Biochemical and Biophysical Research Communications 435 (2013) 428–433

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Arginase inhibition reduces interleukin-1β-stimulated vascular smooth muscle cell proliferation by increasing nitric oxide synthase-dependent nitric oxide production

Jeongyeon Yoon, Sungwoo Ryoo *

Department of Biology, College of Natural Sciences, Kangwon National University, Chuncheon, Republic of Korea

ARTICLE INFO

Article history:
Received 26 April 2013
Available online 9 May 2013

Keywords:
Arginase
Interleukin-1β
Inducible nitric oxide synthase
Vascular smooth muscle cells
Proliferation

ABSTRACT

We investigated whether arginase inhibition suppressed interleukin (IL)-1β-stimulated proliferation in vascular smooth muscle cells (VSMCs) and the possible mechanisms involved. IL-1β stimulation increased VSMC proliferation, while the arginase inhibitor BEC and transfection of the antisense (AS) oligonucleotide against arginase I decreased VSMC proliferation and was associated with increased protein content of the cell cycle regulator p21Waf1/Cip1. IL-1β incubation induced inducible nitric oxide synthase (iNOS) mRNA expression and protein levels in a dose-dependent manner, but did not affect arginase I and II expression. Consistent with this data, IL-1β stimulation resulted in increase in NO production that was significantly augmented by arginase inhibition. The specific iNOS inhibitor 1400W abolished IL-1β-mediated NO production and further accentuated IL-1β-stimulated cell proliferation. Incubation with NO donors GSNO and DETA/NO in the presence of IL-1β abolished VSMCs proliferation and increased p21Waf1/Cip1 protein content. Furthermore, incubation with the cGMP analogue 8-Br-cGMP prevented IL-1β-induced VSMCs proliferation. In conclusion, arginase inhibition augmented iNOS-dependent NO production that resulted in suppression of IL-1β-induced VSMCs proliferation in a cGMP-dependent manner.

1. Introduction

Arginases play a critical role in cardiovascular homeostasis by reciprocally regulating nitric oxide (NO) [1] production through the competitive binding of L-arginine to nitric oxide synthase (NOS) [2]. NO inhibits cell proliferation through cGMP-dependent and -independent mechanisms [3–6]. An increase in arginase activity decreases NO production, causing vascular smooth muscle cell (VSMC) proliferation that can lead to vascular pathologies, restenosis after angioplasty, and atherosclerosis. Studies have shown that NO donor agents and NOS gene transfers result in the inhibition of neointimal formation after balloon angioplasty [7–9], whereas adding NOS enhanced intimal formation [9]. Increased arginase activity also contributes to VSMC proliferation by increasing polyamine production [10], and it attenuates inflammatory cytokine secretion in lipopolysaccharide-stimulated VSMCs by reducing the inducible nitric oxide synthase (iNOS) function and inhibiting nuclear factor-kappa B (NF-κB) activation [11]. Conversely, systematic inhibition of arginase activity using a chemical inhibitor reduced the wall thickness in an atherosclerotic mice model [12].

VSMCs are the primary cell type responsible for maintaining vascular integrity. In the healthy vessel, VSMC proliferation is tightly controlled by humoral mediators, cell cycle regulators, and cytokines [13,14]. Interleukin (IL)-1β is a particularly important biologically active mediators synthesized and secreted by VSMCs. IL-1β uses both paracrine and autocrine mechanisms to promote VSMC proliferation and inflammation, which may contribute to intimal hyperplasia, vascular stiffness, and lesion progression in restenosis and atherosclerosis [15]. This indicates preventing VSMCs proliferation is an important therapeutic approach for the vascular disorders. Therefore, the aim of the present study was to determine if arginase inhibition suppresses IL-1β-stimulated VSMCs proliferation and whether the mechanism is cGMP-dependent.

Abbreviations: IL-1β, interleukin-1β; VSMC, vascular smooth muscle cells; iNOS, inducible nitric oxide synthase; cGMP, cyclic guanosine monophosphate; AS, antisense; RT-PCR, reverse transcription polymerase chain reaction; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; DMEM, Dulbecco's modified Eagle medium; RASMC, rat aortic smooth muscle cell; GSNO, S-nitrosoglutathione; DETA NONOate, diethylenetriamine NONOate; 1400W, N-(3-aminomethyl)benzylacetamidine, 2HCl ; BEC, (S)-(2-(boronoethyl)-L-cysteine, HCl ; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one); 8-Br-cGMP, 8-bromoguanosine 3′, 5′-cyclic monophosphate.

* Corresponding author. Address: Department of Biology, Kangwon National University, Kangwondae-gil 1, Chuncheon, Kangwon-do 200-701, Republic of Korea. Fax: +82 033 251 3990.

E-mail address: ryoosw08@kangwon.ac.kr (S. Ryoo).

0006-291X/$ - see front matter Crown Copyright © 2013 Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.05.002

2. Materials and methods

2.1. Materials

GSNO (S-nitrosoglutathione), DETA NONOate (diethylenetriamine NONOate), 1400W (N-(3-aminomethyl)benzylacetamidine, 2HCl), BEC [(S)-(2-(boronoethyl)-L-cysteine,HCl), ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one)] and 8-Br-cGMP (8-bromoguanosine 3′,5′-cyclic monophosphate) were purchased from Calbiochem. Co. (La Jolla, CA). Polymerase chain reaction (PCR) primers and PCR premix were purchased from Bioneer (Daejeon, Korea). Anti-serum against p21Waf1/Cip1, arginase I and arginase II were from Santa Cruz Biotechnology. β-Tubulin and iNOS antiserum was from BD Biosciences (San Diego, CA). All reagents were purchased from Sigma unless otherwise stated.

2.2. Isolation of rat aortic smooth muscle cells (RASMCs)

RASMCs were isolated from the thoracic and upper parts of the abdominal aorta in 10- to 12-week-old male Sprague–Dawley rats using a modification of a technique previously described [16]. Briefly, the stripped aorta was prepared from the anesthetized rat, cut into 2-mm pieces, treated with type-II collagenase (1 mg/ml, Gibco) for 1 h to remove the endothelial cells, and washed with culture medium [Dulbecco's modified Eagle medium (DMEM) plus 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml)]. The de-endothelialized aortic pieces were incubated with culture medium on gelatin (0.1%)-coated culture dishes for approximately 10 days. RASMCs were identified by their 'spindle-shaped' pattern and further confirmed by double staining using platelet endothelial cell adhesion molecule-1, a specific marker for endothelial cells, and α-smooth muscle actin, a specific marker for SMC. All the cells were stained with anti α-smooth muscle actin antibodies. The RASMCs were routinely used between the fourth and seventh passage. For all experiments, confluent cells were maintained with medium (DMEM plus 0.1% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml) for 24 h.

2.3. NO assay

Intracellular NO production was evaluated by measuring nitrite levels (Calbiochem Co.) after incubation of cells with BEC or 1400W [17].

2.4. Cell proliferation assay

Cells (1×10^4 cells/well) were incubated in 96-well plates, starved, and stimulated with IL-1β in the presence or absence of inhibitor for 24 h. Cell proliferation was measured using WST-1 reagent (Roche) according to the supplier's protocol.

2.5. RT-PCR analysis

Total RNAs were isolated with Trizol reagent according to the supplier's protocol (Gibco), and cDNA was synthesized using AMV reverse transcriptase (Invitrogen). PCR amplification was performed with specific primer sets for rat *arginase I* forward 5′-GTA GCA GAG ACC CAG AAG AAT G-3′, reverse 5′–GGT TGT CAG CGG AGT GTT G-3′(product, 156 bp); *arginase II* forward 5′-CCA CCT GAG CTT TGA CAT AGA TGC-3′, reverse 5′-CTA CAC TTG TGA GAG TGC ACA-3′(product, 460 bp); *iNOS* forward 5′-TGT GCG GAG TGT CAG TGG CTT CC-3′, reverse 5′-TCA CTG TCA TTT TAT TTA GGG CCA-3′(product, 500 bp). Primer set for β-actin (Roche) was amplified as a control.

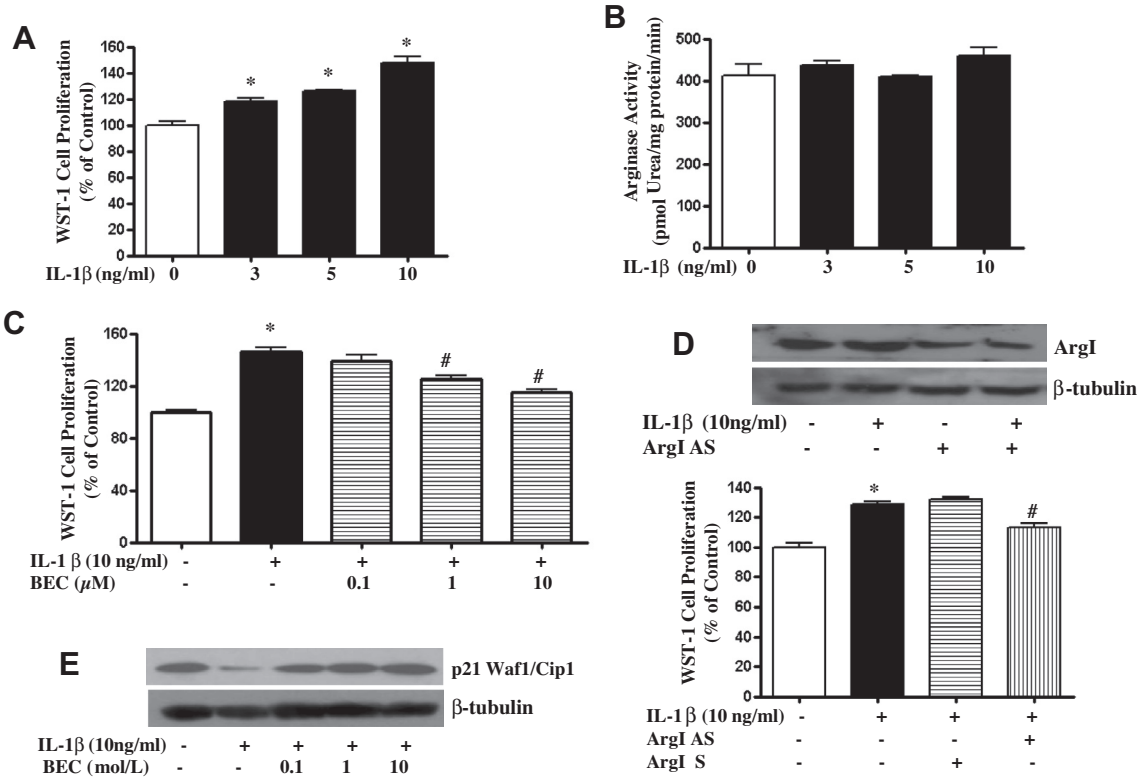

Fig. 1. Effect of arginase inhibition on IL-1β-dependent VSMCs proliferation. (A) VSMC cell proliferation and (B) arginase activity in response to 24 h incubation with IL-1β. (C) Cell proliferation of VSMCs pretreated with the arginase inhibitor BEC for 30 min before IL-1β incubation (10 ng/ml). Knockdown of arginase I (D, upper) and suppression of IL-1β-induced proliferation (D, lower) in response to transfection of phosphorothioated antisense (AS, 100 nM) and sense (S, 100 nM) oligonucleotides against arginase I (E). The p21Waf1/Cip1 protein content in response to IL-1β and BEC. $^*p < 0.05$ compared to control, $^\#p < 0.05$ compared to IL-1β treated.

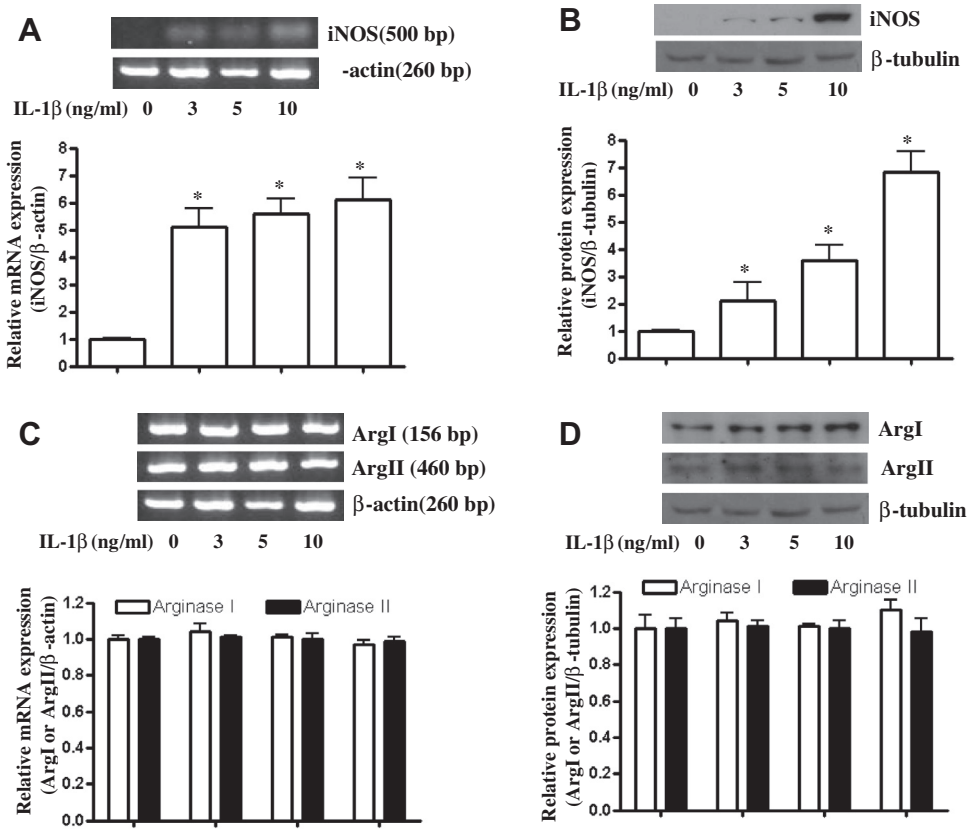

Fig. 2. IL-1β stimulation induced iNOS but not arginase expression. The iNOS mRNA expression (A) and protein content (B), and arginase I and II mRNA expression (C) and protein content (D) after 24 h IL-1β incubation. $^{*}p < 0.01$ compared to control.

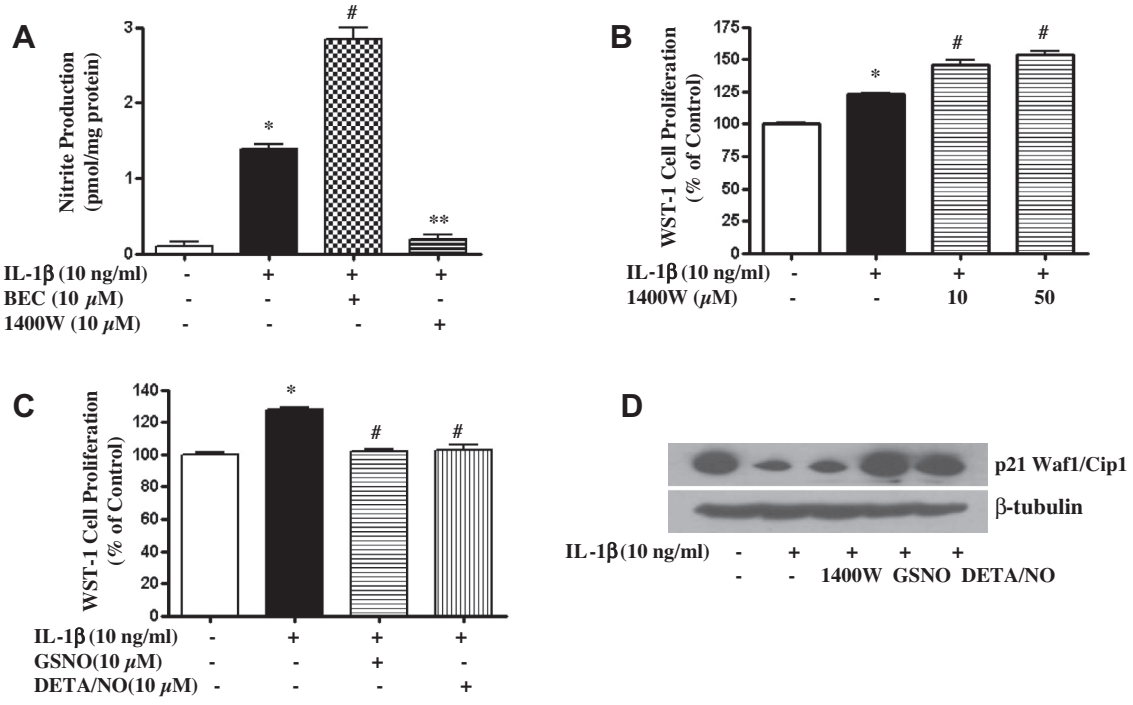

Fig. 3. Arginase inhibition enhanced iNOS-dependent NO signaling, resulting in reduced VSMCs proliferation. (A) Intracellular nitrite generation after 24 h IL-1β incubation with or without BEC preincubation ($^{*}p < 0.01$ compared to control, $^{\#}p < 0.01$ compared to IL-1β). (B) Cell proliferation in response to 1 h preincubation with the iNOS inhibitor 1400 W, followed by 24 h IL-1β incubation ($^{**}p < 0.01$ compared to IL-1β, $^{*}p < 0.01$ compared to control, $^{\#}p < 0.01$, compared to IL-1β). (C) VSMC cell proliferation in response to no stimulation, IL-1β only, IL-1β and GSNO, and IL-1β and DETA/NO, and ($^{*}p < 0.01$, compared to control, $^{\#}p < 0.01$, compared to IL-1β). (D) Protein levels of p21Waf1/Cip1 in response to incubation of IL-1β-stimulated cells with 1400 W (10 μM), GSNO (10 μM) and DETA/NO (10 μM).

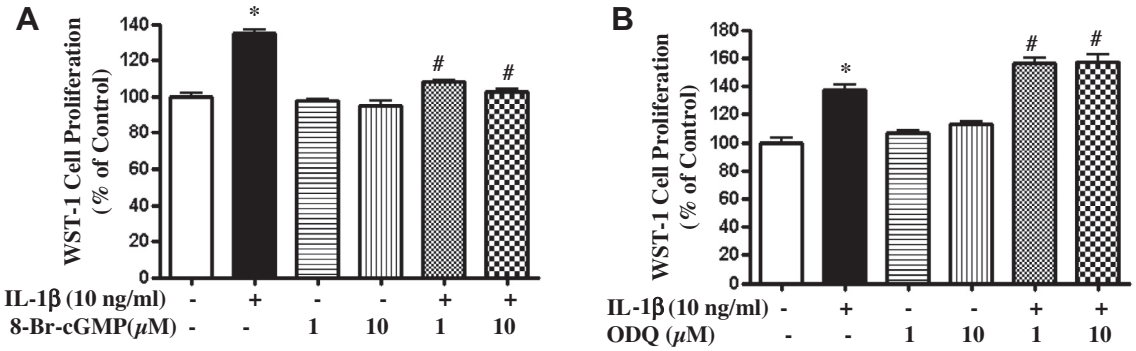

Fig. 4. Soluble guanylyl cyclase activity in response to IL-1β-stimulated cell proliferation. Cell proliferation in response to 8-Br-cGMP (A), and ODQ (B), in the presence or absence of IL-1β stimulation ($^{*}p < 0.01$, compared to control, $^{\#}p < 0.01$, compared to IL-1β).

2.6. Transfection of rat arginase I antisense (AS)

VSMCs were transfected using antisense (AS) and sense (S) oligonucleotides [18] against rat arginase I using Oligofectamine reagent (Invitrogen) following supplier's protocol. In brief, 8 μl of oligofectamine was added to 17 μl Opti-MEM I reduced serum media (Gibco), incubated for 5 min at room temperature, mixed with 180 μl Opti-MEM medium containing oligonucleotide (6 μl of a 10 μM stock oligonucleotide), and further incubated for 15 min. The oligonucleotide-oligofectamine complex was then overlaid on cells (each well, 1 ml serum-free media, in a 6 well culture dish). After incubation for 6 h, the serum concentration in growth medium was returned to a 1× serum concentration by adding 3× media of 0.5 ml per well. Transfected cells were then recovered for 36 h and then serum-starved for 24 h prior to stimulation with IL-1β for 24 h.

2.7. Western blot analysis

Cell lysates prepared in sodium dodecyl sulfate-sample buffer were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes (Bio-Rad), and analyzed with antibodies according to the supplier's protocol, and visualized using chemiluminescence (ECL kit).

2.8. Arginase activity measurement

Arginase activity was measured by quantifying urea amount generated from L-arginine using previously described methods [17].

2.9. Statistics

All data are presented as the mean ± standard deviation of at least three independent experiments. An unpaired Student's *t*-test was used to determine significance. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Arginase inhibition suppressed proliferation in IL-1β-stimulated vascular smoothmuscle cells and was associated with increased p21Waf1/Cip1 protein content

To test whether IL-1β could induce the proliferation of VSMCs, serum-starved cells were incubated with different amount of IL-1β for 24 h. Stimulation of VSMCs with IL-1β resulted in a significant increase in cell proliferation (Fig. 1A), although arginase activity did not change (Fig. 1B). Pretreating cells with BEC, a specific inhibitor of arginase isoforms, significantly suppressed IL-1β-induced proliferation in a dose-dependent manner (Fig. 1C).

To downregulate arginase expression, VSMCs were transfected with an AS sequence against arginase I because arginase I is an abundant isoform in RASMCs [19]. Transfection with the AS sequence resulted in a significant decrease in cell proliferation compared to IL-1β-stimulated cells (Fig. 1D). The effect of arginase inhibition on VSMCs proliferation was further tested by measuring the protein level of p21Waf1/Cip1, which regulates progression through the G1-S transition checkpoint in cell division [1]. As shown in Fig. 1E, IL-1β induced a significant decrease in the protein level of p21Waf1/Cip1, whereas incubation with the arginase inhibitor increased p21Waf1/Cip1 protein content level.

3.2. IL-1β stimulation induced iNOS, but not arginase expression

We next examined whether IL-1β stimulation increases iNOS, arginase I, and arginase II expression in VSMCs. IL-1β increased iNOS mRNA expression (Fig. 2A) and protein content (Fig. 2B) in a dose-dependent manner. The iNOS expression was undetectable in cells not stimulated with IL-1β. Neither arginase isoform showed changes in mRNA (Fig. 2C) or protein (Fig. 2D) levels in response to IL-1β incubation. As indicated in Fig. 2D, arginase I was the dominant isoform in VSMCs.

3.3. Arginase inhibition augmented NO signaling pathway that resulted in inhibition of VSMC proliferation

With our results showing IL-1β induced iNOS expression and previous reports demonstrating arginase isoforms reciprocally regulated NO production in endothelial cells [12,17], we measured NO production in VSMCs. IL-1β incubation significantly increased NO production, which was further augmented in the presence of BEC (Fig. 3A). However, inhibition of iNOS activity by 1400W abolished NO production (Fig. 3A). We tested whether iNOS-dependent NO production influences IL-1β-induced proliferation. Inhibition of iNOS activity with 1400W further increased IL-1β-stimulated cell proliferation (Fig. 3B). Preincubating the cells with the NO donors GSNO and DETA/NO completely abolished IL-1β-dependent VSMC proliferation (Fig. 3C). Protein content of p21Waf1/Cip1 decreased in response to 1400W incubation and increased in the presence of NO donors (Fig. 3D).

3.4. The cGMP analogue inhibited IL-1β-induced proliferation

We next wished to investigate whether NO-dependent inhibition of cell proliferation acts through a cGMP-dependent mechanism. Incubation with the cGMP analogue 8-Br-cGMP suppressed

IL-1β-induced VSMCs proliferation (Fig. 4A), whereas the soluble guanylyl cyclase inhibitor ODQ had no effect on VSMC proliferation (Fig. 4B), suggesting enhanced NO production by arginase inhibition prevented IL-1β-dependent VSMC proliferation in a cGMP-dependent manner.

4. Discussion

In the present study, we demonstrate that arginase inhibition increases iNOS-dependent NO production in a cGMP-dependent manner, reducing IL-1β-induced proliferation of VSMC. The arginase inhibition was also associated with increased content of p21Waf1/Cip1, indicating that cell cycle regulation was involved in the reduced proliferation of VSMCs. Our findings suggest that inhibition of arginase activity may have beneficial effects on prevention of VSMCs proliferation in pathophysiological conditions such as restenosis and atherosclerosis.

Each of the two arginase isoforms – the hepatic isoform arginase I, and the extrahepatic isoform arginase II – are encoded by a distinct gene [20,21]. Although VSMCs constitutively expressed arginases at low levels, their expression was further induced by IL-4 and IL-13 [21], transforming growth factor-β [2], lysophosphatidylcholine [22], and mechanical strain [23]. High arginase and ornithine carboxylase activities also increased polyamine synthesis, which also contributes to cell proliferation [16]. Together with cGMP-independent mechanisms, our results suggest that arginase inhibition suppress IL-1β-induced VSMC proliferation in a cGMP-dependent manner.

Although the etiology of atherosclerosis is multifactorial, inflammatory cytokines such as IL-1β [24], IL-4 [25], and IL-8 [12,26] likely play a role because of their involvement in inflammation and proliferation. Cytokines have growth-promoting and chemotactic effects in VSMCs, and they increase the expression of leukocytes adhesion molecules on VSMCs and endothelial cells. Therefore, reducing the effects of the cytokines effects may help prevent vascular diseases such as atherosclerosis and restenosis.

As shown in Fig. 2, IL-1β stimulation induced iNOS expression, but did not influence the expression of either arginase isoform. These results are consistent with previous findings that interferon-γ, another inflammatory cytokine, induces iNOS but does not affect arginase mRNA expression [27]. The cGMP analogue 8-Br-cGMP induced both arginases but not iNOS in murine macrophage cells [27]. IL-1β activated transcription factor NF-κB, resulting in iNOS induction [28]; however, the transcription factor activating protein-1 was responsible for the induction of arginase I expression in rat aortic endothelial cells [29].

NO is produced from L-arginine by endothelial NOS (eNOS), neuronal NOS (nNOS), and iNOS. It has been demonstrated that iNOS is present in atherosclerotic legions [30,31]. Although the physiological significance of iNOS expression in atherosclerotic vessels is not completely clear, it may produce NO to support eNOS function in VSMCs. Expression of iNOS plays antiatherogenic and vasculoprotective roles by exerting vasorelaxation and inhibiting leukocyte adhesion, platelet aggregation, low-density lipoprotein oxidation, VSMC proliferation and migration [32,33]. However, other studies demonstrate opposite effects of iNOS [34,35], which may be due to the different concentration and exposure times of NO in the respective studies. In one study, arginase I activity increases through its interaction with iNOS protein and the subsequent modification of S-nitrosylation in aged rat model [36]. Furthermore, the increased arginase activity itself can induce VSMCs proliferation as suggested by previous report [10]. Therefore, our results also suggest that alternative means of modulating iNOS, such as arginase inhibition, should be useful for the treatment of cardiovascular diseases.

Acknowledgments

This work was supported by the Basic Science Research Program of the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2012-0006812) and by supported by 2010 Research Grant from Kangwon National University.

References

[1] Y. Xiong, G.J. Hannon, H. Zhang, D. Casso, R. Kobayashi, D. Beach, P21 is a universal inhibitor of cyclin kinases, Nature 366 (1993) 701–704.
[2] W. Durante, L. Liao, S.V. Reyna, K.J. Peyton, A.I. Schafer, Transforming growth factor-beta(1) stimulates L-arginine transport and metabolism in vascular smooth muscle cells: role in polyamine and collagen synthesis, Circulation 103 (2001) 1121–1127.
[3] F. Blachier, V. Robert, M. Selamnia, C. Mayeur, P.H. Duee, Sodium nitroprusside inhibits proliferation and putrescine synthesis in human colon carcinoma cells, FEBS Lett. 396 (1996) 315–318.
[4] G.M. Buga, L.H. Wei, P.M. Bauer, J.M. Fukuto, L.J. Ignarro, NG-hydroxy-L-arginine and nitric oxide inhibit Caco-2 tumor cell proliferation by distinct mechanisms, Am. J. Physiol. 275 (1998) R1256–64.
[5] U.C. Garg, A. Hassid, Inhibition of rat mesangial cell mitogenesis by nitric oxide-generating vasodilators, Am. J. Physiol. 257 (1989) F60–6.
[6] U.C. Garg, A. Hassid, Nitric oxide-generating vasodilators inhibit mitogenesis and proliferation of BALB/C 3T3 fibroblasts by a cyclic GMP-independent mechanism, Biochem. Biophys. Res. Commun. 171 (1990) 474–479.
[7] P.H. Groves, A.P. Banning, W.J. Penny, A.C. Newby, H.A. Cheadle, M.J. Lewis, The effects of exogenous nitric oxide on smooth muscle cell proliferation following porcine carotid angioplasty, Cardiovasc. Res. 30 (1995) 87–96.
[8] M. Laitinen, S. Yla-Herttuala, Vascular gene transfer for the treatment of restenosis and atherosclerosis, Curr. Opin. Lipidol. 9 (1998) 465–469.
[9] G.R. De Meyer, H. Bult, L. Ustunes, M. Kockx, F.H. Jordaens, L.L. Zonnekeyn, A.G. Herman, Vasoconstrictor responses after neo-intima formation and endothelial removal in the rabbit carotid artery, Br. J. Pharmacol. 112 (1994) 471–476.
[10] L.H. Wei, G. Wu, S.M. Morris Jr., L.J. Ignarro, Elevated arginase I expression in rat aortic smooth muscle cells increases cell proliferation, Proc. Natl. Acad. Sci.USA 98 (2001) 9260–9264.
[11] X.P. Wang, Y.G. Chen, W.D. Qin, W. Zhang, S.J. Wei, J. Wang, F.Q. Liu, L. Gong, F.S. An, Y. Zhang, Z.Y. Chen, M.X. Zhang, Arginase I attenuates inflammatory cytokine secretion induced by lipopolysaccharide in vascular smooth muscle cells, Arterioscler Thromb Vasc. Biol. 31 (2011) 1853–1860.
[12] S. Ryoo, G. Gupta, A. Benjo, H.K. Lim, A. Camara, G. Sikka, J. Sohi, L. Santhanam, K. Soucy, E. Tuday, E. Baraban, M. Ilies, G. Gerstenblith, D. Nyhan, A. Shoukas, D.W. Christianson, N.J. Alp, H.C. Champion, D. Huso, D.E. Berkowitz, Endothelial arginase II: a novel target for the treatment of atherosclerosis, Circ. Res. 102 (2008) 923–932.
[13] A.C. Newby, Matrix metalloproteinases regulate migration, proliferation, and death of vascular smooth muscle cells by degrading matrix and non-matrix substrates, Cardiovasc. Res. 69 (2006) 614–624.
[14] K. Rakesh, D.K. Agrawal, Cytokines and growth factors involved in apoptosis and proliferation of vascular smooth muscle cells, Int. Immunopharmacol. 5 (2005) 1487–1506.
[15] C.A. Dinarello, Biologic basis for interleukin-1 in disease, Blood 87 (1996) 2095–2147.
[16] Y. Wang, K.A. Lindstedt, P.T. Kovanen, Mast cell granule remnants carry LDL into smooth muscle cells of the synthetic phenotype and induce their conversion into foam cells, Arterioscler. Thromb. Vasc. Biol. 15 (1995) 801–810.
[17] S. Ryoo, C.A. Lemmon, K.G. Soucy, G. Gupta, A.R. White, D. Nyhan, A. Shoukas, L.H. Romer, D.E. Berkowitz, Oxidized low-density lipoprotein-dependent endothelial arginase II activation contributes to impaired nitric oxide signaling, Circ. Res. 99 (2006) 951–960.
[18] A.R. White, S. Ryoo, D. Li, H.C. Champion, J. Steppan, D. Wang, D. Nyhan, A.A. Shoukas, J.M. Hare, D.E. Berkowitz, Knockdown of arginase I restores NO signaling in the vasculature of old rats, Hypertension 47 (2006) 245–251.
[19] K.J. Peyton, D. Ensenat, M.A. Azam, A.N. Keswani, S. Kannan, X.M. Liu, H. Wang, D.A. Tulis, W. Durante, Arginase promotes neointima formation in rat injured carotidarteries, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 488–494.
[20] J. Steppan, S. Ryoo, K.H. Schuleri, C. Gregg, R.K. Hasan, A.R. White, L.J. Bugaj, M. Khan, L. Santhanam, D. Nyhan, A.A. Shoukas, J.M. Hare, D.E. Berkowitz, Arginase modulates myocardial contractility by a nitric oxide synthase 1-dependent mechanism, Proc. Natl. Acad. Sci. USA 103 (2006) 4759–4764.
[21] L.H. Wei, A.T. Jacobs, S.M. Morris Jr., L.J. Ignarro, IL-4 and IL-13 upregulate arginase I expression by cAMP and JAK/STAT6 pathways in vascular smooth muscle cells, Am. J. Physiol. Cell Physiol. 279 (2000) C248–56.
[22] W. Durante, L. Liao, K.J. Peyton, A.I. Schafer, Lysophosphatidylcholine regulates cationic amino acid transport and metabolism in vascular smooth muscle cells. Role in polyamine biosynthesis, J. Biol. Chem. 272 (1997) 30154–30159.
[23] W. Durante, L. Liao, S.V. Reyna, K.J. Peyton, A.I. Schafer, Physiological cyclic stretch directs L-arginine transport and metabolism to collagen synthesis in vascular smooth muscle, Faseb J. 14 (2000) 1775–1783.

[24] A. Qamar, D.J. Rader, Effect of interleukin 1beta inhibition in cardiovascular disease. Curr. Opin. Lipidol. 23 548–553.

[25] Y.W. Lee, A.A. Hirani, Role of interleukin-4 in atherosclerosis, Arch. Pharm. Res. 29 (2006) 1–15.

[26] H.K. Lim, S. Ryoo, Native low-density lipoprotein-dependent interleukin-8 production through pertussis toxin-sensitive G-protein coupled receptors and hydrogen peroxide generation contributes to migration of human aortic smooth muscle cells. Yonsei Med J 52 413–419.

[27] S.M. Morris Jr., D. Kepka-Lenhart, L.C. Chen, Differential regulation of arginases and inducible nitric oxide synthase in murine macrophage cells, Am. J. Physiol. 275 (1998) E740–7.

[28] B. Jiang, S. Xu, P. Brecher, R.A. Cohen, Growth factors enhance interleukin-1 beta-induced persistent activation of nuclear factor-kappa B in rat vascular smooth muscle cells, Arterioscler. Thromb. Vasc. Biol. 22 (2002) 1811–1816.

[29] W. Zhu, U.M. Chandrasekharan, S. Bandyopadhyay, S.M. Morris, Jr., P.E. DiCorleto, V.S. Kashyap, Thrombin induces endothelial arginase through AP-1 activation. Am. J. Physiol. Cell Physiol. 298 C952–C960.

[30] G.A. Joly, V.B. Schini, P.M. Vanhoutte, Balloon injury and interleukin-1 beta induce nitric oxide synthase activity in rat carotid arteries, Circ. Res. 71 (1992) 331–338.

[31] J.S. Luoma, S. Yla-Herttuala, Expression of inducible nitric oxide synthase inmacrophages and smooth muscle cells in various types of human atherosclerotic lesions, Virchows Arch. 434 (1999) 561–568.

[32] K. Egashira, A. Takeshita, Nitric oxide activity at the site of coronary spasm: deficient or preserved?, Circulation 96 (1997) 1048–1050

[33] Y. Fukumoto, H. Shimokawa, T. Kozai, T. Kadokami, K. Kuwata, Y. Yonemitsu, T. Kuga, K. Egashira, K. Sueishi, A. Takeshita, Vasculoprotective role of inducible nitric oxide synthase at inflammatory coronary lesions induced by chronic treatment with interleukin-1beta in pigs in vivo, Circulation 96 (1997) 3104–3111.

[34] L.D. Buttery, D.R. Springall, A.H. Chester, T.J. Evans, E.N. Standfield, D.V. Parums, M.H. Yacoub, J.M. Polak, Inducible nitric oxide synthase is present within human atherosclerotic lesions and promotes the formation and activity of peroxynitrite, Lab. Invest. 75 (1996) 77–85.

[35] K.Y. Chyu, P. Dimayuga, J. Zhu, J. Nilsson, S. Kaul, P.K. Shah, B. Cercek, Decreased neointimal thickening after arterial wall injury in inducible nitric oxide synthase knockout mice, Circ. Res. 85 (1999) 1192–1198.

[36] L. Santhanam, H.K. Lim, V. Miriel, T. Brown, M. Patel, S. Balanson, S. Ryoo, M. Anderson, K. Irani, F. Khanday, L. Di Costanzo, D. Nyhan, J.M. Hare, D.W. Christianson, R. Rivers, A. Shoukas, D.E. Berkowitz, Inducible NO synthase dependent S-nitrosylation and activation of arginase1 contribute to age-related endothelial dysfunction, Circ. Res. 101 (2007) 692–702.