International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.9, No.3, pp 134-143, 2016

The Effect of Hypertension on ET-1 Signaling Pathway Activation in Trabecular Meshwork of Hypertension Rat Model

Seskoati Prayitnaningsih[1]*, Hidayat Sujuti[1], Aulia Abdul Hamid[1], Nur Permatasari[1], Mohammad Aris Widodo[2]

[1]Department of Ophthalmology, Faculty of Medicine, Brawijaya University, Indonesia
[2]Laboratorium of Pharmacology, Faculty of Medicine, Brawijaya University, Indonesia

Abstract: This study aims to evaluate the effect of hypertension model induced by Deoxycorticoacetate (DOCA) on ratio of endothelin (ET-1)/eNOS, expression of ET-1 Receptor A (ETRA) and ET-1 Receptor B (ETRB), expression of phosphorylated Myosin Light Chain Kinase (MLCK) and Caldesmon (CaD) in endothelial cells of TM. Experimental study was performed on 20 male rats, divided into control group (1) and hypertension group (2-4): DOCA subcutaneous 10 mg/kg BW twice a week + NaCl 0.9% for 2, 6, and 10 weeks. Blood pressure was measured by BP analyzer. ET-1 signaling were evaluated by immunofluorescent staining under confocal microscope observation. Data were analyzed by one way Anova or Kruskal Wallis Test and Mann Whitney Test. Blood pressure was significantly increased in all of hypertension groups compare to control ($p = 0.001$). The average ratio of ET-1/eNOS were highest in 2 weeks (1.31±0.025). The ETRA were significantly increased in 2 and 6 weeks after treatment (1476.3±20.9 Au and 1209.7±6.1 Au), while ETRB only in 2 weeks (1160.5±18.2 Au). The highest average of MLCK (1916.68±6.41 Au) and CaD (1676.37±7.72 Au) were also found in 2 weeks of hypertension. Hypertension induced by DOCA-salt stimulation activated ET-1 signaling pathway in TM. Activation peak was achieved at 2 weeks hypertension, as a development phase of hypertension.
Keywords: Deoxycorticoacetate-salt, ET-1 signaling pathway, hypertension, trabecular meshwork.

Introduction

Glaucomatous optic neuropathy (GON) is a progressive optic neuropathy characterized by retinal ganglion cell (RGC) apoptosis and degeneration of its axons that can result in permanent blindness. The prevalence of glaucoma in the world is 60.5 million in 2010 which will increase to 79.6 million by 2020, of which 74% is primary open-angle glaucoma (POAG). This has driven many research to be carried out on the risk factors of GON, one of which is hypertension[1,2].

The relationship of hypertension and GON is still controversial, but research that show positive correlation indicates that 47.3% among of POAG patients suffer from hypertension. The Blue Mountain Study also stated a strong correlation between hypertension and POAG, where the prevalence of POAG patients with poor control of hypertension reached 5.4%. The Beaver Dam Study results showed that the increase in systolic blood pressure (SBP) ≥ 10 mmHg led to the increase in IOP by 0.44 mmHg. On the contrary, decrease in SBP ≤ 10 mmHg caused a decrease in IOP by 0.59 mmHg[3-5]. On the other hand, The Indonesia Basic Health Research

(Riskesdas) in 2013 reported that the prevalence of hypertension in the population aged more than 18 years has reached 25.8% [6]. This may lead to possible increase of glaucoma prevalence.

Disruption of endothelial function of TM plays an important role in the occurrence of GON. Endothelium cells will release peptides such as ET-1, nitric oxide (NO), and prostaglandin (PG) after a stimulation. Endothelin-1 (ET-1) has been suggested to be a potential contributor to the pathogenesis of glaucoma. The imbalance of ET-1 and NO production is a typical condition of initial endothelial dysfunction in patients with glaucoma. Study by Ghanem et al., 2011 found levels of ET-1 and NO in aquous humor (AH) was significantly increased[7]. Additionally, levels of ET-1 have been found to be two to three times higher in AH than in the plasma, presumably because it is secreted by the cilliary epithelium and not derived from plasma[7,8-11].

The involvement of ET-1 in GON, is through the paracrine effect of ET-1 on the ocular area reaching ciliary muscles, TM, optic nerve astrocytes, lamina cribrosa cells and RGC. Activation of ET-1 signaling pathway is initiated with activation of ET-1 receptor in the endothelial cells. Study of bovine tissue with specific ETR_A and ETR_B antagonist found that ETR_A contribute more to contractility TM than ETR_B. On the other hand, several studies found evidence for the role of ET-1 and specifically ETR_B in RGC death[7,10,12,13].

Significant evidence exists for the role of the smooth muscle-like properties of the TM in the regulation of AH outflow and hence, control of IOP. Myosin light chain kinase (MLCK) as the regulator of the contraction affects the TM contractility. Study by Rao et al., 2005 found the significant role of Rho/Rho-kinase pathway-mediated MLC phosphorylation in modulating AH outflow through the TM[14]. Meanwhile, Grosheva et al, 2006 showed overexpression of CaD would induce changes in contractility and actin cytoskeleton of TM cells[14-16].

DOCA-salt hypertensive model is a good model of ET-1 dependent hypertension. Deoxycorticoacetate (DOCA) is a precursor of aldosterone that is involved in the Renin-Angiotensin-Aldosteron System (RAAS) for inducing hypertension. Research by Grobe et al. (2011) on angiotensinergic signaling in the brain, and the involvement of RAAS in the brain, on sympathetic nerve activity, Vasopressin and ET-1 through ATR1 activation in DOCA-salt administration[17,18].

Angiotensinergic signaling in brain does not depend on BP magnitude change, it is an act as a metabolic consequence from DOCA-salt. DOCA-salt hypertension evolves at several phases of cells, whereas at the developmental phase that occur at the second and sixth week. Sympathetic nerve role is balance with humoral role which shown by the increase of [VP] pl and [ET-1] pl[17,18]. Therefore, this study will determine the influence of hypertension on the activity of ET-1 in TM, and will be evaluated according to the duration of hypertension, not the grade of its severity.

There were still no patobiomolecular researches that studyout the effect of hypertension on GON. This study is aimed to determine the effects of hypertension duration on the ET-1, eNOS, ETR_A, ETR_B, MLCK and CaD in TM

Experimental

The experimental design which used in this study was true experimental-post only with control. This study was conducted in June to October 2014 at Pharmacology Laboratory-Faculty of Medicine of Brawijaya University and Central Laboratory of Biological Sciences - Brawijaya University.

Male Spraque Dawley rats, with 4-5 months of age and 250-350 grams of weight were obtained from Animal Model Laboratory, Biomedical Center and Health Basic Technology (Jakarta, Indonesia). Total number of 20 animal models were divided into four groups. Animal care and all experiments were conducted in accordance with the approved standard guidelines for animal experimentation of the Health Research Ethics Committe of the Faculty of Medicine, Brawijaya University and adhered to the ARVO statements for the Use of Animals in Ophthalmic and Vision Research.

DOCA-salt hypertension Induction

Rats were injected with DOCA 10 mg/kg dissolved in corn oil subcutaneously 2 times a week and had been administered 0.9% of NaCl solution for 2 weeks (group 2), 6 weeks (group 3) and 10 weeks (group 4). Rats were categorized as hypertensive if their BP were reach > 150/90 mmHg. Control group (group 1) received corn oil administration 2 times a week subcutaneously and were given aqua bidest ad libitum. Blood pressure was measured by animal BP analyzer. Systolic BP value was calculated from the average of 3 lowest values from 5 SBP recorded values[18].

Trabecular Meshwork (TM) tissue sampling

Trabecular Meshwork tissues were taken under the operating microscope (DECA 21, Inami Co Ltd), by enucleation. After being enucleated, they were directly put into bottles with 4% of paraformaldehyde. Eyeballs were cut transversely on the equator. After lenses were removed, anterior segments were divided into four quadrants, which allow to ease in obtaining TM tissues through paraffin blocking process[19].

ET-1, ETRA, ETRB eNOS, and phosphorylated MLCK and CaD expression assays

Immunofluorescence (IF) staining was started with deparaffinization process. Primary antibodies which used were anti-rat ET-1Ab, eNOS-activated Ab, anti rat ETR_A antibody (Bioss, USA), anti rat ETR_B antibody (Bioss, USA), phospho-specific MLC serine 19 Ab, phospho-specific CaD Ab (pT730), and Smoothelin Ab (Bioss, USA). Secondary antibodies which used were FITC for eNOS, ETR_B, and CaD while TRITC for ET-1, ETR_A and, MLCK. Furthermore, slides were immediately observed with laser confocal microscope (LSCM) type Olympus fluo FV 10-ASW 1.7, with 400X of magnification with 5 fields of view. Color intensity analysis were using image of Olympus Image Binary Format was in the form of numerical data in arbitrary units (Au).

Data analysis

Data were analyzed using One Way Anova or Kruskal Wallis Test and Mann Whitney Test. All calculations were performed with SPSS software for Windows 19.0.

Results

Administration of DOCA-salt led to the significantly increase in final SBP average according to the duration of hypertension ($p < 0.05$).

The effect of Hypertension on expression of ET-1 and eNOS in TM endothelial cells

Figure 1 shows qualitatively that the most significant increase in ET-1 expression was found in 2-week group. Meanwhile, the 6-week and 10-week groups showed a decrease in expression compared with 2-week group.

Kruskal Wallis and Mann Whitney tests of ET-1 and eNOS expressions were significant difference in endothelial cells of TM between hypertension group and control group ($p < 0.05$). The highest increase in ET-1 expression was in the 2^{nd} week, then decreased in the 6^{th} week, even in the 10^{th} week was lower than the control group although it was not significant. Meanwhile, in the eNOS expression, there was no significant difference among the hypertension group.

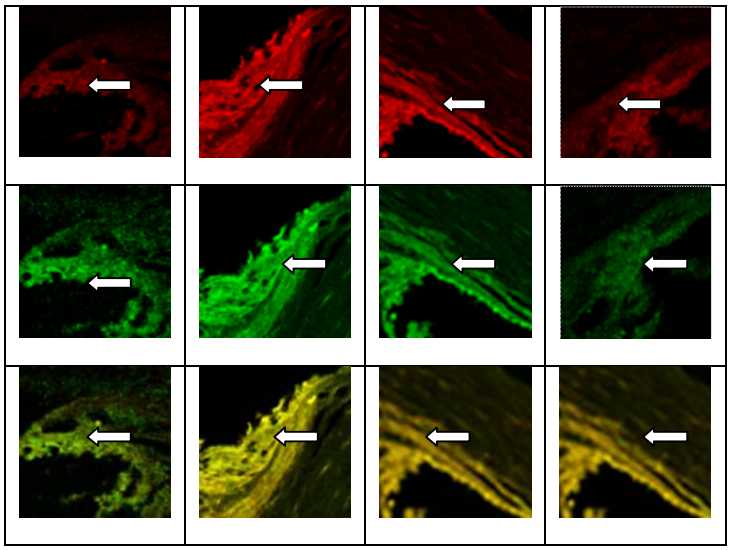

Fig 1. ET-1's expression at the endothelial TM. The expression of ET-1 shown by the TRITC staining (red fluoresence), while the endothelial cell by FITC (green fluoresence). There is an increase of ET-1 expression in two weeks (B), six weeks (C) and ten weeks (10) groups compared to the control (A).

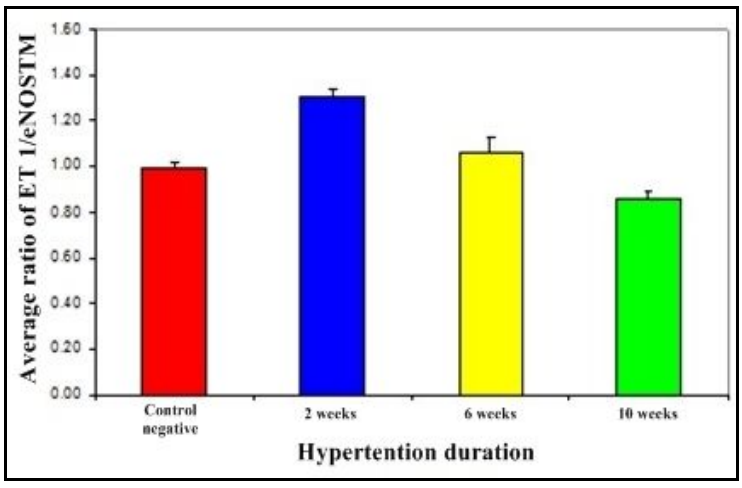

Fig 2. Comparison mean ratio of ET-1: eNOS in TM endothelial cell. The significantly increase in mean ratio of ET-1: eNOS in TM endothelial cell (p = 0.001) in the second week (1.31±0.025) and sixth week groups (1.06±0.074), which followed by the decrease in the tenth week group (0.86±0.036) compared to control group (0.99±0.021).

Figure 2 shows there was significant difference between ET-1 and eNOS in TM endothelial cell (p = 0.001). The significant increase of ET-1 and eNOS ratio occured at the second week. This imbalance is the beginning of ischemia and will still occurs until the sixth week. The decrease in ratio also happened in the tenth week which lower than the control group.

The effect of Hypertension on ETR_A and ETR_B expression in TM endothelial cells

The results showed significant differences in hypertension duration effects on ETR_A and ETR_B expression in TM endothelial cells ($p < 0.05$). In TM endothelial cells, the highest ETR_A and ETR_B expression was seen in 2-week hypertension group. Significant decrease in ETR_A occurred in the 10th week while the ETR_B occurred in the 6th week (Figure 3).

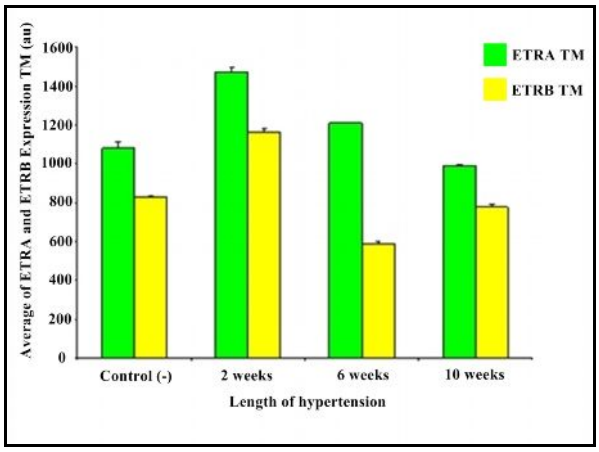

Fig 3. Comparison of hypertension duration effects on ETR_A and ETRB expression in TM endothelial cells. The highest increasing of ETR_A (1476.3±20.9 Au) and ETR_B expression (1160.5 ± 18.2 Au) was in endothelial cells of TM in the 2nd week.

The effect of Hypertension on phosphorylated of MLCK and CaD expression in TM endothelial cells

The results showed significant differences of hypertension duration effects on phosphorylated MLCK and CaD expression in TM endothelial cells ($p < 0.05$). In TM endothelial cells, the highest MLCK and CaD was seen in 2-week hypertension groups. Significant decrease of MLCK occurred in the 6th week while the CaD occurred in the 10th week (Figure 4).

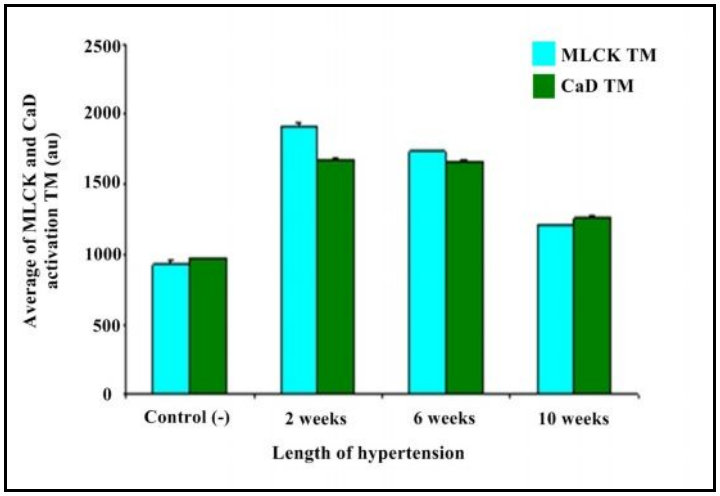

Fig 4. Comparison of hypertension duration effects on MLCK and CaD in TM endothelial cells. The highest activity of MLCK (1916.68±6.41Au) and CaD (1676.37±7.72 Au) was seen in the 2nd week of hypertension. Significantly decreasing level in MLCK occurred in the 6th week group (1742.02±20.44 Au), while CaD occurred in the 10^{th} week of hypertension (1260.91±17.29 Au).

Discussion

Approximately 90% of cases of hypertension are primary (essential), in which most likely results from a disregulation of various renal, hormonal, and cellular processes in conjunction with environmental factor such as diet and exercise. These process include abnormal sodium transport, increased sympathetic nervous system activity, abnormal vasodilatation, excess TGFβ, and renin-angiotensin aldosterone system. This research showed significant increase in the final BP according to hypertension duration ($p = 0.001$). Administration of DOCA-salt induced neurohumoral activation and increased blood volume, as well as the occurrence of hypertonicity in the brain through stimulation of vasopressin secretion and stimulation of sympathetic nervous system[17,18].

The effect of Hypertension on the ratio of ET-1 and eNOS expression in TM

The effects of hypertension on ET-1 and eNOS expression in TM was alleged due to the effects of RAS system activation in brain and TM tissues which was more dominant than in kidney (Figure 6). DOCA-salt administration increased angiotensin receptor density, especially in the region of brain which control cardiovascular vessels. The increasing of ET-1 expression was found starting from week 2, it corresponds with study by Grobe et al. (2010) on angiotensinergic signaling in brain through ATR1 activation which would increase the activity of sympathetic nerve, vasopressin, and ET-1[17]. The increasing of metabolic rate was also found after 3 weeks of DOCA-salt administration. Sharif (2015), mentioned that the effect of RAS on TM was shown in ATR1 activity that mediated signaling for the acumulation of extraceluler matrix and TGFβ on TM tissue that lead increase of IOP occurred[17,20,21].

Hypertension effects of DOCA-salt model were alleged due to the increased glucocorticoid receptor (GR). This condition would increase TM cells intercellular and extracellular matrix volume. Stokes et al. (2000) has found the high expression of GR mRNA and mineralocorticoid 11β-HSD that interact with mineralocorticoid receptor (MR) which has role in the mechanism of ion and water transport, including the secretion of AH. Also known that MR on TM is well-bonded with cortisol and aldosterone. Mineralocorticoid effects on eyes were mediated by epithelial sodium channels (EnaC), which involved in the increasing of AH formation and organize TM resistance through the regulation of TM cell volume[22,23].

Significantly increased activation of eNOS in all hypertension groups was in line with research by Yusuf et al. (2012), where Ang 1-7 through Mas receptor mediation significantly inhibited the effects of ET-1, while NO production and ETR_B expression increased[24]. After the ischemia and hypoxia, NO reacted as a neurodegenrative factor or conversely as a neuroprotective, it depends on included NOS isoform and the number of formed NO. NO that was formed from eNOS and nNOS on the perivascular nitrergic neurons played a role in keeping the ocular blood flow and the decrease infarct size[8,24,25].

The incresing of ET-1 and eNOS expressions in TM started in the 2nd week which could be described by the theory that the DOCA-salt hypertension model developed through several stages. In the developmental stage occured at the second until sixth weeks of DOCA adminstration, the role of sympathetic nerves was in balance with humoral role that was shown by the increase of [VP] pl and [ET-1] pl[17,18,26].

The change of ratio between ET-1 and eNOS in TM was once increased at the second week then decreased at the sixth week. The eNOS level became higher than ET-1 in the tenth week which can be explained by the impairment of NO constitutive production which acts as an etiological factor of glaucoma. On the other hand, the increase of the synthetis and release of NO by NO constitutive is to limit the progresitivity of the glaucoma through vasodilatation, increase of blood flow, and the relaxation of smooth muscle and also the negative modulation of pro inflammatory action and mitogenic from ET-1 through the crosstalk bertween Mas and ETR_B and also the increase of the production of NO[8,13,24,25].

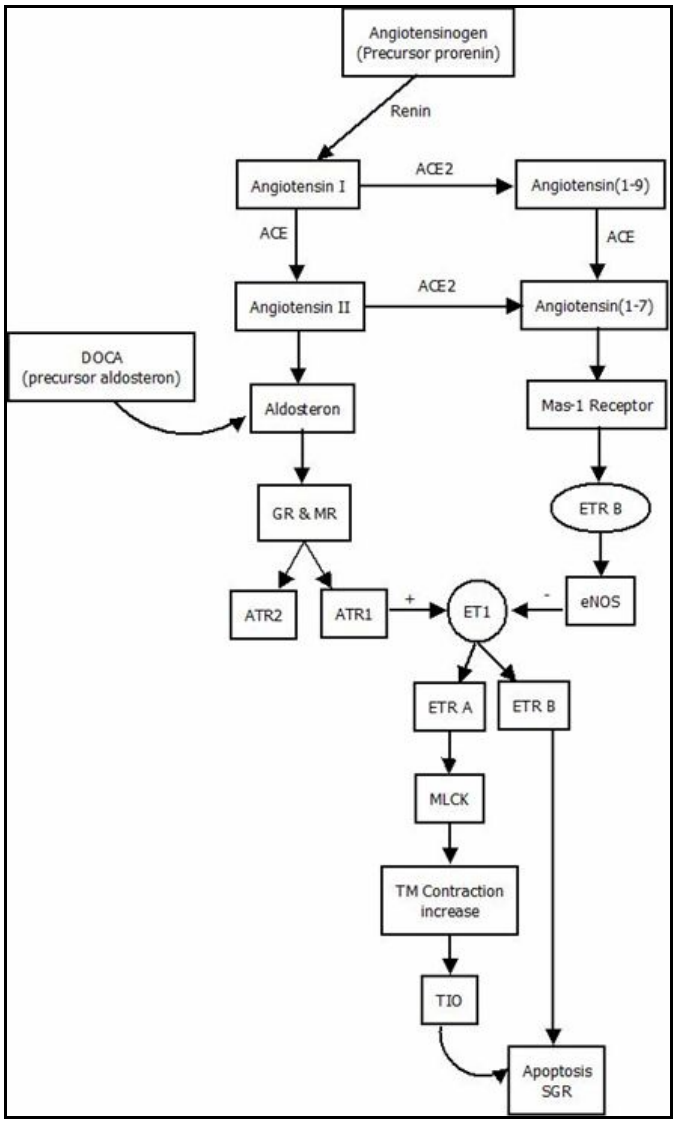

Fig 6. RAS system shows Ang-I, Ang-II, and Ang (1-7) production and their preferred receptor subtype. There is hypothesis and functional data that mentioned about activataion ET-1 signaling pathway involvement of IOP elevation in mice. Mas-1 and AT-1 receptors mediate signaling for activation of MLCK that responsible to raise IOP. Ang= angiotensin; ATR1= angiotensin reseptor 1; IOP=intraocular pressure; RAS= renin-angitensin. Modified Sharif (2015)[21].

Hypertension effect on ETR_A and ETR_B expression in TM

This study shown that DOCA-salt increased the expression of ETR_A and ETR_B which indicated by interaction between ET-1 with ETR_A and ETR_B. Some study about TM contractility showed that the effect of ET-1 on IOP is dominantly mediated by ETR_A, although both receptor is expressed in TM. Rosenthal et al. (2011) result has shown that TM contraction is induced by charbacol (10-6 mol.L-1) and ET-1 (10-8 mol.L-1). After BQ-123 (ETR_A antagonist) addition there was indication of IOP decrease, while BQ-788 (ETR_B antagonist) addition did not give similar effects. The same result was obtained in a study using BQ-485 (ETR_A antagonist)[16,28].

A study conducted by Minton et al. (2012), resulted in the increasing of ETR_B in nerve fiber layer, inner plexiform layer, outer plexiform layer, RGC layer after IOP increase in the second and fourth weeks[29]. While Krishnamoorthy et al. (2008) has shown that RGC cell line that given with BQ 788, indicated the activity of ocular ET-1 through ETR_B that mediated RGC apoptosis 27, 28[30]. The increasing of ETR_A expression was occur in the second and sixth weeks of treatment, although the increasing of ETR_B was found in the second week. This condition indicates more persistant activity of the ETR_A compared to ETR_B in TM endothelial cell.

Heterologous ETR_A entered the recycling pathway, but ETR_B was targeted by lysosome which caused ET_B response were temporary[29-31].

Result of this study also related to Yusuf et al. (2012) which shown that Ang 1-7 increases the NO production and the expression of ETR_B[24]. It can be concluded that the Ang-1 modulates negative pro-inflammatory and mitogenic action of ET-1 through crosstalk between Mas and ETR_B. The activation of ET_B receptor on the endothelial cell by ET-1 restricted contraction response by inducing NO release from endothel[24,32].

Hypertension effect on phosphorylated MLCK and CaD expression in TM

Results showed there were significant increase of MLCK and CAD in hypertension groups. Study by Rao et al., (2005) identified potential physiological effect of MLCP as a regulator in human TM cells and there were significant role of MLC mediated Rho/Rho-kinase pathway in HA outflow modulation[14]. The highest increase in MLCK expression was in the 2nd week, which followed by the decrease in its expression since the 6th week. ET-1 interaction with ETR_A contributed to TM contractility which stimulated by MLC phosphorylation[14,33,34].

CaD overexpression induced reorganization of actin cytoskeleton which associated with focal damage and fibrillar cell matrix adhesion that caused destabilization of cell-cell adherent junctions. This condition induced change in TM contractility in glaucomatous eyes. Caldesmon (CaD) expression lasted up to the 10th week, while MLCK only up to the 6th week. This condition was expected caused by the increasing of CaD expression induced by ETR_A which longer than MLCK induction. MLCK activation through IP3 pathway expected was required Ca2+ and Calmodulin complex formation while CaD activation did not depend on Ca^{2+} [14,35].

Conclusion

Hypertension model induced by DOCA-salt significantly affects on the activation of ET-1 signaling pathway in TM endothelial cells. Peak of ET-1 activation occurs in the 2nd week of hypertension as a development phase of hypertension.

References

1. Qiugley H.A., Broman A.T., The number of people with glaucoma worldwide in 2010 and 2020, Br. J. Ophthalmol., 2006, 90(3), 262-267. DOI: 10.1136/bjo.2005.081224.
2. Agarwal R., Krasilnikova A.V., Raja I.S., Agarwal P., Mohd Ismail N., Mechanism of angiotensin converting enzyme inhibitor-induced IOP reduction in Normotensive Rats, Eur. J. Pharmacol., 2014, 730, 8-13. DOI: 10.1016/j.ejphar.2014.02.021.
3. Mitchell P., Lee A.J., Rochtchina E., Wang J.J., Open angle glaucoma and systemic hypertension: the blue mountain eye study, Am. J. Ophthalmol., 2004, 13(4), 319-326. PMID: 15226661.
4. Klein B.E.K., Klein R., Knudtson M.D., Intraocular pressure and systemic blood pressure: longitudinal perspective the beaver dam study. Br. J. Ophthalmol., 2005, 89(3), 284-287. DOI: 10.1136/bjo.2004.048710.
5. Dave A., Bali S.J., Sharma R., Khurana A.K., Gupta R., Dada T., Prevalence of diabetes mellitus and hypertension among Indian glaucoma patients and evaluation of systemic therapy. Int. Ophthalmol., 2013, 33(5), 527-532. DOI: 10.1007/s10792-013-9737-3.
6. Tim Penelitian dan Pengembangan Kemenkes RI (2013): Riset Kesehatan Dasar. Jakarta: Kemenkes RI 126-127.
7. Ghanem A.A., Elewa A.M., Arafa L.F., Endothelin-1 and nitric oxide levels in patients with glaucoma. Ophthalmic. Res., 2011, 46(2), 98-102. DOI: 10.1159/000323584.
8. Toda N., Nakanishi-Toda M., Nitric Oxide: ocular blood flow, glaucoma, and diabetic retinopathy. Prog. Retin. Eye Res., 2007, 26(3), 205-238. PMID: 17337232.

9. Agarwal R.D., Gupta S.K., Agarwal P., Saxena R., Agrawal S.S., Current concepts in the pathophysiology of glaucoma, Indian J. Ophthalmol., 2009, 57(4), 57-66. DOI: 10.4103/0301-4738.53049.
10. Good J.T., Kahook Y.M., The role of endothelinin the pathophysiology of glaucoma, Expert. Opin. Ther. Targets, 2010, 14(6), 647-654.DOI: 10.1517/14728222.2010.487065.
11. Salvatore S., Vingelo E.M., Endothelin-1 role in human eye: a review. J. Ophthalmol., 2010, 354645. DOI: 10.1155/2010/354645. PMID: 21461356.
12. Kapakos G., Bouallegue A., Daou G.B., Srivastava A.K., Modulatory role of nitric oxide/cGMP system in endothelin-1-induced signaling responses in vascular smooth muscle cell. Curr. Cardiol. Rev., 2010, 6(4), 247-254. DOI: 10.2174/157340310793566055.
13. Rocha-Sousa A., Rodrigues-Araujo J., Gouvela P., Barbosa-Breda J., Azevedo-Pinto S., Pereira-Silva P., Leite-Moreira A., New therapeutic targets for intraocular pressure lowering. ISRN Ophthalmol., 2013, 261386. DOI: 10.1155/2013/261386.
14. Rao P.V., Deng P., Sasaki Y., Epstein D.L., Regulation of myosin light chain phosphorylation the trabecular meshwork: role in aqueous humour outflow facility, Exp. Eye Res., 2005, 80(2), 197-206. PMID: 15670798.
15. Grosheva I., Vittitow J.L., Goichberg P., Gabelt B.T., Kaufman P.L., Borras T., Geiger B., Bershadsky A.D, Caldesmon effects on the actin cytoskeleton and cell adhesion in cultured HTM cells. Exp. Eye Res., 2006, 26(6), 945-958. PMID: 16679125.
16. Rosenthal R., Fromm M., Endothelin antagonism as an active principle for glaucoma therapy, Br. J. Pharmacol., 2011, 162(4), 806-816. DOI: 10.111/j.1476-5381.2010.01103.x.
17. Grobe J.L., Buehrer B.A., Hilzendeger A.M., Liu X., Davis D.R., Xu D., Sigmund C.D., Angiotensinergic signaling in the brain mediates metabolic effects of deoxycorticosterone (DOCA)-salt in C57 mice., Hypertension, 2011, 57(3), 600-607. DOI: 10.1161/HYPERTENSIONAHA.110.165829.
18. Prahalathan P., Kumar S., Raja B., Effect of morin, a flavonoid against DOCA-salt hypertensive dependent study, Asian Pac. J. Trop., 2012, 2(6), 443-448. DOI: 10.1016/S2221-1691(12)60073-2.
19. Tektas O.Y., Lutjen-Drecoll E., Structural changes of the trabecular meshwork in different kinds of glaucoma. Exp. Eye Res., 2009, 88(4), 769-775. DOI: 10.1016/j.exer.2008.11.025.
20. de Kloet A., Wang L., Summers C., Krause E., Activation of brain angiotensin type 2 receptors reverse DOCA-salt hypertension in mice. J. Federation Am. Soc. Exp. Biol., 2015, 29(1), 971-977.
21. Sharif N.A., Novel potential treatment modalities for ocular hypertension: focus on angiotensin and bradykinin system axes. J. Ocul. Pharmacol. Ther., 2015, 31(3), 131-145. DOI: 10.1089/jop.2014.0114.
22. Stokes J., Noble J., Brett L., Philips C., Seckl J.R., O'brien C., Andrew R., Distribution of glucocorticoid and mineralocorticoid receptors and 11β-hydroxysteroid dehydrogenases in human and rat ocular tissues. Invest. Ophthalmol. Vis. Sci., 2000, 41(7), 1629 – 1638.
23. May A.C., Could mineralocorticoids play a role in the pathophysiology of open angle glaucoma?, J. Ophthalmol., 2012, 1-5. DOI: 10.1155/2012/196418.
24. Yusuf H, Montezano AC, Callera GE, Din Cat AN, Santos RA, Castro CH, Touyz RM. Angiotensin 1-7 attenuates endothelin-1-induced endothelial cell inflammation and growth through nitric oxide production and activation of mas and endothelin b receptors. Hypertention 2012(60), A258.
25. Vaajanen A., Kalesnykas G., Vapaatalo H., Uusitalo H., The expression of mas-receptor of the renin-angiotensin system in the human eye. Graefes Arch. Clin. Exp. Ophthalmol., 2015, 253(7), 1053-1059. DOI: 10.1007/s00417-0152952-z.
26. Yemane H., Busauskas M., Burris S.K., Knuepfer M.M.., Neurohumoral mechanism in deoxycorticosterone acetate (DOCA)-salt hypertension in rats. Exp. Physiol., 2010, 95(1), 51-55. DOI: 10.1113/expphysiol.2008.046334.
27. Toshihide KKurihara, T., Ozawa, Y., Ishida, S., Okano, H., Tsubota, K., Renin-angiotensin system hyperactivation can induce inflammation and retinal neural dysfunction. Int. J. Inflam., 2012, 1-15. DOI: 10.1155/2012/581695.
28. Prasanna G., Narayan S., Krishnamoorty R.R., Yorio T., Eyeing endothelins: a cellular perspective, Mol. Cell Biochem., 2003, 253(1-2), 71-88. PMID: 14619958.
29. Minton A.Z., Phatak N.R., Stankowska D.L., He S., Ma H.Y., Mueller B.H., Jiang M., Luedtke R., Yang S., Brownlee C., Khrishnamoorthy R.R., Endothelin B receptors contribute to retinal ganglion cell loss in a rat model of glaucoma, Plos ONE, 2012, 7(8), e43199.
DOI: 10.1371/journal.pone.0043199.

30. Krishnamoorthy R.R., Rao V.R., Dauphin R., Prasanna G., Johnson C., Yorio T., Role of the ET_B receptor in retinal ganglion cell death in glaucoma. Can. J. Physiol. Pharmacol., 2008, 86(8), 380-393. DOI: 10.1139/Y08-040.
31. Mazzuka M.Q., Khalil R.A., Vascular endothelin receptor type B: structure, function and dysregulation in vascular disease. Biochem. Pharmacol., 2012, 84(2), 147-162. DOI: 10.1016/j.bcp.2012.03.020.
32. Vaajanen A., Vapaatalo H., Kautiainen H., Oksala O., Angiotensin (1-7) reduces intraocular pressure in the normotensive rabbit eye. Invest. Ophthalmol. Vis. Sci., 2008, 49(6), 2557-2562. DOI: 10.1167/iovs.07-1399.
33. Inoue T., Tanihara H., Rho-associated kinase inhibitors: a novel glaucoma therapy. Prog. Retin. Eye Res., 2013, 37, 1-12. DOI: 10.1016/j.preteyeres.2013.05.002.
34. Kuespert S., Junglas B., Braunger M.B., Tamm E.R., Fuchshofer R., The regulation of connection tissue growth factor expression influences the viability of human trabecular meshwork cells. J. Cell Mol. Med., 2015, 19(5), 1010-1020. DOI: 10.1111/jcmm.12492.
35. Lin J.J., Li Y., Eppinga R.D., Wang Q., Jin J.P., Roles of caldesmon in cell motility and actin cytoskeleton remodeling. Int. Rev. Cell Mol. Biol., 2009, 274, 1-68. DOI: 10.1016/S1937-6448(08)02001-7.

*****