

ORIGINAL RESEARCH

Medicine Science 2019;8(3):537-40

Arginine, symmetric and asymmetric dimethylarginine levels in the molsidomine treatment of experimental ischemia-reperfusion retinopathy**Nihat Polat¹, Murat Atabey Ozer², Hakan Parlakpınar³, Zeynep Aksungur⁴, Onural Ozhan³, Yusuf Turkoz⁴**¹*Inonu University Faculty of Medicine, Department of Ophthalmology, Malatya, Turkey*²*Giresun University Faculty of Medicine, Department of Ophthalmology, Giresun, Turkey*³*Inonu University Faculty of Medicine, Department of Pharmacology, Malatya, Turkey*⁴*Inonu University Faculty of Medicine, Department of Biochemistry, Malatya, Turkey*

Received 11 January 2019; Accepted 04 February 2019

Available online 06.03.2019 with doi:10.5455/medscience.2019.08.9008

Copyright © 2019 by authors and Medicine Science Publishing Inc.

Abstract

This study aimed to evaluate the mean changes in Arginine, Asymmetric Dimethylarginine (ADMA) and Symmetric Dimethylarginine (SDMA) levels in the ischemia/reperfusion (I/R) retinopathy and efficacy of treatment with molsidomine by these levels. Experiments were performed on the New Zealand white rabbits each weighing approximately 2.5 kg. 28 rabbits were assigned to the following 4 groups, group 1 consisted sham, group 2 consisted I/R, group 3 consisted I/R+ treatment with molsidomine, group 4 consisted prophylaxis with molsidomine +I/R. In the group 2, 3 and 4, ischemia was induced by raising the intraocular pressure to 150 mmHg for 60 minutes. After 60 min, the IOP was returned to normal pressure. 4 mg/kg/day molsidomine was administered intraperitoneally four days after I/R in group 3, one day before I/R and three days after I/R in group 4. Arginine, ADMA and SDMA levels were measured on the aqueous humor. The mean arginine levels were 12.3±4.8 µmol/L in group 1, 12.4±1.4 µmol/L in group 2, 13.2±2.4 µmol/L in group 3 and 13.7±4.3 µmol/L in group 4. No difference was present between the groups (p=0.807). The mean ADMA levels were 2.6±0.8 µmol/L, 7.3±2.7 µmol/L, 0.5±0.5 µmol/L and 2.5±1.0 µmol/L respectively. Significant increase was present in the group 2 and significant decrease was present in the group 3 (p=0.001). The mean SDMA levels were 1.0±0.3 µmol/L, 1.8±0.2 µmol/L, 0.3±0.3 µmol/L and 1.0±0.4 µmol/L respectively. Significant increase was present in the group 2 and significant decrease was present in the group 3 (p=0.001). L-Arginine levels were kept steady, ADMA and SDMA values decreased with molsidomine. Four days treatment with molsidomine after I/R may be beneficial more than prophylaxis and three days treatment.

Keywords: Aqueous humor, arginine, asymmetric dimethylarginine, ischemia-reperfusion retinopathy, molsidomine, symmetric dimethylarginine**Introduction**

Retinal ischemia/reperfusion (I/R) injury may occur in such conditions as retinal vascular occlusion, acute angle-closure glaucoma, diabetic retinopathy, and retinopathy of prematurity [1]. Some mechanisms, including inflammation, oxidative stress, excitotoxicity, and apoptosis, are implicated in I/R-associated damage [2]. Retinal I/R insult leads to degeneration in neuronal cells and activation of inflammatory cells [3]. Damage caused by lack of oxygen and substrate during ischemia is growing more because of excessive reactive radical production during reperfusion

[4]. Nitric oxide (NO) maintains proper blood flow, and perfusion also plays a critical role by blocking platelet activation and leukocyte adhesion, preventing smooth muscle cell proliferation, and increasing the survival of endothelial cells in tissue [5]. NO may prevent the release of inflammatory products (leukotrienes, cytokines, and prostaglandins) with cytotoxic vasoconstrictor actions and could have a direct cytoprotective effect on endothelial cells during the inflammatory process [6].

Molsidomine (MOL) is a prodrug that decarboxylates enzymatically to form 3-morpholinosydnonimine (SIN-1) in the liver, which spontaneously releases NO [7]. Some studies report that SIN-1 shows beneficial effects on I/R injury by decreasing the release of neutrophils, and reducing vascular resistance and shows the protective effect on endothelial functions [1]. In our previous

*Corresponding Author: Nihat Polat, Inonu University Faculty of Medicine, Department of Ophthalmology, Malatya, Turkey, E-mail: drnihatpolat@gmail.com

study we found that MOL protected the retina from I/R injury by enhancing anti-oxidative effects and inhibiting apoptosis of retinal cells [8]. Asymmetric Dimethylarginine (ADMA), Symmetric Dimethylarginine (SDMA), and L-arginine are involved in the production of NO, which is a key player in microvascular damage pathogenesis [9]. ADMA and SDMA are produced by methylation of the arginine in protein pathway. SDMA and ADMA are physiological isomers. ADMA is an endogenous inhibitor of nitric oxide synthase (NOS), which the key endothelial enzyme that catalyses the conversion of L-arginine to L-citrulline and NO [10]. SDMA does not directly inhibit NOS but appears to interfere with the cellular transport of arginine and some other amino acids [11]. Increased ADMA levels have been found in patients with retinal venous occlusive disease [12]. The result of the inhibition of NOS by ADMA is the reduction of NO bioavailability.

We aimed to evaluate the mean changes in L-arginine, ADMA and SDMA levels in the I/R retinopathy and levels of these, under treatments with molsidomine.

Material and Methods

Study design

This experimental animal study was designed according to Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines [13]. The authors confirm adherence to the Association for Research in Vision and Ophthalmology (ARVO) statement for the Use of Animals in Ophthalmic and Vision Research. The experimental protocol in the current study was approved by the Ethics Committee on Animal Research of İnönü University (reference number: 2016/A-16) and the Guidelines for Animal Research from the National Institutes of Health were followed in all experimental procedures.

Animals and groups

Experiments were performed on healthy adult New Zealand white rabbits, each weighing approximately 2.5 kg. Animals were obtained from the İnönü University Laboratory Animals Research Center and placed in a temperature (21±2°C)- and humidity (60±5%)-controlled room in which a 12:12 h light/dark cycle was maintained. The animals were assigned to the following 4 groups, group 1 consisted sham, group 2 consisted I/R, group 3 consisted I/R+ Treatment with molsidomine, group 4 consisted prophylaxis with molsidomine +I/R.

Induction of ischemia/reperfusion

The animals were anesthetized by an intramuscular injection of ketamine (35 mg/kg) + xylazine (5 mg/kg). Corneal anesthesia was established by 1–2 drops of proxymetacaine hydrochloride 0.5% (Alcaine, Alcon, USA). Induction of ischemia/reperfusion was done as previously reported [4]. Briefly, the anterior chamber

was cannulated with a 20-gauge needle connected to a container of sterile normal saline, and the reservoir was elevated to achieve 150 mmHg intraocular pressure for 60 min. Retinal ischemia was established when there was whitening of the anterior segment and loss of the red reflex of the retina. After 60 min, the cannula was removed from the anterior chamber to termination of ischemia. Retinal reperfusion was confirmed by reflow of the anterior segment and retina.

Drugs

The doses of MOL (intraperitoneal 4 mg/kg/d, Molsidomin, Sigma Chemical Co., St. Louis, MO, USA) was chosen dependent on the previous dose-response studies that have been reported to have found marked anti-oxidative effects [14]. MOL was administered intraperitoneally four days after I/R in group 3, one day before I/R and three days after I/R in group 4.

Measurement of L-Arginine, ADMA, and SDMA

The levels of L-Arginine, ADMA, and SDMA in the aqueous humor samples were measured using HPLC (Shimadzu Corporation, Kyoto, Japan) with fluorescence detection. For ADMA, SDMA, L-Arginine analysis, it was used Eureka brand HPLC kit (Code Z58010, Eureka Lab Division) and Watersbr and analytical column (4.6x250 mm, spherisorb 5 m phenyl). All analysis procedures were performed according to the manufacturer's instructions. Fluorescence detection was performed at the excitation and emission wavelengths of 340 and 450 nm, respectively.

Statistical analysis

The sample sizes necessary for a power of 0.80 were estimated using NCSS (LLC, Kaysville, UT, USA) software. All data were analyzed with a commercially available statistical software package (SPSS for Windows v. 22.0, Chicago, IL, USA). For all parameters, one-way ANOVA was performed with post hoc tukey test. Results were presented as mean ± SD. P <0.05 was regarded as statistically significant for all data.

Results

The results are presented in Table 1. Briefly, the mean arginine levels were 12.3±4.8 µmol/L in group 1, 12.4±1.4 µmol/L in group 2, 13.2±2.4 µmol/L in group 3 and 13.7±4.3 µmol/L in group 4. No difference was present between the groups (p=0.807). The mean ADMA levels were 2.6±0.8 µmol/L, 7.3±2.7 µmol/L, 0.5±0.5 µmol/L and 2.5±1.0 µmol/L respectively. Significant increase was present in the group 2 and significant decrease was present in the group 3 (p=0.001). The mean SDMA levels were 1.0±0.3 µmol/L, 1.8±0.2 µmol/L, 0.3±0.3 µmol/L and 1.0±0.4 µmol/L respectively. Significant increase was present in the group 2 and significant decrease was present in the group 3 (p=0.001).

Table 1. Arginine, asymmetric dimethylarginine and symmetric dimethylarginine levels in the aqueous humor

	Group 1	Group 2	Group 3	Group 4	P
L-Arginine (µmol/L)	12.3±4.8	12.4±1.4	13.2±2.4	13.7±4.3	0.807
ADMA(µmol/L)	2.6±0.8 ^{b,c}	7.3±2.7 ^{a,c,d}	0.5±0.5 ^{a,b,d}	2.5±1.0 ^{b,c}	0.001
SDMA(µmol/L)	1.0±0.3 ^{b,c}	1.8±0.2 ^{a,c,d}	0.3±0.3 ^{a,b,d}	1.0±0.4 ^{b,c}	0.001

^a Statistically different from Group 1, ^b Statistically different from Group 2, ^c Statistically different from Group 3, ^d Statistically different from Group 4

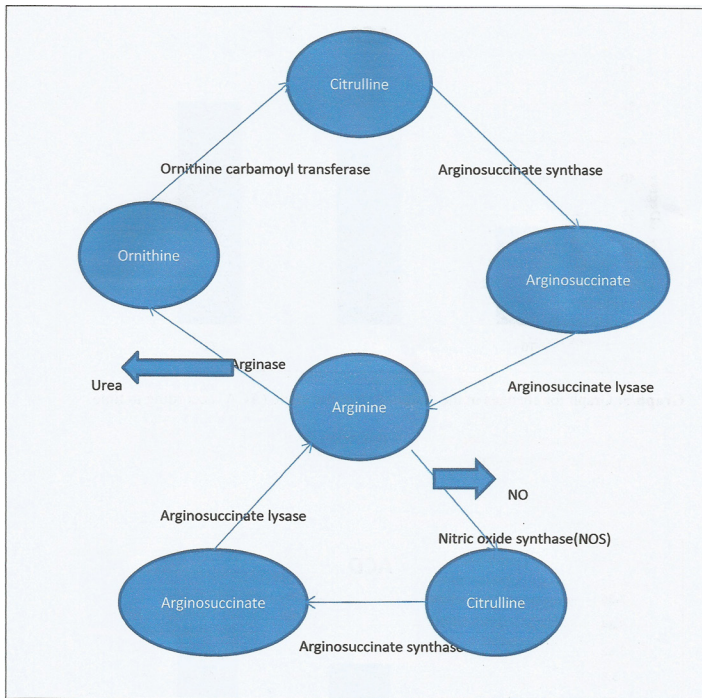


Figure 1. L- Arginine pathways

Discussion

The results of the present study show the importance of L-arginine and its metabolites ADMA and SDMA (both of which inhibit the generation of NO by NOS), in the I/R retinopathy and under the treatments of with molsidomine.

Figure 1 shows the L- Arginine pathways. L-Arginine is converted to urea by arginase or converted to NO with O₂ by NOS. NOS have three isoforms: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). Each isoform is produced by different cell types. Endothelial cells especially produce eNOS. Arginase activity increases in ischemic retinopathy conditions [15]. Arginase compete with NOS for their common substrate, L-arginine. If the arginine needed for NOS activity is insufficient, NOS can become uncoupled [16, 17]. Therefore that is leading to further decrease in NO and increased production of superoxide by NOS [18]. L-arginine activates the arginase and induces expression of arginase [19].

According to our results the L-arginine levels kept constant in the I/R conditions even under MOL treatments. ADMA and SDMA levels were found increase in the I/R conditions. The result of the inhibition of NOS by ADMA is the reduction of NO bioavailability. The inhibition of NOS by ADMA causes to more production of ADMA from arginine. ADMA and SDMA levels were found decrease in MOL treatment group and kept steady in prophylaxis group. Free radicals are produced excessive amounts in tissues in I/R conditions especially at reperfusion period. This causes the formation of oxidative stress in tissues and tissue damage. In the case of I/R injury superoxide (O₂⁻) radical can react with NO to form a peroxynitrite radical (NOO⁻), which is very toxic for tissues and also reduces the amount of useful NO [15]. If NO is increased by MOL, NO can react with O₂⁻ and so O₂⁻ decreases. Therefore, it limits the formation of hydrogen peroxides (H₂O₂) and hydroxyl radicals (•OH) due to a lack of O₂⁻. Besides,

H₂O₂ and peroxynitrite have been shown to activate arginase in endothelial cells [20, 21], and these actions may be prevented by MOL. We speculate that this is a mechanism of the protective effect of MOL in the I/R conditions.

To date, there has been limited information concerning the role of ADMA and its structural isomer, SDMA, in the retinopathies [22]. ADMA concentrations in the aqueous have also been previously reported by other investigators in diabetes patients [23] and in patients with uveitis [24, 25], and in patients with exfoliation syndrome [26]. Such retinopathies are associated with elevated serum ADMA, SDMA, and L-arginine [27]. After the acute event of ischemic stroke, levels of ADMA and SDMA are elevated through augmentation of protein methylation and oxidative stress [28]. ADMA released from the ischemic tissue during the reperfusion period competes with arginine for the substrate-binding site in the active center of NOS [29, 30]. Increased ADMA concentrations have been shown to be related to increased reactive oxygen species production and oxidative stress [26]. On the other hand, oxidative stress has been shown to increase levels of ADMA [31]. Thus, ADMA levels may be used as a marker of oxidative stress [32]. According to our results MOL treatment inhibits production of ADMA and SDMA in I/R retinopathy. This may be due to regular NOS activity by MOL. That can be associated with anti-oxidative property of MOL. Also, the blockage of NOS by these molecules was prevented. Therefore production of NO may increase.

Our study is a preliminary study so that have some limitations. Firstly, conventional oxidative and anti-oxidative parameters were not investigated. Secondly, histopathological investigation wasn't done. Thirdly, some important biochemical investigations like NO levels, L-Citrulline levels, and Arginase activity were not investigated.

As a conclusion L-Arginine levels was kept steady, ADMA and SDMA values decreased with molsidomine therapy in the I/R. Four days treatment with molsidomine after I/R may be beneficial more than prophylaxis and three days treatment. Further studies are required.

Acknowledgments

Preliminary results of this study were presented as a free paper at the 16th Euretina congress (Copenhagen/Denmark-2016).

Competing interests

The authors declare that they have no competing interest.

Financial Disclosure

All authors declare no financial support.

Ethical approval

Consent of ethics was approved by the local ethics committee.

Nihat Polat ORCID: 0000-0002-1735-1363

Murat Atabey Ozer ORCID: 0000-0003-1807-6911

Hakan Parlakpınar ORCID: 0000-0001-9497-3468

Zeynep Aksungur ORCID: 0000-0002-9002-6604

Onural Ozhan ORCID: 0000-0001-9018-7849

Yusuf Turkoz ORCID: 0000-0001-5401-0720

References

- Schmid H, Renner M, Dick HB, et al. Loss of inner retinal neurons after retinal ischemia in rats. Invest Ophthalmol Vis Sci. 2014;55:2777-87.
- Zhang XY, Xiao YQ, Zhang Y, et al. Protective effect of pioglitazone on

- retinal ischemia/reperfusion injury in rats. *Invest Ophthalmol Vis Sci.* 2013;54:3912-21.
3. Osborne NN, Casson RJ, Wood JP, et al. Retinal ischemia: mechanisms of damage and potential therapeutic strategies. *Prog Retin Eye Res.* 2004;23:91-147.
 4. Riazi-Esfahani M, Kiumehr S, Asadi-Amoli F, et al. Effects of intravitreal morphine administered at different time points after reperfusion in a rabbit model of ischemic retinopathy. *Retina.* 2009;29:262-8.
 5. Loscalzo J, Welch G. Nitric oxide and its role in the cardiovascular system. *Prog Cardiovasc Dis.* 1995;38:87-104.
 6. Johnson G, Tsao PS, Lefer AM. Cardioprotective effects of authentic nitric oxide in myocardial ischemia with reperfusion. *Crit Care Med.* 1991;19:244-52.
 7. Kukovetz WR, Holzmann S. Cyclic GMP as the mediator of molsidomine-induced vasodilatation. *Europ J Pharmacol.* 1986;122:103-9.
 8. Polat N, Ozer MA, Parlakpinar H, et al. Effects of molsidomine on retinal ischemia/reperfusion injury in rabbits. *Biotech Histochem.* 2018;93:188-97.
 9. Toda N, Nakanishi-Toda M. Nitric oxide: ocular blood flow, glaucoma, and diabetic retinopathy. *Prog Retin Eye Res.* 2007;26:205-38.
 10. Javadiyan S, Burdon KP, Whiting MJ, et al. Elevation of serum asymmetrical and symmetrical dimethylarginine in patients with advanced glaucoma. *Invest Ophthalmol Vis Sci.* 2012;53:1923-7.
 11. Tran CT, Leiper JM, Vallance P. The DDAH/ADMA/NOS pathway. *Atheroscler Suppl.* 2003;44:33-40.
 12. Zinellu A, Pinna A, Sotgia S, et al. Increased plasma asymmetric dimethylarginine (ADMA) levels in retinal venous occlusive disease. *Clin Chem Lab Med.* 2008;46:387-92.
 13. Colak C, Parlakpinar H. Hayvan Deneyleri: In vivo Denemelerin Bildirimi: ARRIVE Kılavuzu-Derleme. *J Turgut Ozal Med Cent.* 2012;19:128-31.
 14. Bentli R, Parlakpinar H, Polat A, et al. Molsidomine Prevents Cisplatin-induced Hepatotoxicity. *Arch Med Res.* 2013;4:521-8.
 15. Narayanan SP, Rojas M, Suwanpradid J, et al. Arginase in retinopathy. *Prog Retin Eye Res.* 2013;36:260-80.
 16. Romero MJ, Platt DH, Tawfik HE, et al. Diabetes-induced coronary vascular dysfunction involves increased arginase activity. *Circ Res.* 2008;102:95-102.
 17. White AR, Ryoo S, Li D, et al. Knockdown of arginase I restores NO signaling in the vasculature of old rats. *Hypertension.* 2006;47:245-51.
 18. Kaesemeyer WH, Ogonowski AA, Jin L, et al. Endothelial nitric oxide synthase is a site of superoxide synthesis in endothelial cells treated with glyceryl trinitrate. *Br J Pharmacol.* 2000;131:1019-23.
 19. Morris SM Jr. Regulation of enzymes of urea and arginine synthesis. *Annu Rev Nutr.* 1992;12:81-101.
 20. Thengchaisri N, Hein TW, Wang W, et al. Upregulation of arginase by H₂O₂ impairs endothelium-dependent nitric oxide-mediated dilation of coronary arterioles. *Arterioscler Thromb Vasc Biol.* 2006;26:2035-42.
 21. El-Remessy AB, Tawfik HE, Matragoon S, et al. Peroxynitrite mediates diabetes-induced endothelial dysfunction: possible role of Rho kinase activation. *Exp Diabetes Res.* 2010;2010:247861.
 22. Chen YH, Xu X, Sheng MJ, et al. Effects of asymmetric dimethylarginine on bovine retinal capillary endothelial cell proliferation, reactive oxygen species production, permeability, intercellular adhesion molecule-1, and occludin expression. *Mol Vis.* 2011;17:332-40.
 23. Sugai M, Ohta A, Ogata Y, et al. Asymmetric dimethylarginine (ADMA) in the aqueous humour of diabetic patients. *Endocr J.* 2007;54:303-9.
 24. Yoshida Y, Yamagishi S, Ueda S, et al. Aqueous humor levels of asymmetric dimethylarginine (ADMA) are correlated with pigment epithelium-derived factor (PEDF) in patients with uveitis. *J Int Med Res.* 2007;35:892-5.
 25. Yoshida Y, Yamagishi S, Ueda S, et al. Aqueous humor levels of asymmetric dimethylarginine (ADMA) are positively associated with monocyte chemoattractant protein-1 (MCP-1) in patients with uveitis. *Br J Ophthalmol.* 2008;92:93-4.
 26. Tosun M, Erdurmus M, Bugdayci G, et al. Aqueous humor and serum concentration of asymmetric dimethyl arginine in pseudoexfoliation syndrome. *Br J Ophthalmol.* 2012;96:1137-40.
 27. Abhary S, Kasmeridis N, Burdon KP, et al. Diabetic retinopathy is associated with elevated serum asymmetric and symmetric dimethylarginines. *Diabetes Care.* 2009;32:2084-6.
 28. Chen S, Li N, Deb-Chatterji M, et al. Asymmetric dimethylarginine as marker and mediator in ischemic stroke. *Int J Mol Sci.* 2012;13:15983-6004.
 29. Martín-Sanz P, Olmedilla L, Dulin E, et al. Presence of methylated arginine derivatives in orthotopic human liver transplantation: relevance for liver function. *LiverTranspl.* 2003;9:40-8.
 30. Vallance P, Leiper J. Cardiovascular biology of the asymmetric dimethyl arginine: dimethylarginine dimethyl amino hydrolase pathway. *Arterioscler Thromb Vasc Biol.* 2004;24:1023-30.
 31. Sydow K, Munzel T. ADMA and oxidative stress. *Atheroscler Suppl.* 2003;4:41-51.
 32. Yonem A, Duran C, Unal M, et al. Plasma apelin and asymmetric dimethylarginine levels in type 2 diabetic patients with diabetic retinopathy. *Diabetes Res Clin Pract.* 2009;84:219-23.