Actin cytoskeleton modulates ADMA-induced NF-kappaB nuclear translocation and ICAM-1 expression in endothelial cells

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Guo Wei-Kang[ABCDEFG]*, **Zhang Dong-Liang**[ABCDEFG]*, **Wang Xin-Xin**[B], **Kong Wei**[B], **Zhang Qi-Dong**[B], **Zhang Yu**[B], **Liu Wen-Hu**[AG]

* Wei-Kang Guo and Dong-Liang Zhang contributed equally to this work

Department of Nephrology, Affiliated Beijing Friendship Hospital, Faculty of Kidney Diseases, Capital Medical University, Beijing, Peoples Republic of China

Summary

Background: Asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase (NOS) inhibitor, increases the activity of NF-κB (NF-κB) and then induces the expression of intercellular adhesion molecule-1 (ICAM-1). However, the mechanisms regulating ADMA-induced NF-κB activation are unknown. This study investigated the function of actin cytoskeleton for ADMA-induced NF-κB activation and ICAM-1 expression in endothelial cells.

Material/Methods: Human umbilical vein endothelial cells (HUVEC) were cultured and left untreated or challenged for 24h with 100 μM ADMA in the absence and presence of 5 μM cytochalasin D (Cyt D), or 1 μM Jasplakinolide (Jas). The form of actin cytoskeleton, the translocation of NF-κB, NF-κB DNA binding activity, and the expression of ICAM-1 were determined.

Results: ADMA increased the formation of stress fiber in endothelial cells, and Cyt D clearly induced destabilization of the actin filaments. Either stabilizing or destabilizing the actin cytoskeleton prevented ADMA-induced NF-κB activation. It also showed that the inhibition of NF-κB activity was due to the impaired NF-κB nuclear translocation. Further, stabilizing or destabilizing the actin cytoskeleton inhibited the expression of the NF-κB target protein, ICAM-1.

Conclusions: Actin cytoskeleton may be engaged in modulated ADMA-induced NF-κB activation and thereby ICAM-1 expression in endothelial cells.

key words: ADMA • NF-κB • ICAM-1 • endothelial cell • actin cytoskeleton

Author's address: Wen-Hu Liu, Department of Nephrology, Affiliated Beijing Friendship Hospital, Faculty of Kidney Diseases, Capital Medical University, Beijing 100050, Peoples Republic of China, e-mail: liuwh2002@yahoo.cn

BACKGROUND

Asymmetric dimethylarginine (ADMA) has been described as a uremic toxin in chronic kidney disease (CKD) patients and a critical factor of cardiovascular diseases (CVD) [1,2]. On the basis of many experimental and clinical studies, it is postulated that ADMA is not only a marker but also a potent mediator of endothelial dysfunction, CVD and mortality of CKD [3–11]. However, the precise molecular mechanism underlying ADMA-induced impaired endothelial functions is not fully understood.

Recent studies demonstrated that NF-κB may play a critical role in ADMA-induced endothelial dysfunction through inflammatory cytokines such as ICAM-1 [12,13], an inducible endothelial adhesive protein that serves as a counter-receptor for β2-integrins (CD11/CD18) present on the surface of leukocytes. Interaction of ICAM-1 with β2-integrins enables polymorphonuclear leukocytes to adhere firmly and stably to the vascular endothelium, and to migrate across the endothelial barrier [14–17].

NF-κB dimers, typically a heterodimer of p50 and RelA/p65 subunits, are primarily sequestered in the cytoplasm by inhibitory protein IκBα, belonging to the IkBs family, which masks the nuclear localization signal (NLS) of RelA/p65 [18,19]. Upon stimulation, IκBα is phosphorylated by IκB kinase (IKK) complex, ubiquitinated by the ubiquitin ligase, and then degraded by the 26S proteasome [20–23]. The released NF-κB dimers undergo rapid nuclear translocation and subsequent binding to NF-κB responsive elements to activate transcription of target genes, including ICAM-1 [24–27].

Despite ADMA increases the NF-κB activity, the mechanisms underlying it are unclear. A recent study showed that the destabilization or stabilization of actin filaments inhibited NF-κB activation [28]. Hence, the present study was designed to investigate the role of actin cytoskeleton in modulating ADMA-induced NF-κB activation and ICAM-1 expression in endothelial cells.

MATERIAL AND METHODS

Materials

HUVEC was purchased from Sciencell Inc (Carlsbad, CA). A monoclonal antibody to ICAM-1 was obtained from Santa Cruz Biotechnology Inc (Santa Cruz, CA). Monoclonal antibody to p65/RelA, Texas Red Goat anti-Rabbit IgG and polyclonal antibodies to β-actin were obtained from Abcam Inc (Cambridge, MA). In addition, cytochalasin D, fluorescein isothiocyanate-phalloidin and Hoechst 33342 were obtained from Sigma Inc (Louis, MO). Jasplakinolide was purchased from Enzo Life Sciences Inc (Plymouth Meeting, PA).

Cell culture

HUVEC was cultured as described [29] in gelatin-coated flasks using endothelial cell medium (Sciencell Inc) with bullet kit additives (Sciencell Inc) and 5% fetal bovine serum (Sciencell Inc). HUVECs used in the experiments were between 3 and 6 passages.

Cell Lysis and Immunoblotting

Cells were harvested in RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.25 mM EDTA, pH 8.0, 1% deoxycholic acid, 1% Triton X-100, 5 mM NaF, 1 mM sodium orthovanadate) containing protease inhibitors. For immunoblotting, protein samples were subjected to 10% SDS-PAGE and transferred to nitrocellulose membranes (Amersham International PLC, Cardiff, UK) electrophoretically. The membrane was preincubated in 5% (w/v) nonfat dry milk in TBST (10 mM Tris, pH 8.0, 150 mM NaCl, 0.1% Tween 20) with shaking for 2.5 h at room temperature to block the residual binding sites, followed by an overnight incubation with the indicated prime antibodies at 4°C and a second antibody at room temperature for 1 h with shaking. The membranes were processed using the enhanced chemiluminescence (ECL) kit (Millipore Co, Billerica, MA) and exposed to Kodak X-OMAT film.

Immunofluorescence

Cells grown on coverslips were fixed in 3.7% paraformaldehyde/PBS for 30 min and permeabilized with 0.1% Triton X-100 for 10 min. Then permeabilized cells were incubated in 1% bovine serum albumin/PBS for 30 min at room temperature to remove nonspecific binding of the antibody. Cells were incubated with fluorescein isothiocyanate-phalloidin (FITC-phalloidin) for 60 min at room temperature to localize F-actin filaments. RelA/p65 was detected by a rabbit monoclonal anti-RelA/p65 antibody for 60 min at 37°C and a secondary antibody conjugated to Texas Red for 60 min at room temperature. Nuclei were stained using Hoechst 33342 in PBS. The coverslips were mounted on the slide using mounting media. Images were obtained using a fluorescence microscope (Leica DM 4000B, Germany).

Cytoplasmic and nuclear extract

Preparation

After treatments, cells were washed 2 times with ice-cold Tris-buffered saline and resuspended in 400 µl of bufferA (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1mMEDTA, 0.1 mMEGTA, 1mM dithiothreitol, 0.5mM phenylmethylsulfonyl fluoride). After 15 min, Nonidet P-40 was added to a final concentration of 0.6%. Samples were centrifuged to collect the supernatants containing cytosolic proteins. The pelleted nuclei were resuspended in 50 µl of buffer B (20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). After 30 min at 4°C, lysates were centrifuged and supernatants were transferred to new vials containing nuclear protein.

Electrophoretic Mobility Shift Assay (EMSA)

Ten µg of nuclear extract was incubated with 1 µg of poly(dI-dC) in a binding buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.5mM dithiothreitol, 10% glycerol, 20-µl final volume) for 15 min at room temperature. Then end-labeled double-stranded oligonucleotides containing a NF-κB site (30,000 cpm each) were added and the reaction mixtures were incubated for 15 min at room
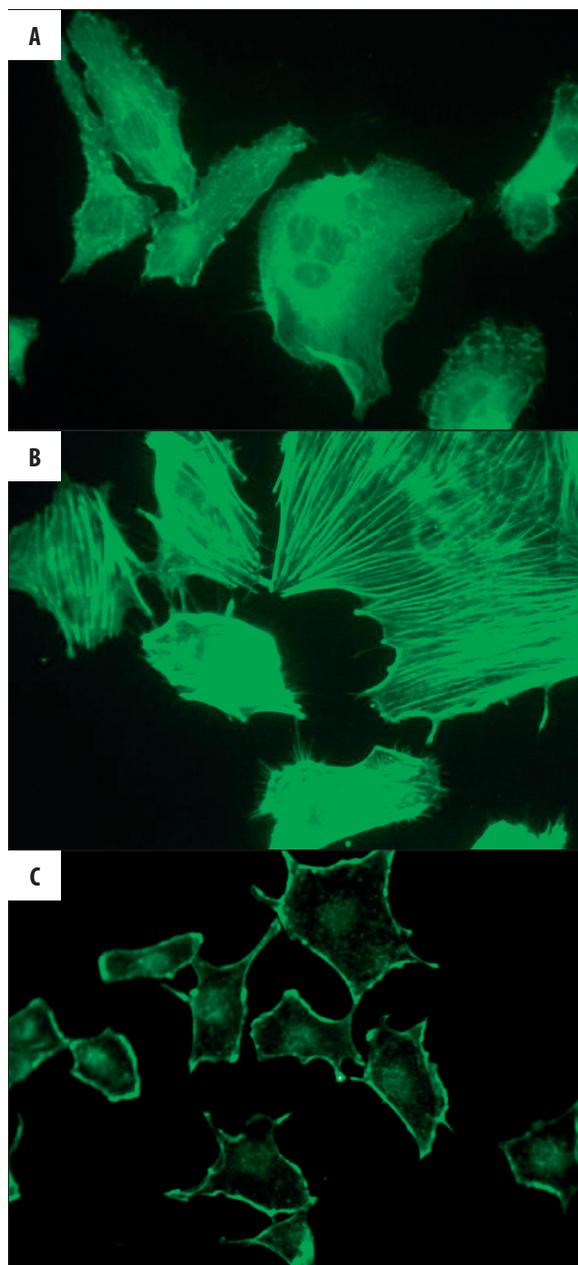
Figure 1. Effects of Cyt D on ADMA-induced actin stress fiber formation. Confluent HUVEC monolayers were (**A**) conrol, (**B**) ADMA, (**C**) ADMA + Cyt D.

temperature. The DNA-protein complexes were resolved in 5% native polyacrylamide gel electrophoresis in low ionic strength buffer (0.25× Tris borate/EDTA). The oligonucleotide used for the gel shift analysis was NF-κB 5'-AGTTGAGGGGACTTTCCCAGGC-3'. The sequence motifs within the oligonucleotides are underlined.

Statistical analysis

Data are expressed as mean ±S.E. Comparisons between experimental groups were made by T test. Differences in mean values were considered significant at $p<0.05$.

RESULTS

ADMA increased the formation of stress fiber in endothelial cells and Cyt D induced obvious destabilization of the actin filaments

HUVEC was treated with or without 100 uM ADMA for 24h and stained with FITC-phalloidin to visualize F-actin by immunofluorescence microscopy. Before the treatment, F-actin was found mostly in the cortical regions comprising the cortical actin bands (Figure 1A), while after treatment with ADMA these bands rearranged into stress fibers (Figure 1B). Pretreatment of cells with 5 μM Cyt D, the prototypic actin depolymerizing drug [30], induced obvious destabilization of the actin filaments and then prevented the formation of stress fibers induced by ADMA (Figure 1C).

Stabilization and destabilization of the actin filaments prevented ADMA-induced nuclear accumulation of RelA/p65

Immunofluorescence results showed that RelA/p65 was predominantly localized in the cytoplasm in unstimulated cells (Figure 2A). Stimulation of cells with 100 μM ADMA resulted in nuclear accumulation of RelA/p65 as indicated by the pink versus blue nuclei in the control cells (Figure 2B). Pretreatment of cells with 5 μM Cyt D for 30 min decreased RelA/p65 nuclear localization induced by ADMA (Figure 2C). Unlike Cyt D, Jas is a drug that induces actin polymerization [30], but it was found that cells pretreated with 1 μM Jas for 30 min also inhibited RelA/p65 nuclear localization induced by ADMA(Figure 2D).

Stabilization and destabilization of actin cytoskeleton inhibited ADMA-induced DNA binding of NF-κB

The EMSA revealed that the control cells show a faint shift, while the cells exposed to 100 μM ADMA for 24h produced a strong shift. In subsequent experiments, it was found that 5 μM Cyt D and 1 μM Jas both reduced the DNA binding of RelA/p65 in response to ADMA challenge (Figure 3A, B). The reduction is significant in that the shift induced by Cyt D and Jas are almost equal to the control cells.

Stabilization and destabilization of actin cytoskeleton inhibited the ICAM-1 expression in endothelial cells

Western blot analysis showed that control cells expressed a low level of ICAM-1. ADMA(100 μM) challenge of HUVECs resulted in increased level of ICAM-1 protein expression; this response was significantly inhibited in cells pretreated with of Cyt D(5 μM) or Jas(1 μM) for 30 min (Figure 4).

DISCUSSION

The results suggest that actin cytoskeleton modulated ADMA-induced NF-κB activation and the expression of ICAM-1 in endothelial cells and demonstrate that ADMA altered the actin cytoskeleton in endothelial cells. Interfering with these alterations, whether by stabilizing or destabilizing the actin filaments, prevented ADMA-induced NF-κB activation and ICAM-1 expression. The effects were associated with impaired nuclear translocation of RelA/p65,
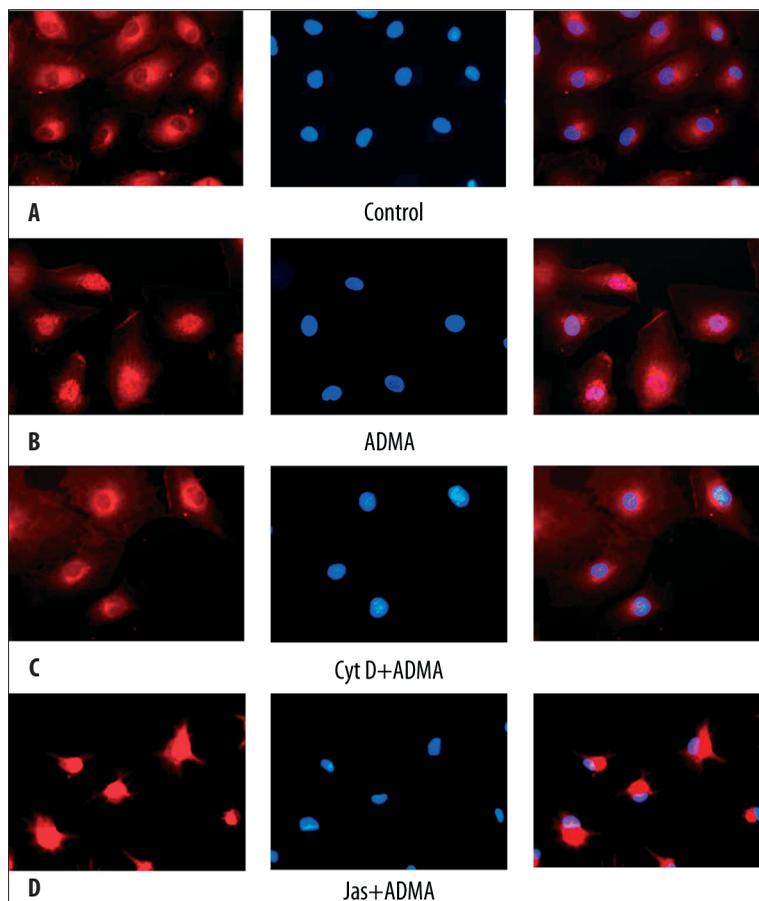
Med Sci Monit, 2011; 17(9): BR242-247

Guo W-K et al – Actin cytoskeleton modulates ADMA-induced NF-kappaB...

BR

Figure 2. Effects of Cyt D and Jas on ADMA-induced nuclear accumulation of RelA/p65. Confluent HUVEC monolayers grown on coverslips were left untreated (**A**) or challenged (**B**) for 24 h with 100 µM ADMA in the absence and presence of 5 µM Cyt D (**C**) or 1 µM Jas (**D**). Cells were then fixed, permeabilized, and stained with anti-RelA/p65 antibody and a secondary antibody conjugated with Texas Red. DNA was stained using Hoechst 33342 to visualize the nucleus. Results were analyzed by fluorescence microscopy.
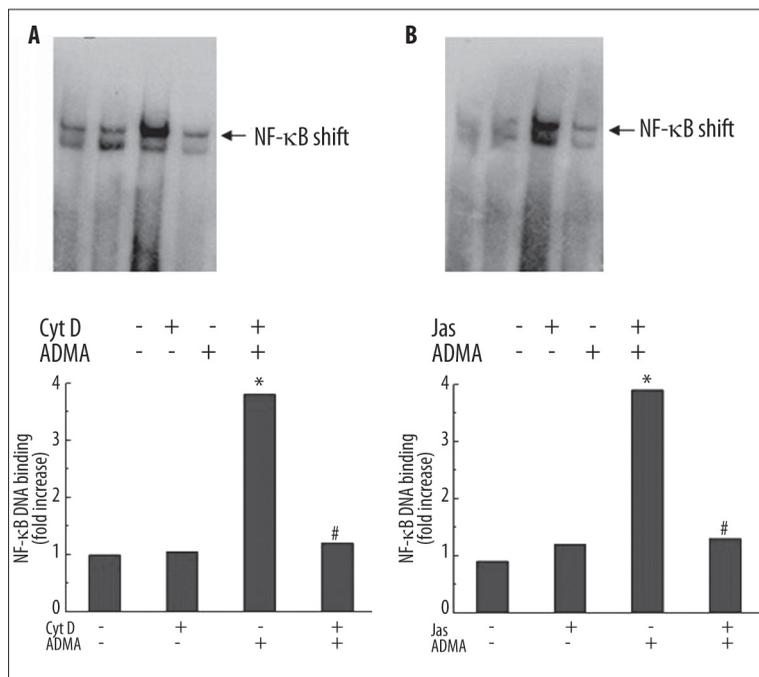

Figure 3. Effects of stabilization and destabilization of actin filaments on ADMA-induced DNA binding of NF-κB. HUVECs were pretreated with 5 µM Cyt D or 1 µM Jas for 30 min prior to challenge with ADMA (100 µM) for 24 h. Nuclear extracts were prepared and assayed for DNA binding of NF-κB by EMSA. The bar graphs represent the effect of 5 µM Cyt D (**A**) or 1 µM Jas (**B**) on ADMA induced DNA binding of NF-κB. NF-κB DNA binding is expressed as -fold increase relative to the untreated control. Data are mean ±S.E. (n=3–4 for each condition). *, different from controls (p<0.05); #, different from ADMA-stimulated controls (p<0.05).

thus suggesting a novel role for the actin cytoskeleton in modulating ADMA-induced nuclear translocation of RelA/p65, and thereby promoting ICAM-1 expression in endothelial cells.

It has been reported that ADMA induces NF-κB activation [13], but mechanisms underlying the activation of NF-κB are poorly understood. Previous reports demonstrated that stabilization and destabilization of the actin filaments
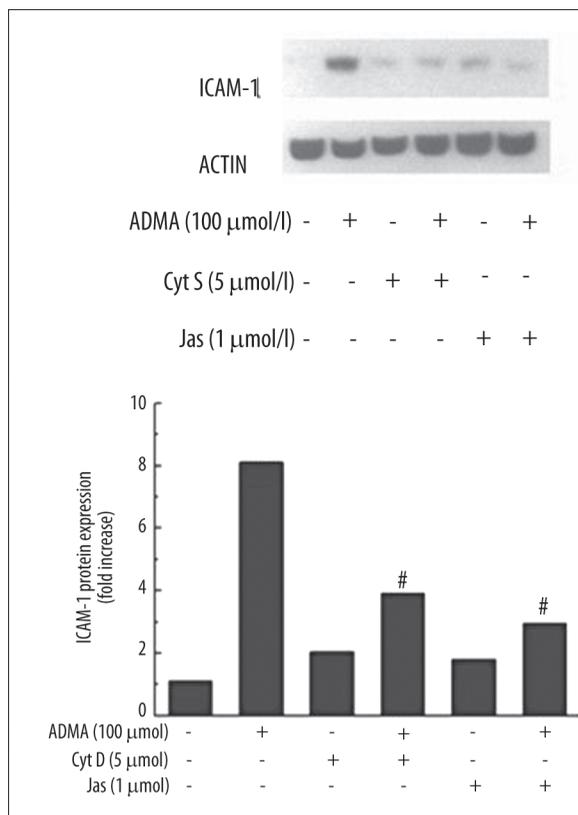
Figure 4. Effects of stabilization and destabilization of actin filaments on ADMA-induced ICAM-1 protein expression. HUVECs were pretreated with 5 µM Cyt D or 1 µM Jas and then challenged with 100 µM ADMA for 24 h. Total cell lysates were immunoblotted with an antibody to ICAM-1. Actin levels were used to monitor loading. The bar graphs represent the effect of 5 µM Cyt D or 1 µM Jas on ADMA-induced ICAM-1 protein expression. ICAM-1 protein expression normalized to actin level is expressed as -fold increase relative to the untreated control. Data are mean ±S.E. (n=3–4 for each condition). *, different from controls (p<0.05); #, different from ADMA-stimulated controls (p<0.05).

prevented thrombin-induced NF-κB activation in endothelial cells [28]. We therefore hypothesized that cytoskeleton dynamics are essential for ADMA-induced NF-κB activation in endothelial cells. Therefore, 2 reciprocal approaches were employed to address the role of actin cytoskeleton in the mechanism of ADMA-induced NF-κB activation in endothelial cells. These included stabilization and destabilization of the actin filaments to perturb the alterations in actin cytoskeleton that occurred in response to ADMA challenge of endothelial cells. Cyt D is used to destabilize the actin filaments, while Jas could induce actin filaments stabilization [30]. ADMA increased the formation of stress fiber (Figure 1B), which is associated with the higher permeability of the endothelial barrier, and Cyt D induced obvious destabilization of the actin filaments (Figure 1C). Because Jas competitively inhibits the binding of Phalloidin to F-actin [31], we did not test the effect of Jas on the actin filaments induced by ADMA.

Nuclear translocation is an important step for NF-κB activation, thus we supposed that Cyt D may prevent the

NF-κB nuclear accumulation in response to ADMA challenge. Indeed, ADMA induced NF-κB nuclear transport (Figure 2B) and Cyt D impaired ADMA-induced nuclear translocation of NF-κB (Figure 2C). Intriguingly, stabilizing the actin cytoskeleton by Jas also inhibited ADMA-induced nuclear translocation of NF-κB, thus indicating the importance of actin dynamics in modulating RelA/p65 nuclear transport following ADMA challenge of endothelial cells.

Further, the effect of Cyt D on the NF-κB DNA binding activity in response to ADMA challenge was evaluated. ADMA increased the DNA binding of NF-κB, and stabilizing and destabilizing the actin filaments both reduced the DNA binding of NF-κB in response to ADMA challenge (Figure 3). Considered together, these data raise the possibility that specific reorganization of the actin cytoskeleton as induced by ADMA in endothelial cells is essential for NF-κB activation.

ICAM-1 is involved in the recruitment of leucocytes to sites of inflammation at the endothelium and is thus involved in the pathogenesis of atherosclerosis [32]. Studies have shown that activation of NF-κB is essential for ICAM-1 expression in endothelial cells after stimulation with ADMA [12]. The effect of destabilizing or stabilizing the actin filaments on ADMA-induced ICAM-1 protein expression was then determined. Western blot analysis showed that ADMA challenge of HUVEC resulted in increased ICAM-1 protein expression and that this response was inhibited in cells pretreated with Cyt D or Jas (Figure 4). A low dose of 5 µM Cyt D or 1 µM Jas was sufficient to inhibit ICAM-1 protein expression by ADMA.

It is consistent with the confirmed reports that disturbing the dynamic of the cytoskeleton affects the activity of NF-κB in other cell sites [33–37]. In addition, actin cytoskeleton plays a regulating role in several other signaling pathways, such as the extracellular signal-regulated protein kinase (ERK) pathways [38], p38MAPK pathway [39], and the regulation of serum response factor (SRF) [40,41]. The mechanisms by which actin cytoskeleton regulates these signaling pathways have been explored, but remain to be detailed.

CONCLUSIONS

In summary, we found that ADMA increased the formation of stress fiber, and then promoted the NF-κB nuclear translocation, DNA binding to NF-κB and thereby ICAM-1 expression in endothelial cells. Disruption of ADMA-induced alterations in actin cytoskeleton, either by stabilizing or destabilizing the actin filaments, impaired the nuclear uptake of NF-κB, interfered with the DNA binding to NF-κB, and prevented the expression of ICAM-1. Thus, the specific targeting of actin cytoskeleton may be a useful strategy for preventing ADMA-activated inflammatory responses.

REFERENCE:

1. Vallance P, Leone A, Calver A et al: Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet, 1992; 339(8793): 572–75

2. Kielstein JT, Boger RH, Bode-Boger SM et al: Asymmetric dimethylarginine plasma concentrations differ in patients with end-stage renal disease: relationship to treatment method and atherosclerotic disease. J Am Soc Nephrol, 1999; 10(3): 594–600

3. Caglar K, Yilmaz MI, Sonmez A et al: ADMA, proteinuria, and insulin resistance in non-diabetic stage I chronic kidney disease. Kidney Int, 2006; 70: 781–87

4. Kielstein JT, Martens-Lobenhoffer J, Vollmer S et al: L-Arginine, ADMA, SDMA, creatinine, MDRD formula: detour to renal function testing. J Nephrol, 2008; 21: 959–61

5. Panichi V, Mantuano E, Paoletti S et al: Effect of simvastatin on plasma asymmetric dimethylarginine concentration in patients with chronic kidney disease. J Nephrol, 2008; 21: 38–44

6. Zoccali C, Benedetto FA, Maas R et al: Asymmetric dimethylarginine, C-reactive protein, and carotid intima-media thickness in end-stage renal disease. J Am Soc Nephrol, 2002; 13: 490–96

7. Fliser D, Kronenberg F, Kielstein JT et al: Asymmetric dimethylarginine and progression of chronic kidney disease: the mild to moderate kidney disease study. J Am Soc Nephrol, 2005; 16: 2456–61

8. Ravani P, Tripepi G, Malberti F et al: Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. J Am Soc Nephrol, 2005; 16: 2449–55

9. Boger RH, Maas R, Schulze F et al: Asymmetric dimethylarginine (ADMA) as a prospective marker of cardiovascular disease and mortality–an update on patient populations with a wide range of cardiovascular risk. Pharmacol Res, 2009; 60(6): 481–87

10. Kielstein JT, Zoccali C: Asymmetric dimethylarginine: a novel marker of risk and a potential target for therapy in chronic kidney disease. Curr Opin Nephrol Hypertens, 2008; 17(6): 609–15

11. Boger RH, Zoccali C: ADMA: a novel risk factor that explains excess cardiovascular event rate in patients with end-stage renal disease. Atheroscler Suppl, 2003; 4(4): 23–28

12. Jiang JL, Wang S, Li NS et al: The inhibitory effect of simvastatin on the ADMA-induced inflammatory reaction is mediated by MAPK pathways in endothelial cells. Biochem Cell Biol, 2007; 85(1): 66–77

13. Xin HY, Jiang DJ, Jia SJ et al: Regulation by DDAH/ADMA pathway of lipopolysaccharide-induced tissue factor expression in endothelial cells. Thromb Haemost, 2007; 97(5): 830–38

14. Smith CW, Rothlein R, Hughes BJ et al: Recognition of an endothelial determinant for CD 18-dependent human neutrophil adherence and transendothelial migration. J Clin Invest, 1988; 82(5): 1746–56

15. Smith CW, Marlin SD, Rothlein R et al: Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils *in vitro*. J Clin Invest, 1989; 83(6): 2008–17

16. Springer TA: Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell, 1994; 76(2): 301–14

17. Doerschuk CM, Tasaka S, Wang Q: CD11/CD18-dependent and -independent neutrophil emigration in the lungs: how do neutrophils know which route to take. Am J Respir Cell Mol Biol, 2000; 23(2): 133–36

18. Baldwin AS Jr: The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu Rev Immunol, 1996; 14: 649–83

19. Whiteside ST, Israel A: I kappa B proteins: structure, function and regulation. Semin Cancer Biol, 1997; 8(2): 75–82

20. Ghosh S, Karin M: Missing pieces in the NF-kappaB puzzle. Cell, 2002; 109(Suppl.): S81–96

21. Yamamoto Y, Gaynor RB: IkappaB kinases: key regulators of the NF-kappaB pathway. Trends Biochem Sci, 2004; 29(2): 72–79

22. Karin M, Ben-Neriah Y: Phosphorylation meets ubiquitination: the control of NF-kappaB activity. Annu Rev Immunol, 2000; 18: 621–63

23. Ben-Neriah Y: Regulatory functions of ubiquitination in the immune system. Nat Immunol, 2002; 3(1): 20–26

24. Rahman, A, Bando, M, Kefer J et al: Protein Kinase C-Activated Oxidant Generation in Endothelial Cells Signals Intercellular Adhesion Molecule-1 Gene Transcription. Mol Pharmacol, 1999; 55: 575–83

25. Ledebur HC, Parks TP: Transcriptional Regulation of the Intercellular Adhesion Molecule-1 Gene by Inflammatory Cytokines in Human Endothelial Cells. J Biol Chem, 1995; 270: 933–43

26. Minami T, Aird WC: Endothelial cell gene regulation. Trends Cardiovasc Med, 2005; 15: 174–84

27. Collins T, Read MA, Neish AS et al: Transcriptional regulation of endothelial cell adhesion molecules: NF-kappa B and cytokine-inducible enhancers. FASEB J, 1995; 9(10): 899–909

28. Fazal F, Minhajuddin M, Bijli KM et al: Evidence for actin cytoskeleton-dependent and -independent pathways for RelA/p65 nuclear translocation in endothelial cells. J Biol Chem, 2007; 282(6): 3940–50

29. Wagner DD, Olmsted JB, Marder VJ: Immunolocalization of von Willebrand protein in Weibel-Palade bodies of human endothelial cells. J Cell Biol, 1982; 95(1): 355–60

30. Kustermans G, Piette J, Legrand-Poels S: Actin-targeting natural compounds as tools to study the role of actin cytoskeleton in signal transduction. Biochem Pharmacol, 2008; 76(11): 1310–22

31. Bubb MR, Senderowicz AM, Sausville EA et al: Jasplakinolide, a cytotoxic natural product, induces actin polymerization and competitively inhibits the binding of phalloidin to F-actin. J Biol Chem, 1994; 269(21): 14869–71

32. Price DT, Loscalzo J: Cellular adhesion molecules and atherogenesis. Am J Med, 1999; 107(1): 85–97

33. Eswarappa SM, Pareek V, Chakravortty D: Role of actin cytoskeleton in LPS-induced NF-kappaB activation and nitric oxide production in murine macrophages. Innate Immun, 2008; 14(5): 309–18

34. Kustermans G, El Mjiyad N, Horion J et al: Actin cytoskeleton differentially modulates NF-kappaB-mediated IL-8 expression in myelomonocytic cells. Biochem Pharmacol, 2008; 76(10): 1214–28

35. Babakov VN, Petukhova OA, Turoverova LV et al: RelA/NF-kappaB transcription factor associates with alpha-actinin-4. Exp Cell Res, 2008; 314(5): 1030–38

36. Nemeth ZH, Deitch EA, Davidson MT et al: Disruption of the actin cytoskeleton results in nuclear factor-kappaB activation and inflammatory mediator production in cultured human intestinal epithelial cells. J Cell Physiol, 2004; 200(1): 71–81

37. Kustermans G, El Benna J, Piette J et al: Perturbation of actin dynamics induces NF-kappaB activation in myelomonocytic cells through an NADPH oxidase-dependent pathway. Biochem J, 2005; 387(Pt 2): 531–40

38. Irigoyen JP, Besser D, Nagamine Y: Cytoskeleton reorganization induces the urokinase-type plasminogen activator gene via the Ras/extracellular signal-regulated kinase (ERK) signaling pathway. J Biol Chem, 1997; 272(3): 1904–9

39. Kim SJ, Hwang SG, Kim IC et al: Actin cytoskeletal architecture regulates nitric oxide-induced apoptosis, dedifferentiation, and cyclooxygenase-2 expression in articular chondrocytes via mitogen-activated protein kinase and protein kinase C pathways. J Biol Chem, 2003; 278(43): 42448–56

40. Sotiropoulos A, Gineitis D, Copeland J et al: Signal-regulated activation of serum response factor is mediated by changes in actin dynamics. Cell, 1999; 98(2): 159–69

41. Posern G, Miralles F, Guettler S et al: Mutant actins that stabilise F-actin use distinct mechanisms to activate the SRF coactivator MAL. EMBO J, 2004; 23(20): 3973–83