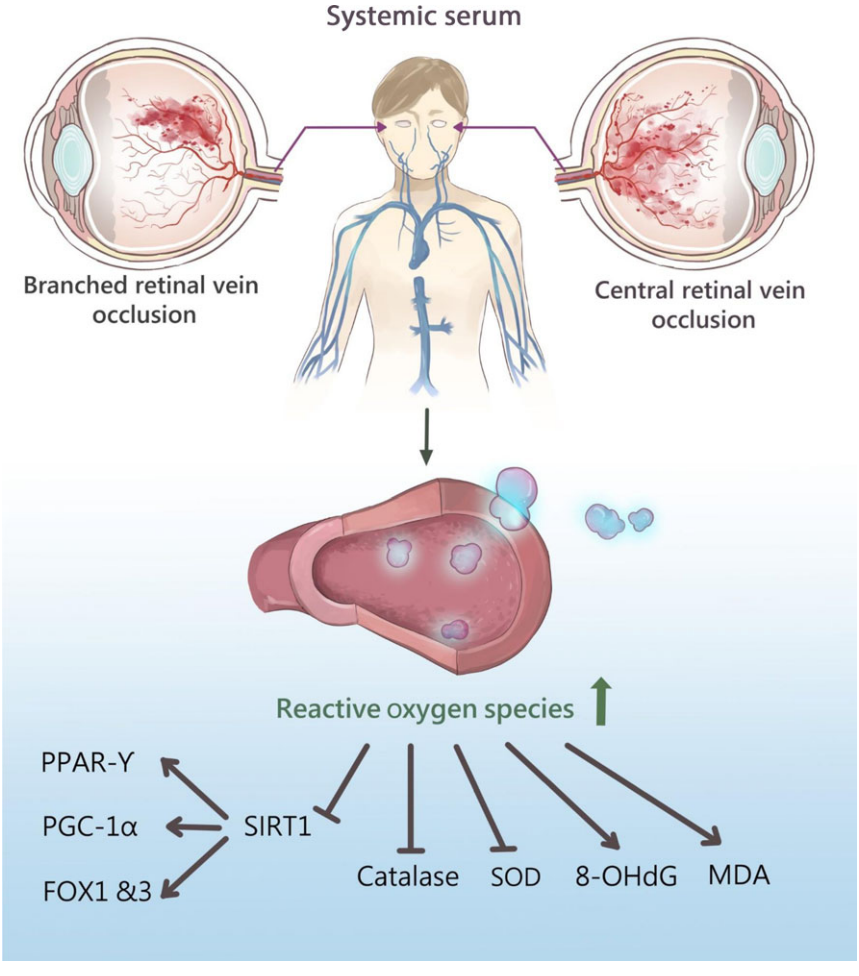

Fig. 5. Schematic diaphragm showed retina vein occlusion cause elevation of oxidative stress within serum and scavenge downstream antioxidant enzymes. Retinal vein occlusion causes elevation of reactive oxygen species and subsequent MDA and 8-ohdg. Antioxidant enzyme such as SIRT1, catalase and superoxide dismutase was decreased due to the serum oxidative stress scavenge by those enzymes. Downstream enzyme of antioxidant enzyme such as PPAR-r, PGC-1α and FOXO1&3 was also decreased.

size was relatively small to stratify these patients into different severity of vessel occlusions. Third, we cannot exactly confirm the causality of our findings that are these elevations caused by vein occlusion, or just predisposing factors for RVO. Further studies should be carried out with a larger sample size and series exams before and after intervention to explore the real relationship between RVO and oxidative stress.

Conclusions

We demonstrated that the serum level of MDA, 8OHdG and hydrogen peroxide is increased in patients with RVO. Among these, the elevation of MDA, 8OHdG and hydrogen peroxide suggests the increasing of serum oxidative stress in RVO patients. All enzymes related reactive oxygen species scavenge were decreased. Thus, focal RVO may increase systemic oxidative stress within serum.

References

Eisinger K, Bauer S, Schaffler A, Walter R, Neumann E, Buechler C, Muller-Ladner U & Frommer KW (2012): Chemerin induces CCL2 and TLR4 in synovial fibroblasts of patients with rheumatoid arthritis and osteoarthritis. Exp Mol Pathol **92**: 90–96.

Giacco F & Brownlee M (2010): Oxidative stress and diabetic complications. Circ Res **107**: 1058–1070.

Golestaneh N, Chu Y, Cheng SK, Cao H, Poliakov E & Berinstein DM (2016): Repressed SIRT1/PGC-1alpha pathway and mitochondrial disintegration in iPSC-derived RPE disease model of age-related macular degeneration. J Transl Med **14**: 344.

Hu Y, Atik A, Yu H et al. (2017): Serum heparanase concentration and heparanase activity in patients with retinal vein occlusion. Acta Ophthalmol **95**: e62–e66.

Kirkegaard K, Heegaard S & Hvas AM (2017): No evidence for thrombophilia in patients with retinal venous occlusion: a systematic GRADE-based review. Acta Ophthalmol **95**: 12–19.

Kuhli-Hattenbach C, Miesbach W, Luchtenberg M, Kohnen T & Hattenbach LO (2017): Elevated lipoprotein (a) levels are an independent risk factor for retinal vein occlusion. Acta Ophthalmol **95**: 140–145.

Li X (2013): SIRT1 and energy metabolism. Acta Biochim Biophys Sin (Shanghai) **45**: 51–60.

Mendes B, Silva P, Mendonca I, Pereira J & Camara JS (2013): A new and fast methodology to assess oxidative damage in cardiovascular diseases risk development through eVol-MEPS-UHPLC analysis of four urinary biomarkers. Talanta **116**: 164–172.

Murdoch CE, Zhang M, Cave AC & Shah AM (2006): NADPH oxidase-dependent redox signalling in cardiac hypertrophy, remodelling and failure. Cardiovasc Res **71**: 208–215.

O'Neill LA & Bowie AG (2007): The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev Immunol **7**: 353–364.

Orimo M, Minamino T, Miyauchi H, Tateno K, Okada S, Moriya J & Komuro I (2009): Protective role of SIRT1 in diabetic vascular dysfunction. Arterioscler Thromb Vasc Biol **29**: 889–894.

Rodrigues GA, Maurier-Mahe F, Shurland DL et al. (2011): Differential effects of PPARgamma ligands on oxidative stress-induced death of retinal pigmented epithelial cells. Invest Ophthalmol Vis Sci **52**: 890–903.

Sabri A, Hughie HH & Lucchesi PA (2003): Regulation of hypertrophic and apoptotic signaling pathways by reactive oxygen species in cardiac myocytes. Antioxid Redox Signal **5**: 731–740.

Saint-Geniez M, Jiang A, Abend S, Liu L, Sweigard H, Connor KM & Arany Z (2013): PGC-1alpha regulates normal and pathological angiogenesis in the retina. Am J Pathol **182**: 255–265.

Salminen A, Kaarniranta K & Kauppinen A (2013): Crosstalk between oxidative stress and SIRT1: impact on the aging process. Int J Mol Sci **14**: 3834–3859.

Simic MG (1992): Urinary biomarkers and the rate of DNA damage in carcinogenesis and anticarcinogenesis. Mutat Res **267**: 277–290.

Song Y, Gong YY, Xie ZG, Li CH, Gu Q & Wu XW (2008): Edaravone (MCI-186), a free radical scavenger, attenuates retinal ischemia/reperfusion injury in rats. Acta Pharmacol Sin **29**: 823–828.

Wu LL, Chiou CC, Chang PY & Wu JT (2004): Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin Chim Acta **339**: 1–9.

Zheng Z, Chen H, Wang H et al. (2010): Improvement of retinal vascular injury in diabetic rats by statins is associated with the inhibition of mitochondrial reactive oxygen species pathway mediated by peroxisome proliferator-activated receptor gamma coactivator 1alpha. Diabetes **59**: 2315–2325.

Received on November 19th, 2017.
Accepted on July 18th, 2018.

Correspondence:
Chi-Hsien Peng MD, PhD
Department of Ophthalmology
Shin Kong Wu Ho-Su Memorial Hospital and Fu-Jen Catholic University
Taipei
Taiwan
Tel: +886 2 28332211
Fax: +886 2 28389343
Email: chpeng1008@gmail.com
and
De-Kuang Hwang MD, PhD
Department of Ophthalmology
Taipei Veterans General Hospital
No. 201, Sec. 2, Shih-Pai Rd.
Taipei 11217
Taiwan
Tel: +886 2 28757325#7502
Fax: +886 2 23595046
Email: m95gbk@gmail.com

This study was supported in part by the intramural grants from the Taipei Veterans General Hospital (Grant No. V103C-166, V104C-175, V104E14-003-MY3-2, V105C-189), Ministry of Science and Technology of Taiwan (106-2314-B-075A-001) and grants in aid from the Research Foundation of Cardiovascular Medicine (100-01-010), Taipei, Taiwan. All author contributed to the conception and design of this study. Data were collected by. Data were analysed by Chen KH, the manuscript was drafted by Chen KH, Wang CT, Chou YC, Chang YL, Hwang DK and Hsu MY. All authors read and approved the final manuscript.