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Angiotensin II Promotes Atherogenesis through upregulating the Expression of Connexin 43 in Dendritic Cells

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Abstract

It is known, for a long time, that angiotensin II (Ang II) could contribute to atherogenesis (AS) and plaque vulnerability, however the underlying mechanisms are poorly understood. Dendritic cells (DCs) are critical for the development of both inflammation and atherogenesis. In the present study, we tried to investigate the influence of AngII on the expression of connexin43 (Cx43) in DCs, as well as the effect of AngII on AS. After mouse bone marrow-derived dendritic cells (BMDCs) were treated by Ang II with or without Valsartan, the expression of Cx43 was quantified by Western Blots. The expression of Cx43 and CD40 (one marker of DCs) of DCs derived from AS plaques of ApoE-/- mice was detected by immunohistochemistry double staining. The morphology of atherosclerotic plaque was indicated by immunohistochemistry staining of smooth muscle cells. The expression of Cx43 ($P < 0.05$) was increased significantly in mouse BMDCs after treatment with AngII. In atherosclerotic plaques from $ApoE^{-/-}$ mice expressing high levels of endogenous AngII, upregulation of Cx43 ($P < 0.01$) and CD40 ($P < 0.01$) was observed. The upregulation and pro-atherogenesis effect of Cx43 could be blocked by the AngII type 1 receptor blocker Valsartan, both in *vitro* and in *vivo*. AngII may promote atherosclerosis and plaque vulnerability by increasing the expression of Cx43 in DCs and inducing the maturation of DCs through the angiotensin II type 1 receptor.

Key words: Angiotensin II, dendritic cells, connexin 43, atherosclerosis.

Introduction

It is well known that atherosclerosis (AS) is a chronic inflammatory disease. For the past several years, dendritic cells (DCs), one kind of the most powerful antigen presenting cells, have drawn much attention for its irreplaceable role in the pathogenesis of AS. DCs reside in the intima, acting as a constituent of vascular-associated lymphoid tissues (VALT), and play a critical role in the initiation, progression and rupture process of AS (1-10). After activation of atherogenic risk factors such as low-density lipoprotein (LDL), oxidized LDL (oxLDL), glycosylation end product and shear stress, DCs can present antigen to naïve T lymphocytes and activate them by acting with co-stimulatory molecules, such as CD40, CD80 and CD86, thus initiating the immuno-inflammatory reaction, which ultimately trigger and accelerate AS course (11, 12) Recently, it was reported that patients with acute coronary syndrome (ACS) or angiogrophically documented coronary artery disease (CAD) showed reduced circulating blood-derived DCs precursors and plasmacytoid DCs, compared to healthy controls, which might be as a result of excessive accumulation of DCs around plaque (13).

Among the connexin (Cx) family, Cx43 is very important for gap junction which mediates intercellular communication in DCs. During the maturation and activation of DCs, the expression of Cx43 is significantly up-regulated; when the expression of Cx43 is downregulated, the maturation and activation of DCs are blocked (14, 15). Moreover, DCs are regulated by renin angiotensin system (RAS). Angiotensin-converting enzyme (ACE)-AngII-angiotensin receptor 1 (AT1) axis and angiotensin-converting enzyme-related carboxypeptidase (ACE2)-Ang-(1-7)-receptor Mas axis have been shown to influence the functions of DCs (16). AngII, the key point of ACE-AT1 axis, has been proved to be important for the differentiation and maturation process of DCs (17-19). Rresearchers also revealed that AngII could regulate the expression of Cx43 in cardiomyocytes and vascular smooth muscle cells (20-23), however whether AngII could regulate the expression of Cx43 in DCs remains largely unknown. We presented here that overexpression of AngII could upregulate the expression of Cx43 in DCs, both in vitro and in vivo, and then promote atherosclerosis and plaque vulnerability.

Materials and methods

This study was approved by the First Affiliated Hospital Committee of Medical College, Zhejiang University on Ethics and Administration of Animal Experiments. All procedures used in this study are strictly in accordance with the guidelines of the First Affiliated Hospital Committee of Medical College, Zhejiang University on Ethics and Administration of Animal Experiments. Animals were housed in the Pathogen-Free Laboratory Animal Center of the First Affiliated Hospital of Medical College, Zhejiang University. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Animals

ApoE-/- mice (C57BL/6, 12- to 14-wk old, male) were purchased from the Laboratory Animal Center of Peking University. C57BL/6 mice (6- to 8-wk old, female) were obtained from the Laboratory Animal Cen-

ter of Zhejiang University. All mice could get regular rodent chow and saline water ad libitum. ApoE-/- mice were divided into three groups: group 1, sham group without medication; group 2, 2K1C+ Valsartan (Val), Valsartan 4mg/kg.d^{-1}, administered by lavage; group 3, 2K1C, administered with the same amount of Valsartan solvent (0.25％sodium bicarbonate) by lavage once per day. Ten weeks later, ApoE-/- mice were sacrificed by cervical dislocation and tissue samples were collected for further investigation.

Preparation and culture of bone marrow-derived dendritic cells (BMDCs)

Bone marrow-derived dendritic cells were generated from C57BL/6 mice as described previously (24-26), with minor modifications. Briefly, bone marrow mononuclear cells were prepared from mouse femur and tibia bone marrow suspensions after depletion of red cells. Cells were allowed to adhere for 2 hours in a density of 1×10^6/ml; later, the suspending cells were washed, while the adhered cells were cultured further in RPMI 1640 supplemented with 2 mM L-glutamine (Invitrogen, USA), supplemented with 10% fetal bovine serum (Invitrogen, USA), 1% of nonessential amino acids (Invitrogen), 20 ng/ml recombinant mouse GM-CSF (Peprotech, China) and 10 ng/ml recombinant mouse IL-4 (Peprotech, China). To isolate the DCs population, cells were collected, suspended in 3 ml complete media and 3ml 30% (v/v) iopamidol and then centrifuged at 1200*g* for 20 min at room temperature. After centrifugation, cells in the interface were collected, washed with complete media three times and then subjected to phenotypic analysis by flow cytometry with phycoerythrin (PE)-labeled anti-CD11c antibody (eBioscience, USA). The population containing ≥ 90% CD11c$^+$ cells was used on day 8–10 without additional purification.

Western Blot

DCs were incubated with either 10^{-6} mol/L Ang II (Sigma, China), 100ng/ml LPS (Sigma, China), Ang II (10^{-6} mol/L) + LPS (100ng/ml), or 10^{-5} mol/L valsartan (Novartis, China) pretreated for 30 min prior to application of Ang II (10^{-6} mol/L) + LPS (100ng/ml). The expression of Cx43 (Sigma, USA) was measured by Western blotting as described previously(14). The quantitative assay of the Western Blotting bands was accomplished by Kodak Digital Science 1D 2.0 Image software.

Mouse models expressing high-level AngII endogenously

The model was generated using 2 kidney-1 clip (2K1C) method as described previously (27). First，mice were anesthetized by Pentobarbital Sodium (2%, 40mg/kg Intraperitoneal Injections). The left kidney was exposed through a small flank incision, externalized, and maintained carefully with an ophthalmic chalazion forceps. For clipping, the renal artery of the left kidney was individualized over a short segment by blunt dissection, and a clip was placed close to the aorta. The kidney was then gently pushed back into the retroperitoneal cavity. The muscle layer was sutured, and the skin incision was closed with surgical staples. A sham procedure, which included the entire surgery with the exception of artery clipping, was applied in sham group mice.

Invasive measurement of blood pressure

Mice were anesthetized by Pentobarbital Sodium. The left carotid artery was exposed through a cervical incision and isolated by blunt dissection. A 24G scalp remaining needle, connected with the RM6240B/C bio-signal collection and processing system by a length of PE-10 tubing, was filled with a solution of physiological saline and heparin (300 IU/mL) and inserted into the vessel. Lidocaine (1%) was used for one minute to prevent spasm. Then, a ligature was tied around the artery to fix the needle. When mice recovered from the anesthesia and emotionally stabled, Blood pressure (BP) and heart rate were recorded continuously for 15 minutes with RM6240B/C bio-signal collection and processing system.

Plasma renin activity (PRA) concentration measurement

PRA concentration were measured using the radioimmunodetection kit (Huaying, China) following the manufacturer`s instructions.

Immunohistostaining

Euthanized mice were perfused at physiological pressure with physiological saline and then with 10% neutral formalin via the arterial remaining needle. The thoraco-abdominal aorta was dissected, from left subclavian artery until iliac bifurcation, fixed in formol and *en face* stained with Oil-red-O (Sigma, China). Pictures of stained aortas were taken with a digital camera and plaque area was analyzed by computerized planimetry using the Image Pro Plus software.

Brachiocephalic artery was serially sectioned and used for evaluation after staining with anti-α-actin (Wuhan Boster, China), and the plaque was analyzed by light microscopy using the Image Pro Plus software.

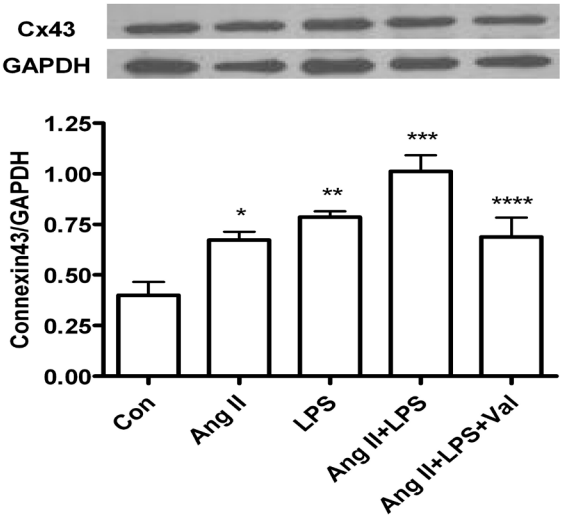

Figure 1. AngII enhances the expression of Cx43 induced by LPS. DCs were treated with either 10^{-6} mol/L Ang II, 100ng/ml LPS, Ang II (10^{-6} mol/L) +LPS (100ng/ml), or 10^{-5} mol/L valsartan pretreated for 30 min prior to application of Ang II (10^{-6} mol/L) + LPS (100ng/ml). Shown are effects on the expression of Cx43, Values are means ± SEM. **P*<0.05 versus control (Con), ***P*<0.01 versus Con, ****P*<0.05 versus LPS, *****P*<0.01 versus Ang II+LPS, n=6.

Table 1. The effect of 2K1C on PRA of ApoE-/- mice.

	SBP (mmHg)	DBP (mmHg)	MBP (mmHg)	HR (bpm)	PRA (ng/ml per h)	BW (g)
Sham	117±4	74±3	88±3	492±13	2.97±0.05	31±0.5
2K1C	165±7**	102±6*	122±6*	525±17	3.69±0.20*	31±0.8
2K1C+Val	163±6**	104±5*	123±5**	519±14	3.83±0.17*	31±1.2

SBP: systolic blood pressure, DBP: diastolic blood pressure, MBP: mean blood pressure, HR: heart rate, PRA: plasma renin activity, BW: body weight. *$P<0.05$ compared with sham, **$P<0.01$ versus sham (n=10).

Sections obtained between the appearance and disappearance of the aortic valve, about 3 millimeters thick, were embedded in paraffin and serially sectioned. The sections were used for immunohistochemisty double staining of anti-S100, anti-CD40 and anti-Cx43 (Abcam, USA), using the Immunohistochemistry double stain kits (ZSGB-BIO, China) following manufacturer`s instructions. Samples were observed with a photomicroscope and pictures were acquired with a high sensitivity color digital camera. Staining positive areas were quantified in each sample by means of the Image Pro Plus software.

Statistical Analysis

Results are presented as means ± SEM. Statistical analysis was performed using GraphPad Prism 6.0. Difference between control and experimental groups was determined using one-way analysis of variance (ANOVA) for multiple groups. Difference between every two groups was determined using Bonferroni post-hoc test. *P* value<0.05 was considered statistically significant.

Results

AngII enhanced the expression of Cx43 induced by LPS

Treatment with either 10^{-6} mol/L Ang II or 100ng/ml LPS alone successfully induced expression of Cx43 in DCs (relative expression: AngII 0.68±0.04 versus Control (Con) 0.40±0.06, $P<0.05$; LPS 0.78±0.03 versus Con 0.40±0.06, $P<0.01$). Treatment with both AngII and LPS further upregulated the expression of Cx43 when compared to LPS treatment alone (relative expression: 1.01±0.08 versus LPS 0.78±0.03, $P<0.05$). Valsartan could attenuate the upregulating effect of Cx43 (relative expression: 0.69±0.09 versus Ang II+LPS 1.01±0.08, $P<0.01$) **(Fig.1, n=6)**.

2K1C increased endogenous PRA and blood pressure

After treatment with 2K1C for ten weeks, the blood pressure and PRA were significantly higher than those in the sham group. Administration of Valsartan (4mg/kg.d^{-1}) had no effect on expression of PRA and BP. Besides, there is no difference in weight and heart rate among all three groups **(Table 1, n=10).**

Endogenous high-level AngII promoted atherogenesis

The plaque burden of thoracoabdominal aorta was detected by Oil-red O staining. Compared to the sham group, the plaque burden in the 2K1C group was significantly higher. Valsartan treatment could relieve the plaque burden **(Fig.2, n=10).**

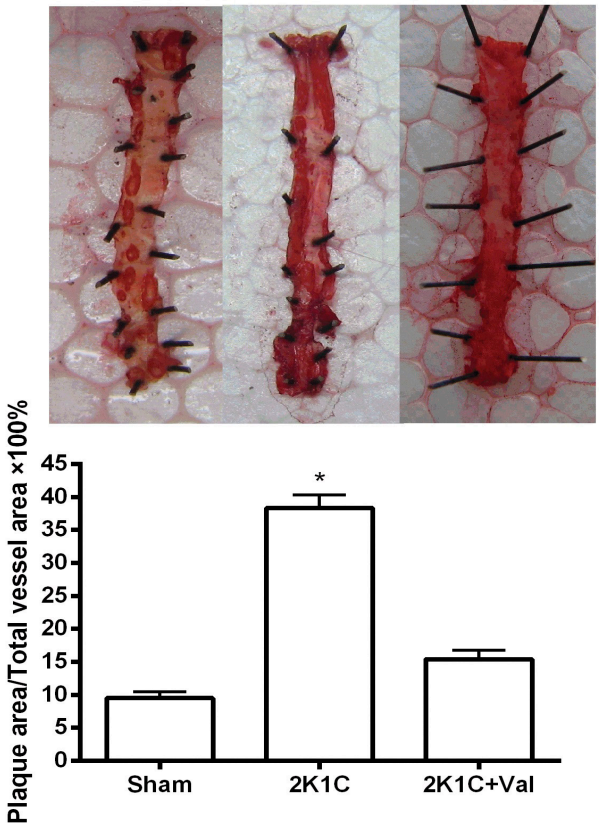

Figure 2. Endogenous AngII promotes atherogenesis. The effect of endogenous AngII on atherogenesis was shown, with a representative Oil-red-O en face stain picture above respectively. Mice were treated for 10 weeks, atherosclerotic plaque burden was quantified by the plaque area/total vessel area×100%. Values were means ± SEM. *$P<0.01$ versus sham and 2K1C+Val (n=10). 2K1C: 2kidney-1clip, 2K1C+Val: 2K1C+ Valsartan 4mg/kg.d-1 administration.

Endogenous high-level AngII induced plaque vulnerability

In the sham group, plaques had intact and integrated fibrous caps with almost intact media, whereas in the 2K1C group, plaques showed vulnerable morphology manifested by thin and even ruptured fibrous caps, huge lipid cores and deteriorated media. Valsartan treatment could stabilize the plaque by keeping the integrity and thickness of fibrous caps **(Fig.3, n=10)**.

Endogenous high-level AngII enhanced Cx43 expression of DCs in plaques

Immunohistochemisty double staining (anti-S100 and anti-Cx43) was applied to detect the Cx43 expression of DCs in plaques. The double positive arears were quantified to indicate Cx43 expression in DCs. The expression of Cx43 was significantly increased in the 2K1C group when compared to the sham group. Valsar-

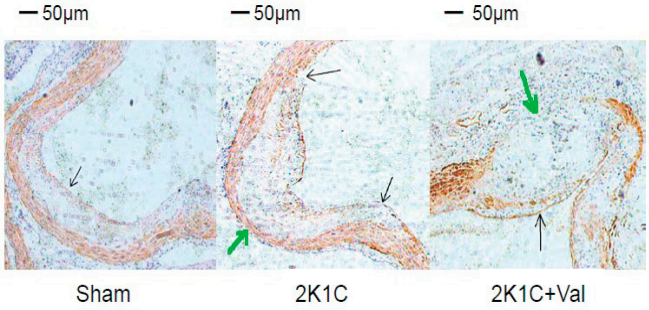

Figure 3. Endogenous AngII switches stable plaques to unstable plaques. Shown was the representative picture of plaque morphology in different groups. In sham group, plaques showed stable phynotype with intact fibrous cap (black arrow) and media without big lipid core, whereas in 2K1C group, there was a typical vulnerable plaque: huge lipid core and ruptured even absent fibrous cap (black arrow) with deteriorated media (green arrow), and in 2K1C+Val group, plaque was still stable with thick and integrated fibrous cap (black arrow) though it had big lipid core and deteriorated media (green arrow). 2K1C: 2kidney-1clip, 2K1C+Val: 2K1C+ Valsartan 4mg/kg.d^{-1} administration.

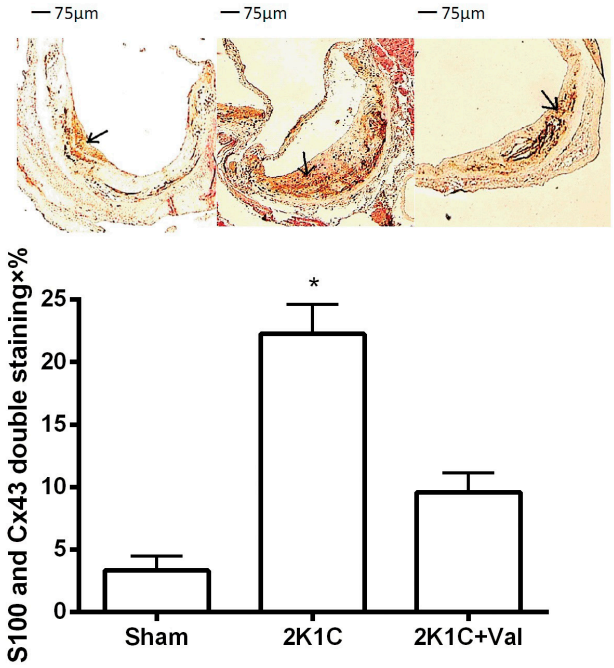

Figure 4. Endogenous AngII enhances Cx43 expression of DCs in plaques. The effect of endogenous AngII on Cx43 expression of DCs in plaques was shown, with a representative picture above respectively. Mice were treated for 10 weeks, the coexpression of S100 and Cx43 was investigated by immunohistochemical double stain. The orange area (black arrow) was the area of S100 and Cx43 positive cells. The expression extent was quantified by coexpression area/plaque area×100%.Values are means ± SEM.*P<0.01 versus sham and 2K1C+Val (n=10). 2K1C: 2kidney-1clip, 2K1C+Val: 2K1C+ Valsartan 4mg/kg.d^{-1} administration.

tan treatment downregulated the expression of Cx43 in DCs **(Fig.4, n=10)**.

Endogenous high-level AngII boosted the maturation of DCs in plaques

Two specific marker, S100 and CD40, were applied to determine matured DCs in plaques. The double positive area was significantly higher in the 2K1C group than that in the Sham group. Valsartan treatment downregulated the number of matured DCs **(Fig.5 n=10)**.

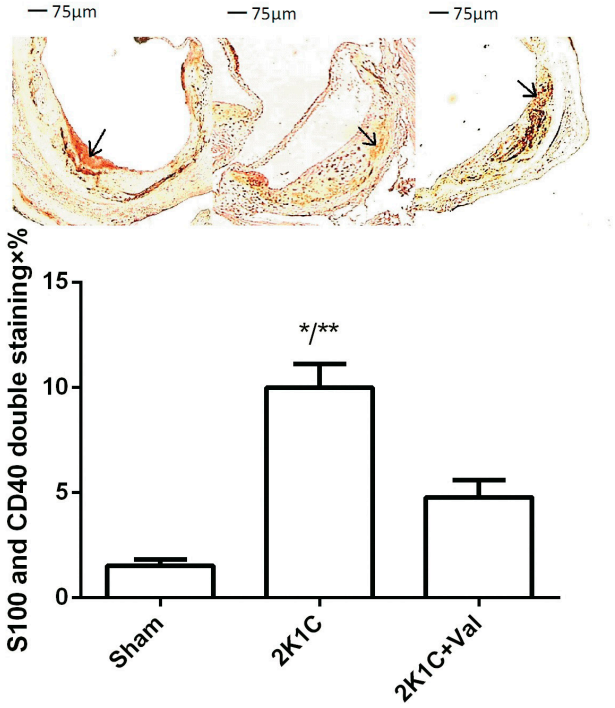

Figure 5. Endogenous AngII boosts the maturation of DCs in plaques. The effect of endogenous AngII on CD40 expression of DCs in plaques was shown, with a representative picture above respectively. Mice were treated for 10 weeks, the coexpression of S100 and CD40 was investigated by immunohistochemical double stain. The orange area (black arrow) was the area of S100 and CD40 positive cells. The maturation extent was quantified by coexpression area/plaque area×100%.Values are means ± SEM. *P<0.01 versus sham, **P<0.05 versus 2K1C+Val (n=10). 2K1C: 2kidney-1clip, 2K1C+Val: 2K1C+ Valsartan 4mg/kg.d^{-1} administration.

Discussion

In the present study, we observed that overexpression of AngII could increase the expression of Cx43 in mouse BMDCs and this effect could be attenuated by AT1 receptor antagonist Valsartan. Lots of investigations have revealed the variable effect of AngII on the expression of Cx43, which depends on cell types and microenvironment. In vascular smooth muscle cells and ventricular myocytes, AngII upregulates Cx43 expression through activating AT1 receptor, in which process p38 and ERK pathways are involved (23, 28-31); while in normal cardiomyocytes, overexpression of AngII could downregulate Cx43 expression, which could then induce ventricular tachycardia (32, 33). Our study demonstrated that overexpression of AngII could upregulate Cx43 expression in mouse BMDCs partially through activating AT1 receptor.

Being the major role of connexons in DCs, Cx43 is essential for the gap junctional intercellular communication. The gap junctional intercellular communication (GJIC) is rare when the immunocyte is in quiescent condition (34, 35). When stimulated by pro-inflammatory factors such as TNF-α, LPS or IFN-γ, the expression of Cx43 in DCs is significantly increased, accompanied by enhanced expression of the co-stimulatory molecules CD40, CD80, CD86, and antigen presentation molecule MHC-II. The maturation inducing effect

of the pro-inflammatory factors could be eliminated by GJIC channel blocker heptanol and Cx43 analogue (15, 36), suggesting that Cx43 and GJIC are very important in the immunological activity of DCs. In the present study, we demonstrated that AngII could not only induce the synthesis of Cx43, but also potentiate the increasing effect of LPS on the expression of Cx43 in DCs, which may partially explain the differentiation and maturation mechanisms of DCs.

High concentration of AngII could result in hypertension through multiple mechanisms, and previous studies revealed that the pro-atherogenic effect of AngII was independent of pro-hypertension effect (37). In the present study, the 4mg/kg.d^{-1} dosage of Valsartan had no effect on blood pressure, which indicated that the inhibitory effect of Valsartan on AS might be dependent not on its hemodynamic effect, but it`s anti-inflammatory effect (38, 39).

The present study demonstrated that, in ApoE deficient mice expressing high-level AngII endogenously, the AS plaque burden was heavy and the plaque showed vulnerable characters with thin fibrous cap, huge lipid core, and deteriorated medium. Valsartan treatment could stabilize the plaque by thickening it`s fibrous cap. Our findings might indicate that AngII enhanced the inflammation and subsequent results in plaque ulcer/rupture through AT1 receptor.

The major finding of the present study was that AngII increased Cx43 expression in DCs in vivo. AngII promoted the aggregation and activation of macrophages in the plaque, accelerated plaque progression, and aggravated the inflammatory reaction in the plaque mainly through AT1 receptor (40, 41). DCs are critical for AS developmen Intercellular communication mediated by the gap junction is a pivotal regulator in DCs` maturation and effective activation. DCs are fully equipped with RAS system (16) and it`s differentiation and maturation is controled by AngII (17-19). Mazzolai *et al*. found that endogenous high-level AngII switched the inflammatory reaction to Th1 response in the plaque of ApoE deficient mice (37). Consistent with this, the present study found that endogenous high-level AngII increased the expression of CD40 in DCs in the plaque of ApoE deficient mice. CD40 is a marker and costimulatory molecule of matured DCs and binds to the CD40 ligand in the surface of T lymphocytes to activate them. Because Cx43 can control the maturation and activation of DCs, so the present study showed that endogenous high-level AngII induced the excessive production of Cx43 in DCs, thereby urged DCs to mature and express more costimulatory molecules such as CD40, enhanced the recruitment of DCs into the plaque, subsequently activated T lymphcytes and macrophages, initiated and aggravated the immunoinflammatory reactions in the plaque, made the plaque became vulnerable, hence the acute coronary syndrome.

This is the first study to dig into the mechanisms of RAS and AS. The results will enrich the theory of atherosclerosis, and may have theraputic implications for other immunoinflammatory diseases.

Acknowledgements

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References

1. Bobryshev, Y.V. and R.S. Lord, S-100 positive cells in human arterial intima and in atherosclerotic lesions. *Cardiovasc Res*. 1995, **29**: 689-696. doi: 10.1016/0008-6363(96)88642-1.

2. Bobryshev, Y.V., Lord RS, Rainer S, Jamal OS, and Munro VF., Vascular dendritic cells and atherosclerosis. *Pathol Res Pract*. 1996, **192**: 462-467. doi: 10.1016/S0344-0338(96)80008-2.

3. Bobryshev, Y.V., and R.S. Lord, Mapping of vascular dendritic cells in atherosclerotic arteries suggests their involvement in local immune-inflammatory reactions. *Cardiovasc Res*. 1998, **37**: 799-810.

4. Waltner-Romen, M., Falkensammer G, Rabl W, and Wick G., A previously unrecognized site of local accumulation of mononuclear cells. The vascular-associated lymphoid tissue. *J Histochem Cytochem*. 1998, **46**: 1347-1350.

5. Ozmen, J., Bobryshev YV, Lord RS, and Ashwell KW., Identification of dendritic cells in aortic atherosclerotic lesions in rats with diet-induced hypercholesterolaemia. *Histol Histopathol*. 2002, **17**: 223-237.

6. Aicher, A., Heeschen C, Mohaupt M, Cooke JP, Zeiher AM, and Dimmeler S., Nicotine strongly activates dendritic cell-mediated adaptive immunity: potential role for progression of atherosclerotic lesions. *Circulation*. 2003, **107**: 604-611. doi: 10.1161/01.CIR.0000047279.42427.6D.

7. Cao, W., Bobryshev YV, Lord RS, Oakley RE, Lee SH, and Lu J., Dendritic cells in the arterial wall express C1q: potential significance in atherogenesis. *Cardiovasc Res*. 2003, **60**: 175-186. doi: 10.1016/S0008-6363(03)00345-6.

8. Yilmaz, A., Lochno M, Traeg F, Cicha I, Reiss C, Stumpf C, Raaz D, Anger T, Amann K, Probst T, Ludwig J, Daniel WG, and Garlichs CD., Emergence of dendritic cells in rupture-prone regions of vulnerable carotid plaques. *Atherosclerosis*. 2004, **176**: 101-110. doi: 10.1016/j.atherosclerosis.2004.04.027.

9. Doherty, T.M., E.A. Fisher, and M. Arditi, TLR signaling and trapped vascular dendritic cells in the development of atherosclerosis. *Trends Immunol*. 2006, **27**: 222-227.

10. Erbel, C., Sato K, Meyer FB, Kopecky SL, Frye RL, Goronzy JJ, and Weyand CM., Functional profile of activated dendritic cells in unstable atherosclerotic plaque. *Basic Res Cardiol*. 2007, **102**: 123-132. doi: 10.1007/s00395-006-0636-x.

11. Zaguri, R., Verbovetski I, Atallah M, Trahtemberg U, Krispin A, Nahari E, Leitersdorf E, and Mevorach D., 'Danger' effect of low-density lipoprotein (LDL) and oxidized LDL on human immature dendritic cells. *Clin Exp Immunol*. 2007, **149**: 543-552.

12. Ge, J.B., Jia Q, Liang C, Luo Y, Huang D, Sun A, Wang K, Zou Y, and Chen H., Advanced glycosylation end products might promote atherosclerosis through inducing the immune maturation of dendritic cells. *Arterioscler Thromb Vasc Biol.* 2005, **25**: 2157-2163. doi: 10.1161/01.ATV.0000181744.58265.63.

13. Van Vre, E.A., Hoymans,V.Y, Bult, H., Lenjou,M., VanBockstaele,D. R, Vrints,C.J, and Bosmans,J.M., Decreased number of circulating plasmacytoid dendritic cells in patients with atherosclerotic coronary artery disease. *Coron Artery Dis.* 2006. **17**: 243-248.

14. Matsue, H., Yao J, Matsue K, Nagasaka A, Sugiyama H, Aoki R, Kitamura M, and Shimada S, Gap junction-mediated intercellular communication between dendritic cells (DCs) is required for effective activation of DCs. *J Immunol.* 2006, **176**: 181-190.

15. Corvalan, L.A., Araya R, Brañes MC, Sáez PJ, Kalergis AM, To-

bar JA, Theis M, Willecke K, and Sáez JC., Injury of skeletal muscle and specific cytokines induce the expression of gap junction channels in mouse dendritic cells. *J Cell Physiol.* 2007, **211**: 649-660.
16. Nie, W.C., Hui Yan, Shan Li, Yun Zhang, Fulin Yu, Weiguo Zhu, Fangyan Fan, and Jianhua Zhu., Angiotensin-(1-7) enhances angiotensin II induced phosphorylation of ERK1/2 In mouse bone marrow-derived dendritic cells. *Mol Immunol*. 2009, **46**: 355-361. doi: 10.1016/j.molimm.
17. Lapteva, N., Nieda M, Ando Y, Ide K, Hatta-Ohashi Y, Dymshits G, Ishikawa Y, Juji T, and Tokunaga K., Expression of renin-angiotensin system genes in immature and mature dendritic cells identified using human cDNA microarray. *Biochem Biophys Res Commun*. 2001, **285**: 1059-1065.
18. Lapteva, N., Ide K, Nieda M, Ando Y, Hatta-Ohashi Y, Minami M, Dymshits G, Egawa K, Juji T, and Tokunaga K., Activation and suppression of renin-angiotensin system in human dendritic cells. *Biochem Biophys Res Commun*. 2002, **296**: 194-200. http://dx.doi.org/10.1016/S0006-291X(02)00855-0.
19. Nahmod, K.A., Vermeulen ME, Raiden S, Salamone G, Gamberale R, Fernández-Calotti P, Alvarez A, Nahmod V, Giordano M, and Geffner JR., Control of dendritic cell differentiation by angiotensin II. *FASEB J*. 2003, **17**: 491-493. doi: 10.1096/fj.02-0755fje.
20. Jia, G.H., Cheng G, Gangahar DM, and Agrawal DK., Angiotensin II and IGF-1 regulate connexin43 expression via ERK and p38 signaling pathways in of coronary artery vascular smooth muscle cells bypass conduits. *J Surg Res.* 2007, **142**: 137-142.
21. Jia, G.H., Cheng G, Gangahar DM, and Agrawal DK., Involvement of connexin 43 in angiotensin II-induced migration and proliferation of saphenous vein smooth muscle cells via the MAPK-AP-1 signaling pathway. *J Mol Cell Cardiol.* 2008,44: 882-890. doi: 10.1016/j.yjmcc
22. Cai, W., Ruan LM, Wang YN, and Chen JZ., Effects of angiotensin II on connexin 43 of VSMCs in arteriosclerosis. *J Zhejiang Univ Sci B*. 2006, **7**: 648-653.
23. Polontchouk, L., Ebelt B, Jackels M, and Dhein S., Chronic effects of endothelin 1 and angiotensin II on gap junctions and intercellular communication in cardiac cells. *FASEB J*. 2002, **16**: 87-89.
24. Inaba, K., Inaba M, Romani N, Aya H, Deguchi M, Ikehara S, Muramatsu S, and Steinman RM., Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med*. 1992, **176**: 1693-1702.
25. Lutz, M.B., Kukutsch N, Ogilvie AL, Rössner S, Koch F, Romani N,and Schuler G., An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J Immunol Methods*. 1999, **223**: 77-92.
26. Son, Y.I., Egawa S, Tatsumi T, Redlinger RE Jr, Kalinski P, and Kanto T., A novel bulk-culture method for generating mature dendritic cells from mouse bone marrow cells. *J Immunol Methods*. 2002, **262**: 145-157.
27. Wiesel, P., Mazzolai L, Nussberger J, and Pedrazzini T., Two-kidney, one clip and one-kidney, one clip hypertension in mice. *Hypertension*. 1997, **29**: 1025-1030.
28. Jia, G.H., Cheng G, Gangahar DM, and Agrawal DK., Angiotensin II and IGF-1 regulate connexin43 expression via ERK and p38 signaling pathways in of coronary artery vascular smooth muscle cells bypass conduits. *J Surg Res.* 2007, **142**: 137-142.
29. Jia, G.H., Cheng G, Gangahar DM, and Agrawal DK., Involvement of connexin 43 in angiotensin II-induced migration and proliferation of saphenous vein smooth muscle cells via the MAPK-AP-1 signaling pathway. *J Mol Cell Cardiol.* 2008,**44**: 882-890. doi: 10.1016/j.yjmcc
30. Dodge, S.M., Beardslee MA, Darrow BJ, Green KG, Beyer EC, and Saffitz JE., Effects of angiotensin II on expression of the gap junction channel protein connexin43 in neonatal rat ventricular myocytes. *J Am Coll Cardiol*. 1998, **32**: 800-807. doi: 10.1016/S0735-1097(98)00317-9.
31. Shyu, K.G., Chen CC, Wang BW, and Kuan P., Angiotensin II receptor antagonist blocks the expression of connexin43 induced by cyclical mechanical stretch in cultured neonatal rat cardiac myocytes. *J Mol Cell Cardiol*. 2001,**33**: 691-698.
32. Mayama, T., Matsumura K, Lin H, Ogawa K, and Imanaga I., Remodelling of cardiac gap junction connexin 43 and arrhythmogenesis. *Exp Clin Cardiol*. 2007, **12**: 67-76.
33. Fischer, R., Dechend R, Gapelyuk A, Shagdarsuren E, Gruner K, Gruner A, Gratze P, Qadri F, Wellner M, Fiebeler A, Dietz R, Luft FC, Muller DN, and Schirdewan A., Angiotensin II-induced sudden arrhythmic death and electrical remodeling. *Am J Physiol Heart Circ Physiol*. 2007, **293**: H1242-1253. doi: 10.1152/ajpheart.01400.2006.
34. Alves, L.A., R. Coutinho-Silva, P. M. Persechini, D. C. Spray, W. Savino, and A. C. Campos de Carvalho., Are there functional gap junctions or junctional hemichannels in macrophages? *Blood*. 1996, **88**: 328-334.
35. Eugenin, E.A., M. C. Branes, J. W. Berman, and J. C. Saez., TNF-α plus IFN-γ induce connexin43 expression and formation of gap junctions between human monocytes/macrophages that enhance physiological responses. *J. Immunol*. 2003, **170**: 1320-1328.
36. Matsue, H., Yao J, Matsue K, Nagasaka A, Sugiyama H, Aoki R, Kitamura M, and Shimada S., Gap junction-mediated intercellular communication between dendritic cells (DCs) is required for effective activation of DCs. *J Immunol.* 2006, **176**: 181-190.
37. Mazzolai, L., Duchosal MA., Korber M, Bouzourene K, Aubert JF, Hao H, Vallet V, Brunner H-R., Nussberger J, Gabbiani G, and Hayoz D., Endogenous Angiotensin II Induces Atherosclerotic Plaque Vulnerability and Elicits a Th1 Response in ApoE-/- Mice. *Hypertension*. 2004,**44**: 277-282.
38. Li Z, Iwai M, Wu L, Liu HW, Chen R, Jinno T, Suzuki J, Tsuda M, Gao XY, Okumura M, Cui TX, and Horiuchi M., Fluvastatin enhances the inhibitory effects of a selective AT1 receptor blocker, valsartan, on atherosclerosis. *Hypertension*. 2004,**44**: 758-763.
39. Yamamoto, T., Sata M, Fukuda D, and Takamoto S., The angiotensin II type 1 receptor blocker valsartan attenuates graft vasculopathy. *Basic Res Cardiol*. 2005, **100**: 84-91.
40. Takata, Y., Chu V, Collins AR, Lyon CJ, Wang W, Blaschke F, Bruemmer D, Caglayan E, Daley W, Higaki J, Fishbein MC, Tangirala RK, Law RE, and Hsueh WA., Transcriptional repression of ATP-binding cassette transporter A1 gene in macrophages: a novel atherosclerotic effect of angiotensin II. *Circ Res*. 2005, **97**: E88-E96.
41. Iwai, M., Chen R, Li Z, Shiuchi T, Suzuki J, Ide A, Tsuda M, Okumura M, Min LJ, Mogi M, and Horiuchi M., Deletion of angiotensin II type 2 receptor exaggerated atherosclerosis in apolipoprotein E-null mice. *Circulation*. 2005, **112**: 1636-1643.