Impaired nitric oxide production in patients with primary open-angle glaucoma
Nitric oxide levels in patients with glaucoma
Diminution de la production du monoxyde d'azote chez des patients atteints de glaucome primitif à angle ouvert

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RÉSUMÉ

Introduction : Le glaucome est une neuropathie optique à étiologie multifactorielle. Le dysfonctionnement vasculaire peut y contribuer.
Objectif : Déterminer l'implication du monoxyde d'azote (NO), un facteur vasodilatateur jouant un rôle dans la régulation du débit sanguin oculaire dans le glaucome primitif à angle ouvert (GPAO). En outre, les taux d'acide urique et du lactate ont été investigués.
Méthodes : Les concentrations du NO, de l'acide urique et du lactate dans le plasma et l'humeur aqueuse (HA) ont été mesurées chez 214 patients tunisiens (100 patients atteints de GPAO et 114 sujets ayant la cataracte). Les métabolites stables du NO, les nitrates et les nitrites (NOx) ont été déterminés à l'aide de la réaction de Griess. Les concentrations du lactate et d'acide urique ont été mesurées à travers des méthodes enzymatiques colorimétriques.
Résultats : Les concentrations du NOx étaient significativement plus faibles chez les patients présentant un GPAO comparés aux sujets atteints de cataracte dans le plasma (5,23±1,55 µmol/L vs 18,35±6,87 µmol/L, p=0,01) et l'HA (20,54±7,41 µmol/L vs 45,25±10,92 µmol /L, p=0,02). En revanche, les taux du lactate et d'acide urique étaient significativement plus élevés chez les patients glaucomateux que chez les sujets témoins au niveau du plasma et de l'HA.
Conclusions: Dans la présente étude, une diminution du NO et une augmentation des taux d'acide urique et du lactate dans l'HA et le plasma ont été observées chez les patients atteints du GPAO, par rapport aux sujets témoins. Ces données suggèrent une possible implication de ces facteurs dans le développement du glaucome.
Mots-clés : GPAO, cataracte, monoxyde d'azote, acide urique, lactate

SUMMARY

Background: Glaucoma is an optic neuropathy induced by many factors. Vascular dysfunction is involved in the mechanism underlying glaucoma.
Aim: To determine the involvement of nitric oxide (NO), which is implicated in the regulation of ocular blood flow, in primary open angle glaucoma (POAG). Furthermore, lactate and uric acid (UA) levels were investigated.
Methods: Concentrations of NO, UA and lactate in plasma and aqueous humor (AH) were measured in 214 Tunisian patients (100 patients with POAG and 114 subjects with cataract as control group). NO metabolites, nitrate and nitrite (NOx) production were determined using the Griess reaction. UA and lactate concentrations were measured using enzymatic- colorimetric methods.
Results: NOx concentrations in patients with POAG were significantly lower compared to cataract group in plasma (5.23±1.55 µmol/L vs 18.35±6.87 µmol/L, p=0.01) and AH (20.54±7.41 µmol/L vs 45.25±10.92 µmol/L, p=0.02). Plasma and AH levels of lactate and UA were significantly higher in glaucoma patients than in control subjects.
Conclusions: In the present study, decreased NO and increased UA and lactate levels were found in the AH and plasma of POAG patients compared to control subjects. These data suggest a possible involvement of these factors in glaucoma.
Keywords: POAG, cataract, nitric oxide, uric acid, lactate

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INTRODUCTION

Glaucoma is a multifactorial optic neuropathy leading to vision loss and blindness (1). Several risk factors are considered in the pathogenesis of glaucoma, but high intraocular pressure (IOP) remains the main risk factor for this disease (2). IOP reflects the balance between aqueous humor (AH) formation and its drainage from the eye through the trabecular meshwork and the Schlemm canal outflow pathway (3). Dysfunction of the outflow pathway increases IOP, which leads to glaucomatous optic neuropathy, but normal IOP is also found in patients with primary open-angle glaucoma (POAG) (4). This suggests that other factors are involved in the mechanisms underlying glaucoma. Among these factors, vascular dysregulation has been implicated in glaucoma pathology. Indeed, the vascular endothelium plays an important role in the regulation of ocular blood flow. In this tissue, nitric oxide (NO) is produced by neuronal and endothelial NO synthase enzyme (eNOS) to maintain a basal vasodilator tone in the optic nerve head (5,6). However, in the pathological conditions, it is well established that endothelial dysfunction of the microvasculature, decreases NO release and reduces blood flow in the optic nerve, which cause the damage seen in glaucoma (4,7,8). *In vivo* study reported an association between altered NO system and reduced blood flow in patients with glaucoma (9). In contrast, other *in vitro* studies have shown enhanced eNOS activity in endothelial cells of glaucomatous optic nerve head (10,11). In addition, genetic studies have identified that eNOS gene variants are associated with POAG in Brazilian and Egyptian populations (12,13).

Furthermore, other factors are directly or indirectly implicated in NO release as uric acid (UA) and lactate, which have been shown to be involved in the dysfunction of endothelial cells. UA, an antioxidant molecule (14), has been suggested to contribute to endothelial alteration in human aortic endothelial cells (15). A study has reported that a high level of UA is associated with decreased NO levels in human plasma (16).

Moreover, the role of lactate, an important source of energy in the optic nerve head (17) has been reported in glaucoma pathogenesis. Indeed, one study has shown that glaucoma may be linked to disturbed lactate homeostasis (18). In this manner, besides high IOP, other mechanisms play a role in glaucomatous optic neuropathy. Clearly, the pathophysiology of glaucoma has not been well-elucidated.

In the present study, in order to investigate the involvement of NO, UA and lactate in glaucoma, we evaluated the levels of these biochemical parameters in the AH and plasma of a sample of Tunisian patients with POAG and compared them with those of a control group of cataract patients.

METHODS

Patients

Patients were recruited from the Ophthalmology Department of Rabta Hospital of Tunis. The study was approved by the Rabta local ethics committee and was conducted in accordance with the Declaration of Helsinki. Consent was obtained from each patient. The study involved 214 patients (100 patients with POAG and 114 patients with cataract). Each patient's medical history and arterial pressure were obtained. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mm Hg or diastolic blood pressure (DBP) ≥ 90 mm Hg (19). For all patients, we measured weight and height to calculate the body mass index (BMI). Obesity was considered on the basis of a BMI ≥ 30 kg/m^2 (20). All subjects underwent ophthalmic examination including best-corrected visual acuity, IOP, slit-lamp examination, gonioscopy and dilated fundoscopic examination. Inclusion criteria for glaucoma patients were: open- angle glaucoma, IOP higher than 21 mm-Hg (without anti-glaucoma drugs), correlated visual field loss and glaucomatous optic nerve head changes criteria. Patients with ophthalmic pathology other than POAG were excluded.

Since, we could not recruit healthy subjects to collect samples of AH, we recruited subjects in whom cataract surgery was indicated as the control group. Patients enrolled in this group had senile cataract and normal IOP, and had not received any topical medications.

Methods

Blood and AH samples were collected from POAG and control patients on the day of surgery. Venous blood samples were drawn after an overnight fast in vials containing lithium heparin for the analysis of UA, NO, calcium, sodium, potassium and chloride. Vials containing dried fluoride-EDTA were used to measure lactate. The samples were immediately placed on ice, centrifuged at 3500 rpm for 15 min, and supernatants were stored at -80°C until analysis. AH samples (0.1-0.2 mL), obtained from each patient requiring either elective glaucoma

surgery or elective cataract surgery, were rapidly collected at the beginning of surgery through paracentesis with a 27-gauge needle on an insulin syringe. Blood contamination was meticulously avoided. Samples were transferred to sterile cryotubes, immediately cooled and stored at -80°C until analysis.

Lactate concentrations were measured following the enzymatic-colorimetric method using a Cobas Integra 400 biochemical analyzer (Roche Diagnostics, Mannheim, Germany). The levels of the more stable NO metabolites, nitrite (NO_2^-) and nitrate (NO_3^-) were measured using a colorimetric assay kit (R&D Systems, Minneapolis, USA), which is based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by colorimetric detection of nitrite as an azo dye product of the Griess Reaction (21). The other parameters: UA, calcium, sodium, potassium and chloride were analyzed with Abbott kits, using the Architect C8000 analyzer (Abbott Laboratories, Abbott Park, IL, USA).

Statistical analysis

Data were analyzed by SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) and presented as mean±SEM. The student t-test was used to detect differences between the two study groups. Chi-square tests were used for categorical variables. A p value less than 0.05 was considered statistically significant.

RESULTS

The demographic and clinical characteristics of the population studied are presented in Table 2. There were no significant differences for age, gender, SBP, DBP and BMI between the two groups (p=0.34; p=0.55; p=0.31; p=0.23 and p=0.74 respectively). The mean cup-to-disc ratio and IOP were significantly higher in the POAG patients compared to the control subjects (p=0.001) (Table 2).

Concentrations of sodium, potassium and chloride in plasma and AH were not significantly different between the two groups (p>0.05) (Table 1). However, calcium levels differed significantly between POAG patients and control subjects in AH, but not in plasma (Table 1). In addition, the results revealed that NO_x concentrations were significantly lower in patients with POAG compared to control subjects in plasma (5.23±1.55 µmol/L vs 18.35±6.87 µmol/L, p=0.01) and AH (20.54±7.41 µmol/L vs 45.25±10.92 µmol/L, p=0.02) (Table 1). Moreover, UA concentrations were significantly higher in glaucoma patients than in control subjects in plasma (323.56±25.76 µmol/L vs 270.84±15.20 µmol/L, p=0.01) and in AH (102.91±23.97 µmol/L vs 62.60±17.35 µmol/L, p=0.04 (Figure 1). UA levels were lower in AH than in plasma. Furthermore, lactate concentrations differed significantly between the POAG group and the control group in plasma (2.55±0.4 mmol/L vs 1.74±0.25 mmol/L, p=0.03) and in AH (5.81±0.61 mmol/L vs 4.71±0.38 mmol/L, p=0.04) (Figure 2). On the other hand, concentrations of lactate and NO were higher in AH than in plasma.

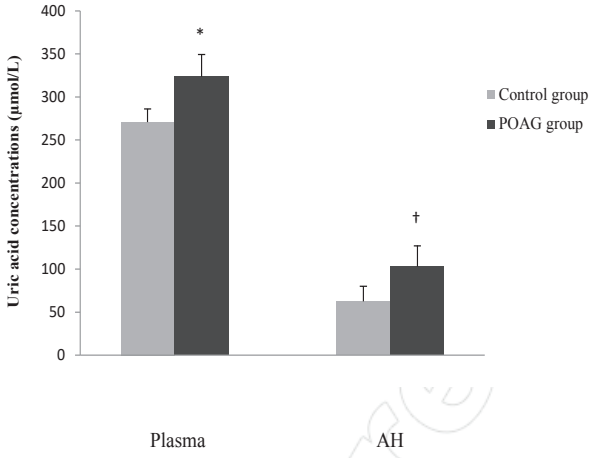

Figure 1. Plasma and aqueous humor (AH) concentrations of uric acid (µmol/L) in primary open-angle glaucoma (POAG) and control groups. Data are expressed as mean±SEM. *p<0.05 in plasma and †p<0.05 in AH

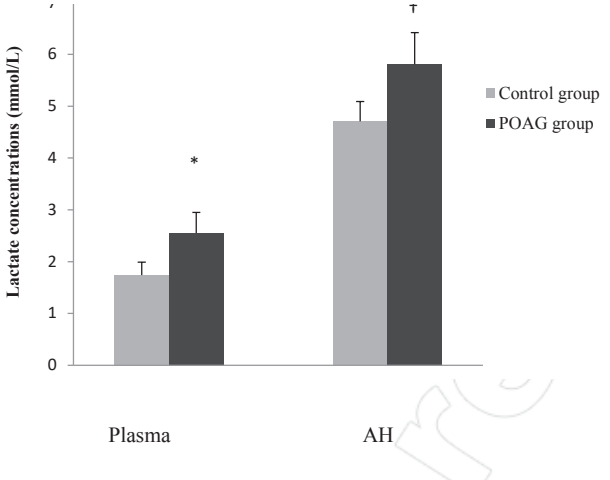

Figure 2. Plasma and aqueous humor (AH) concentrations of lactate (mmol/L) in primary open-angle glaucoma (POAG) and control groups. Data are expressed as mean±SEM. *p<0.05 in plasma and †p<0.05 in AH

Table 1. Biochemical parameters in the plasma and AH of the study groups

Parameters	Plasma			AH		
	Control group (n=114)	POAG group (n=100)	p	Control group (n=114)	POAG group (n=100)	p
Sodium (mmol/L)	137.17±0.59	137.18±0.72	0.99	143.71±1.78	143.57±1.20	0.95
Potassium (mmol/L)	4.54±0.07	4.63±0.09	0.43	3.56±0.17	3.40±0.25	0.59
Chloride (mmol/L)	104.23±0.48	105.31±0.65	0.17	123.25±2.25	126.04±2.42	0.40
Calcium (mmol/L)	2.28 ± 0.01	2.30± 0.02	0.49	1.35 ± 0.03	1.63± 0.09	0.04
NO_x (µmol/L)	18.35±6.87	5.23±1.55	0.01	45.25±10.92	20.54±7.41	0.02

AH: aqueous humor; NO_x: nitric oxide metabolites; POAG: primary open-angle glaucoma. Values are expressed as mean±SEM; p<0.05 is considered significant

Table 2. Demographic and clinical characteristics of the study groups

Parameters	Control group (n=114)	POAG group (n=100)	p
Age (years)	70.18±1.14	68.33±1.44	0.34
Gender, male, n (%)	52 (45.6%)	46 (46.0%)	0.55
Cup-to-disc ratio	0.26±0.03	0.63±0.02	0.001
IOP (mm Hg)	13.29±1.20	20.51±1.27	0.001
SBP (mm Hg)	130.90±3.20	132.80±2.20	0.31
DBP (mm Hg)	80.50±1.40	78.30±1.10	0.23
BMI (kg/m^2)	25.41±0.59	25.15±0.54	0.74

BMI: body mass index; DBP: diastolic blood pressure; IOP: intraocular pressure; POAG: primary open-angle glaucoma; SBP: systolic blood pressure. Values are expressed as mean±SEM; p<0.05 is considered significant

DISCUSSION

In the current study, a significant decrease of NO_x levels was detected in the AH and plasma of glaucoma patients when compared to control subjects. These results are in agreement with previous studies, which have also reported that the NO level in human AH was lower in the POAG group than in the control group (9,22,23). We explain the low NO level by endothelial dysfunction in glaucoma patients (24). Additionally, other reports have shown that NO is implicated in the regulation of AH outflow and IOP (25,26). A decreased NO level in POAG was also reported in genetic studies. Otherwise, eNOS gene variants could predispose the patient to endothelial dysfunction reducing blood flow in the eye and the optic nerve head (12,13). All these data suggest that low NO production may contribute to the elevation of IOP and the progression of glaucoma. Moreover, in both group the data from the present study show that NO_x concentrations were higher in AH than in plasma. This suggests that NO is synthesized in the eye and is not diffused from plasma to AH. This observation indicates that the NO disorder exists locally and systemically in POAG patients.

Furthermore, the data reported herein show that AH and plasma UA levels were significantly higher in POAG patients than in control. These findings are compatible with a previous study

(27) that reported that serum UA level was higher in glaucoma patients compared to healthy controls. UA contributes to the total anti-oxidative status (14,28). Nevertheless, a study showed that UA decreased NO levels in rat endothelial cells culture (29). In addition, another study examining human umbilical vein endothelial cells, reported that a high UA level increased endothelial dysfunction by mediating apoptosis and decreasing NO production under oxidative and endoplasmic reticulum stress through protein kinase C (PKC)-dependent eNOS phosphorylation (30). The mechanism by which UA may reduce NO and induce endothelial dysfunction, particularly under oxidative stress, is also proposed in human plasma and aortic endothelial cell lysates (16). Taken together, we suggest that elevated UA level in AH induces endothelial

cells dysfunction in POAG and reduces NO production.

Lactate and glucose are identified as an important energy source in the optic nerve head (17). In this study, we evaluated the lactate level to determine its possible implication in the development of glaucoma.

Lactate concentrations in AH and plasma were significantly higher in patients with POAG compared to control subjects. These findings are in agreement with a previous study, which reported increased levels of lactate and lactate dehydrogenase in the AH of the POAG patients (31). Disturbed lactate homeostasis may predispose to glaucoma (18), suggesting that mechanisms other than elevated IOP are involved in glaucomatous optic neuropathy. Furthermore, we observed that lactate concentrations were higher in AH than in plasma. These observations confirm that lactate is produced in the eye and the optic nerve head. The elevated lactate concentration in AH reflected the rate of the glycolytic pathway in the eye (32) and its relationship with disturbed mitochondrial function (33,34), which induces the generation of reactive oxygen species (ROS) and contributes to cell death in glaucoma (35). Until now, the relation between lactate and NO remains unclear, but a study reported that hyperglycemia causes impairment of endothelium-dependent NO-mediated dilation by activation of interacting-protein-1/c-Jun N-terminal kinases (JIP1/JNK) signaling in retinal arterioles (36).

Our findings showed that AH calcium concentrations were significantly higher in patients with glaucoma than in control subjects. Calcium dysregulation might be involved in the pathogenesis of glaucoma by increasing AH outflow resistance (37). Hong et al. (38) have reported that hyperuricemia induced endothelial dysfunction by Na^{+}/Ca^{++} exchanger, which mediated calcium overload.

In summary, the present study shows an impairment of NO production and increased UA and lactate levels in the plasma and AH of Tunisian patients with POAG. These data suggest that these factors are involved in glaucoma. Further investigations are needed to study the mechanism underling the UA-induced impairment of NO in POAG.

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REFERENCES

1. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 2006;90:262-7.
2. Kass MA, Heuer DK, Higginbotham EJ et al. The ocular hypertension treatment study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. Arch ophthalmol 2002;120:701-13.
3. To CH, Kong CW, Chan CY, Shahidullah M, Do CW. The mechanism of aqueous humor formation. Clin Exp Optom 2002;85:335-49.
4. Fechtner RD, Weinreb RN. Mechanisms of optic nerve damage in primary open angle glaucoma. Surv Ophthalmol 1994;39:23-42.
5. Haefliger IO, Flammer J, Luscher TF. Heterogeneity of endothelium-dependent regulation in ophthalmic and ciliary arteries. Invest Ophthalmol Vis Sci 1993;34:1722-30.
6. Luksch A, Polak K, Beier C et al. Effects of systemic NO synthase inhibition on choroidal and optic nerve head blood flow in healthy subjects. Invest Ophthalmol Vis Sci 2000;41:3080-84.
7. Flammer J, Orgül S, Costa VP et al. The impact of ocular blood flow in glaucoma. Prog Retin Eye Res 2002;21:359-93.
8. Grieshaber MC, Mozaffarieh M, Flammer J. What is the link between vascular dysregulation and glaucoma? Surv Ophthalmol 2007; 52(2 Suppl):144-54.
9. Polak K, Luksch A, Berisha F, Fuchsjaeger-Mayrl G, Dallinger S, Schmetterer L. Altered nitric oxide system in patients with open-angle glaucoma. Arch Ophthalmol 2007;125:494-8.
10. Neufeld AH, Hernandez MR, Gonzalez M. Nitric oxide synthase in the human glaucomatous optic nerve head. Arch Ophthalmol 1997;115:497-503.
11. Neufeld AH. Nitric oxide: a potential mediator of retinal ganglion cell damage in glaucoma. Surv Ophthalmol 1999; 43(1 Suppl):129-35.
12. Magalhaes da Silva T, Rocha AV, Lacchini R. Association of polymorphisms of endothelial nitric oxide synthase (eNOS) gene with the risk of primary open angle glaucoma in a Brazilian population. Gene 2012;502:142-6.
13. Emam WA, Zidan HE, Abdulhalim BE, Dabour SH, Ghali MA, Kamal AT. Endothelial nitric oxide synthase polymorphisms and susceptibility to high-tension primary open-angle glaucoma in an Egyptian cohort. Mol Vis 2014;20:804-11.
14. Horwath-Winter J, Kirchengast S, Meinitzer A, Wachswender C, Faschinger C, Schmut O. Determination of uric acid concentrations in human tear fluid, aqueous humour and serum. Acta Ophthalmol 2009;87:188-92.
15. Sánchez-Lozada LG, Lanaspa MA, Cristóbal-García M et al. Uric acid-induced endothelial dysfunction is associated with mitochondrial alterations and decreased intracellular ATP concentrations. Nephron Exp Nephrol 2012;121:e71-8.
16. Gersch C, Palii SP, Kim KM, Angerhofer A, Johnson RJ, Henderson GN. Inactivation of nitric oxide by uric acid. Nucleosides Nucleotides Nucleic Acids 2008;27:967-78.
17. Carreras FJ, Aranda CJ, Porcel D, Rodriguez-Hurtado F, Martínez-Agustin O, Zarzuelo A. Expression of glucose transporters in the prelaminar region of the optic-nerve head of the pig as determined by immunolabeling and tissue culture. PLoS One 2015;10:e0128516.

18. Kolko M, Vosborg F, Henriksen UL et al. Lactate transport and receptor actions in retina: Potential roles in retinal function and disease. Neurochem Res 2016;41:1229-36.

19. Guidelines Subcommittee. World Health Organization-International Society of Hypertension Guidelines for the management of hypertension. J Hypertens 1999;17:151-83.

20. World Health Organization Expert Committee. Physical status: the use and interpretation anthropometry. Technical Report Series, no. 854. Geneva: WHO 1995.

21. Giustarini D, Rossi R, Milzani A, Dalle-Donne I. Nitrite and nitrate measurement by griess reagent in human plasma: evaluation of interferences and standardization. Methods Enzymol 2008;440:361-80.

22. Doganay S, Evereklioglu C, Turkoz Y, Er H. Decreased nitric oxide production in primary open-angle glaucoma. Eur J Ophthalmol 2002;12:44-8.

23. Galassi F, Renieri G, Sodi A, Ucci F, Vannozzi L, Masini E. Nitric oxide proxies and ocular perfusion pressure in primary open angle glaucoma. Br J Ophthalmol 2004; 88:757-60.

24. Resch H, Garhofer G, Fuchsjäger-Mayrl G, Hommer A, Schmetterer L. Endothelial dysfunction in glaucoma. Acta Ophthalmol 2009;87:4-12.

25. Schmetterer L, Polak K. Role of nitric oxide in the control of ocular blood flow. Prog Retin Eye Res 2001;20:823-47.

26. Buys ES, Potter LR, Pasquale LR, Ksander BR. Regulation of intraocular pressure by soluble and membrane guanylate cyclases and their role in glaucoma. Front Mol Neurosci 2014;7:38.

27. Yuki K, Murat D, Kimura I, Ohtake Y, Tsubota K. Reduced-serum vitamin C and increased uric acid levels in normal-tension glaucoma. Graefes Arch Clin Exp Ophthalmol 2010;248:243-8.

28. Yu ZF, Bruce-Keller AJ, Goodman Y, Mattson MP. Uric acid protects neurons against excitotoxic and metabolic insults in cell culture, and against focal ischemic brain injury in vivo. J Neurosci Res 1998;1:613-25.

29. Khosla UM, Zharikov S, Finch JL et al. Hyperuricemia induces endothelial dysfunction. Kidney Int 2005;67:1739-42.

30. Li P, Zhang L, Zhang M, Zhou C, Lin N. Uric acid enhances PKC-dependent eNOS phosphorylation and mediates cellular ER stress. A mechanism for uric acid-induced endothelial dysfunction. Int J Mol Med 2016;37:989-97.

31. Jovanovic P, Zoric L, Stefanovic I et al. Lactate dehydrogenase and oxidative stress activity in primary open-angle glaucoma aqueous humour. Bosn J Basic Med Sci 2010;10:83- 8.

32. Zhou L, Lai H, Yew D, Lam KW. Elevation of lactic acid concentration associated with megalophthalmia in Black Moor goldfish. Exp Eye Res 2001;73:897-900.

33. Miller AM, Nolan MJ, Choi J et al. Lactate treatment causes NF-kappa B activation and CD44 shedding in cultured trabecular meshwork cells. Invest Ophthalmol Vis Sci 2007;48:1615-21.

34. Andersen LW, Mackenhauer J, Roberts JC, Berg KM, Cocchi MN, Donnino MW. Etiology and therapeutic approach to elevated lactate levels. Mayo Clin Proc 2013;88:1127- 40.

35. Izzotti A, Bagnis A, Sacca SC. The role of oxidative stress in glaucoma. Mutat Res 2006;612:105-14.

36. Hein TW, Xu W, Kuo L. Dilation of retinal arterioles in response to lactate: role of nitric oxide, guanylyl cyclase, and ATP-sensitive potassium channels. Invest Ophthalmol Vis Sci 2006;47:693-9.

37. He Y, Ge J, Tombran-Tink J. Mitochondrial defects and dysfunction in calcium regulation in glaucomatous trabecular meshwork cells. Invest Ophthalmol Vis Sci 2008;4:4912-22.

38. Hong Q, Qi K, Feng Z et al. Hyperuricemia induces endothelial dysfunction via mitochondrial Na^{+}/Ca^{2+} exchanger-mediated mitochondrial calcium overload. Cell Calcium 2012;51:402-10.