Caduet enhances connexin 43 phosphorylation in left ventricular and thoracic aorta of SH model rats

XIAOYAN HUANG[1], JUNLU YANG[2], BAOGUO SONG[3], NANA WANG[1], MEIJUAN MA[4], HAIFANG WANG[5], SHA WANG[3], SHUANGPING HAO[6] and GONG CHENG[4]

[1]Shaanxi Provincial Key Laboratory of Infection and Immunity Diseases, Shaanxi Provincial People's Hospital, Xi'an, Shaanxi 710068; [2]Department of Cardiology, Baoji Traditional Chinese Medicine Hospital, Baoji, Shaanxi 721000; Departments of [3]Cardiac Surgery and [4]Cardiology, Shaanxi Provincial People's Hospital, Xi'an, Shaanxi 710068; [5]Shaanxi Key Laboratory of Integrated Traditional and Western Medicine for Prevention and Treatment of Cardiovascular Diseases, Shaanxi University of Chinese Medicine, Xi'an, Shaanxi 712046; [6]Department of Cardiology, Guangshui Traditional Chinese Medicine Hospital of Hubei Province, Guangshui, Hubei 432700, P.R. China

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Abstract. Caduet, also known as amlodipine besylate and atorvastatin calcium (AM + AT) tablet, can improve cardiac and vascular remodeling in patients with spontaneous hypertension (SH), but the underlying mechanism remains unknown. The present study aimed to explore whether AM + AT improved hypertensive left ventricular and thoracic aortic remodeling by regulating connexin 43 (Cx43) phosphorylation. A total of 32 male spontaneous hypertension model rats (SHR) were randomly divided into four groups: SHR control group, amlodipine-alone group (SHR-AM), atorvastatin-alone (SHR-AT) and AM + AT group (SHR-AM + AT); 8 Wistar-Kyoto (WKY) rats with normal blood pressure were used as the normal control. Drugs were orally administered for 8 weeks; subsequently, body weight, heart rate (HR), left ventricular mass index (LVMI), blood pressure (BP), plasma lipid levels and morphological changes of myocardial tissue in each group were analyzed. The expression of total (T)-Cx43 and phosphorylated (P)-Cx43 protein in the left ventricular and thoracic aortic tissues was determined using western blotting and immunofluorescence double labeling. The results revealed that AM + AT significantly decreased LVMI and cardiomyocyte cross-sectional area compared with SHR-AM and SHR-AT group. The western blotting results demonstrated that AM + AT could inhibit the expression of T-Cx43 protein, but increased the expression of P-Cx43 in the left ventricular and thoracic aorta. Moreover, immunofluorescence results indicated AM + AT could also decrease the expression T-Cx43, and increase that of P-Cx43 in the left ventricular and thoracic aorta compared with AM and AT alone. Therefore, it was concluded that AM + AT may mitigate left ventricular and thoracic aorta remodeling in SH rats by enhancing Cx43 phosphorylation, and the efficacy of AM + AT was superior to that of AM and AT alone.

Correspondence to: Dr Gong Cheng, Department of Cardiology, Shaanxi Provincial People's Hospital, 256 West Road, Beilin, Xi'an, Shaanxi 710068, P.R. China
E-mail: 119275360@qq.com

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Introduction

Spontaneous hypertension (SH) is one of the most critical factors in leading to cardiovascular diseases (1,2). Persistently elevated blood pressure in patients who are hypertensive results in vascular tone increasement and vascular smooth muscle systolic dysfunction, leading to myocardial ischemia and ventricular and vascular remodeling (3,4). Furthermore, left ventricular and aorta remodeling is a frequent pathological change in hypertension, which contributes to arrhythmia, heart failure and cardiovascular mortality (5-7).

Caduet is a single pill containing a combination of amlodipine besylate and atorvastatin calcium tablet (AM + AT), which is widely used in the clinical treatment of cardiovascular diseases, such as hypertension and coronary heart disease (8). In addition, previous experimental and clinical studies have reported that AM + AT can prevent cardiac hypertrophy and remodeling (9,10).

The gap junction (GJ) channel is the structural basis for cellular electric coupling and signal transmission, which mainly functions though regulating the GJ protein connexin (11,12). Connexin 43 (Cx43)-associated GJ protein in ventricular cardiomyocytes and vascular smooth muscle cells is involved in cardiac and vascular remodeling (13). Moreover, left ventricular remodeling is closely associated with the expression and distribution of Cx43 in cardiomyocytes (9,14), and our previous study revealed that the expression of Cx43 was enhanced in the thoracic aorta of SH model rats (SHR) (15). Previous studies have also reported that Cx43

phosphorylation contributes to ischemia-associated remodeling of Cx43 channels in cardiomyocytes (16,17), and Cx43 dephosphorylation contributes to arrhythmias and cardiomyocyte apoptosis in ischemia/reperfusion hearts (18). Based on these clinical data and laboratory reports, it was hypothesized that Cx43 phosphorylation may be involved in the process of hypertensive left ventricular and thoracic aorta remodeling, and AM + AT may improve this remodeling by enhancing Cx43 phosphorylation.

Therefore, the aim of the present study was to investigate the effect of Cx43 phosphorylation in the process of hypertensive left ventricular and thoracic aorta remodeling, and to examine whether AM + AT improved this remodeling by regulating Cx43 phosphorylation.

Materials and methods

Experimental reagents. A ProteoPrep Total Extraction Sample kit was obtained from Sigma-Aldrich (Merck KGaA), the BCA protein assay kit was from Beyotime Institute of Biotechnology and nitrocellulose membrane was purchased from Thermo Fisher Scientific, Inc. Primary antibody against rat total (T)-Cx43 was obtained from Thermo Fisher Scientific, Inc. (1:1,000 for western blotting; 1:100 for immunofluorescence; cat. no. 3D8A5). Primary antibody against rat phosphorylated (P)-Cx43 was obtained from Cell Signaling Technology (1:1,000 for western blotting; 1:100 for immunofluorescence; cat. no. 52559). Horseradish peroxidase (HRP)-conjugated goat anti-mouse (1:1,000; cat. no. G-21040) and goat anti-rabbit polyclonal (1:1,000; cat. no. 31466) secondary antibodies were obtained from Thermo Fisher Scientific, Inc. ECL reagents were purchased from Thermo Fisher Scientific, Inc. (cat. no. 35055). The FITC-conjugated goat anti-mouse secondary antibody was obtained from Thermo Fisher Scientific, Inc. (1:50; cat. no. 62-6511), and the tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-rabbit secondary antibody was purchased from Thermo Fisher Scientific, Inc. (1:100; cat. no. A16101). Bovine serum albumin (BSA) was from Thermo Fisher Scientific, Inc. (cat. no. 37520).

Drugs and animals. The Chinese drug administration license nos. of AM, AT and AM + AT were H10950224, J20070061 and J20171045, respectively, which were from Betriebsstätte Freiburg, Inc.. All procedures and ethics associated with animal use were reviewed and approved by Medical School of Xi'an Jiaotong University (approval no. 2018-898). In total, 32 male SHR (age, 8 weeks; weight, 210-265 g) were obtained from Weitong Lihua Experimental Animal Technology Co., Ltd, with clean grade certificate [SCXK (Beijing) 2007-0001], and eight Wistar-Kyoto (WKY) rats were purchased from Skerries Laboratory Animal Center, Ltd. as the WKY control group, with clean grade certificate [SCXK (Shanghai) 2007-0005]. The AM group (SHR-AM; n=8), AT group (SHR-AT; n=8) and AM + AT group (SHR-AM + AT; n=8) were orally administered 10 mg/kg/day of AM, AT or AM + AT as treatment groups for 8 weeks (19,20), respectively. The WKY control group (n=8) and SHR control group (n=8) were orally administrated with the same volume of water as the treatment group. All rats received humane care and were raised in the same clean environment, with ambient temperature at 22±1˚C, humidity of 50±5%, 14/10-h light/dark cycle, free access to food and water.

Measurement of body weight, heart rate (HR), left ventricular mass index (LVMI), blood pressure and plasma lipid levels in each group. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) of the rats were measured at 0, 2, 4, 6 and 8 weeks after treatment using the Non-Invasive Blood Pressure system (LE-5001 HX-II tail-cuff small animal blood pressure meter; Panlab S.L.U). After 8 weeks, HR was measured and anal temperature were measured by thermometer. Then the rats were weighed and anesthetized with pentobarbital (40 mg/kg) via intraperitoneal injection. A total of 2 ml blood was collected from heart of every rat, and the plasma lipid levels of each group were measured using a Hitachi 7170 biochemical analyzer (Hitachi, Inc.); these included total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and triglycerides (TG). Then the chest of the rat was opened, the heart was quickly removed and the left ventricular free wall was cut along the atrioventricular ring and weighed as the LVMI (left ventricular mass/body weight; mg/g).

Comparison of pathological changes in cardiac tissues. The free wall of the left ventricle was fixed in 10% formaldehyde for 2 h at room, dehydrated in an ethanol gradient (80, 90, 95 and 100%), then embedded in paraffin. Sections (3 μm thick) were cut and conventionally dewaxed to water in a series, including xylene I, xylene II, 100% alcohol I and 100% alcohol II (10 min each), then a 95, 90, 80, 70% ethanol series (10 min each) and finally distilled water. Sections were stained with hematoxylin for 1 min, rinsed with water once, stained with eosin for 2 min, then dehydrated with conventional gradient alcohol (70, 80, 90 and 95% ethanol, 2 min each; 100% ethanol I, 100% alcohol II, 10 min each), cleared with xylene, and finally mounted with neutral gum and observed with Olympus BX41 fluorescent microscope (magnification, x400; Olympus Corporation). Images were captures and used to quantitatively analyze the myocardial cell cross-sectional area using Image-Pro Plus analysis software 6.0 (Media Cybernetics, Inc.).

Analysis of T-Cx43 and P-Cx43 protein expression levels in the left ventricular and thoracic aortic tissue using western blotting. Western blotting was performed as reported previously (21). Proteins of the left ventricular free wall and thoracic aortic ascending tissue were extracted using ProteoPrep Total Extraction Sample kit and measured using a BCA protein assay kit. Each sample was mixed with 40 μl 1X SDS electrophoresis sample buffer and boiled for 2-3 min. Protein samples (30 μg) were separated by 12% SDS-PAGE gel and transferred onto a nitrocellulose membrane. The membranes were blocked with 5% skimmed milk for 2 h at room temperature, and washed twice with Tris-buffered saline with 0.1% Tween-20. Membranes were incubated with T-Cx43 and P-Cx43 primary antibodies overnight at 4˚C, then probed with HRP-conjugated secondary antibody for 2 h at room temperature. Protein bands were visualized using ECL reagents and imaged using an Alpha Innotech FluorChem FC2 Imaging System (Alpha Innotech Inc.), the densitometric analysis was used ImageJ

Table I. Effects of the different drugs on body weight, HR and LVMI (n=8).

Parameter	Group				
	WKY	SHR	SHR-AM	SHR-AT	SHR-AM + AT
Body weight, g	299.91±9.90	298.33±8.52	292.00±10.58	288.72±10.68	288.72±10.68
HR, bpm	346.61±6.92[a]	384.88±8.26	372.44±8.79	376.50±5.96	360.77±6.56
LVMI, mg/g	2.06±0.43[a,b]	2.92±0.24	2.55±0.28[a,b]	2.65±0.38[a,b]	2.31±0.49[a]

Data are presented as the mean ± SD (n=8). [a]P<0.05 vs. SHR; [b]P<0.05 vs. SHR-AM + AT. AM, amlodipine; AT, atorvastatin; HR, heart rate; LVMI, left ventricular mass; SHR, spontaneous hypertension model rat; WKY, Wistar-Kyoto.

software v1.46 (National Institutes of Health), and β-actin expression was used to normalize the data.

Expression and distribution of T-Cx43 and P-Cx43 in the left ventricular and thoracic aortic tissue using immunofluorescence double labeling. The frozen tissue embedded with optimum cutting temperature compound was cut into 8-μm thick tissue sections and fixed for 15 min with pre-cooled acetone at 4˚C. Then, the samples were blocked with 1% BSA for 2 h at room temperature, and incubated with the primary antibodies against rat T-Cx43 and P-Cx43 at 4˚C overnight, followed by incubation with the FITC-conjugated secondary antibody at 37˚C for 1 h. Then, the sections were incubated with a TRITC-conjugated secondary antibody in 1% fetal bovine serum at 37˚C for 1 h in the dark. After washing three times with PBS (5 min each), five random fields of each section were examined under an Olympus BX41 fluorescent microscope (magnification, x400; Olympus Corporation), and the fluorescence intensity was determined using ImageJ software.

Statistical analysis. The data were analyzed with SPSS 21.0 statistical software (IBM Corp.). Data are presented as the mean ± SD; the experiments were repeated three times. Comparisons between groups were performed using one-way ANOVA followed Tukey's test. P<0.05 was considered to indicate a statistically significant difference.

Results

Comparison of body weight, HR and LVMI in each group. As presented in Table I, no significant differences were identified in the body weight in all groups after 8 weeks of drug treatment (P>0.05). It was demonstrated that HR and LVMI were significantly lower in treatment groups compared with the respective SHR group (P<0.05); and LVMI was especially lower in the SHR-AM + AT group.

Comparison of blood pressure, plasma lipid level and myocardial tissue morphology. After treatment for 8 weeks, SBP and DBP in the SHR-AM and SHR-AM + AT groups were significantly decreased compared with the SHR group (P<0.05; Fig. 1A). However, there was no difference between SHR-AM and SHR-AM + AT group (P>0.05; Fig. 1A).

The plasma lipid results identified that TC, LDL and TG in the SHR-AT + AM group were significantly lower compared with the SHR group (P<0.05), and TC and LDL was lower in the SHR-AM + AT compared with levels in the SHR-AM group (P<0.05; Fig. 1B). Furthermore, there was no significant difference in HDL in all groups (P>0.05).

Compared with the WKY group, hematoxylin and eosin staining demonstrated that the myocardiocytes in the SHR group were hypertrophied, swelled and arranged disorderly, and the cross-sectional area of cardiomyocytes were significantly increased (Fig. 1C). Furthermore, compared with SHR group, the cardiomyocyte was arranged regularly and the cross-sectional area was reduced in the treatment groups (P<0.05), with an enhanced reduction in the SHR-AM + AT group (P<0.05; Fig. 1D).

Comparison of T-Cx43 and P-Cx43 protein expression levels in the left ventricular and thoracic aortic tissue using western blotting in each group. Compared with the WKY group, the expression of T-Cx43 in the free wall of the left ventricle was significantly increased in the SHR group (P<0.05). After treatment, compared with the SHR group, the expression of T-Cx43 was significantly decreased in SHR-AM and SHR-AM+AT (P<0.05), especially in SHR-AM + AT group (Fig. 2A and B). Moreover, compared with the SHR group, the expression of P-Cx43 was significantly increased in treatment groups (Fig. 2A and C). And the P-Cx43/T-Cx43 ratio demonstrated significantly increased in treatment groups compared with the ratio in the SHR group, (P<0.05), especially in SHR-AM+AT (Fig. 2A and D). The results suggested that drug treatment could enhance P-Cx43 protein expression, and that AM + AT was superior to either AM or AT alone.

In thoracic aortic tissue, the expression of T-Cx43 was decreased in the treatment groups compared with the SHR group (P<0.05), especially in SHR-AM + AT group (Fig. 2E and F). In addition, compared with SHR group, P-Cx43 expression was significantly increased in the treatment groups, with the highest upregulation in the SHR-AM + AT group (P<0.05; Fig. 2E and G). The P-Cx43/T-Cx43 ratio was also increased to a greater extent in the SHR-AM + AT group compared with the SHR-AM and SHR-AT groups (P<0.05; Fig. 2E and H).

Expression of T-Cx43 and P-Cx43 in myocardial tissue using immunofluorescence double labeling. Compared with the SHR group, the expression of T-Cx43 in the left ventricular myocardium was significantly decreased in other groups

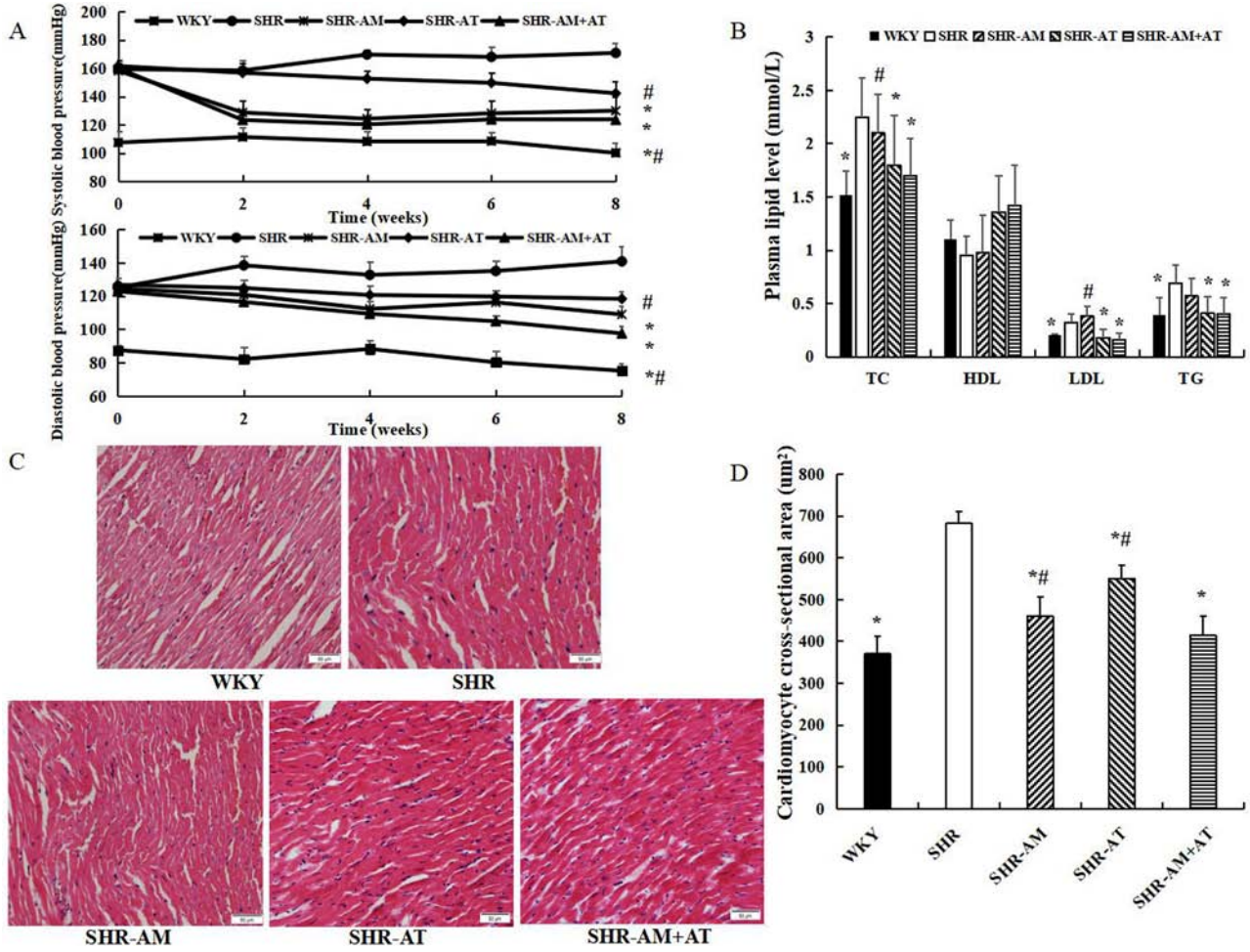

Figure 1. Comparison of blood pressure, plasma lipid level and myocardial tissue morphology in each group. (A) Comparison of systolic blood pressure and diastolic blood pressure in each group. (B) Comparison of plasma lipid levels. (C) Representative micrographs of myocardial tissue using hematoxylin and eosin staining in each group; magnification, x400. (D) Cardiomyocyte cross-sectional area in each group. Data are presented as the mean ± SD; n=8; *P<0.05 vs. SHR; #P<0.05 vs. SHR-AM + AT. AM, amlodipine; AT, atorvastatin; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; SHR, spontaneous hypertension model rat; TC, total cholesterol; TG, triglycerides; WKY, Wistar-Kyoto.

(P<0.05; Fig. 3A and B), whereas the expression of P-Cx43 was significantly increased (P<0.05), particularly in the SHR-AM + AT group (P<0.05; Fig. 3A and C).

Expression and distribution of T-Cx43 and P-Cx43 in the thoracic aorta using immunofluorescence double labeling. Compared with WKY group, T-Cx43 expression was increased and mainly distributed at the inner and medial membrane of the thoracic aorta. Moreover, P-Cx43 expression was decreased and mainly distributed at the medial and outer membrane in the SHR group. Compared with the SHR group, the expression of T-Cx43 was significantly decreased in the treatment groups, especially in the SHR-AM+AT group (all P<0.05; Fig. 4A and B), and the expression of P-Cx43 was significantly increased in treatment groups (P<0.05), especially in the SHR-AM+AT group (P<0.05; Fig. 4A and C).

Discussion

Hypertension is one of the most critical factors to cause left ventricular and vascular remodeling (22,23). Furthermore, ventricular remodeling is a risk factor of various severe arrhythmias, as well as sudden mortality (24). Owing to improved compliance, AM + AT have been used for treating hypertension and hyperlipidemia, and can protect vascular smooth muscle and myocardium from remodeling (25,26). Caduet is a single pill containing AM and AT, which blocks Ca^{2+} transmembrane influx, inhibits β-Hydroxy β-methylglutaryl-coenzyme A reductase, decreases vascular resistance and reverses ventricular remodeling (27). In addition, our previous study revealed that the expression of Cx43 was enhanced in the ventricular and thoracic aorta of SHR (15). However, the exact mechanism of AM + AT improving ventricular and thoracic aorta remodeling by regulating Cx43 phosphorylation it yet to be fully elucidated.

The present results indicated that AM + AT enhanced Cx43 phosphorylation in the left ventricular and thoracic aorta of SHR, and that AM + AT was superior to AM and AT treatment alone.

GJs serve an important role in vascular tone and blood pressure regulation (28). The GJ protein Cx43 is a sensitive pressure receptor that is closely related to the development of hypertension (29). Cardiovascular diseases have been reported to affect the expression and localization of Cx43, and dysregulated Cx43 is related to the loss of cardioprotection (30,31).

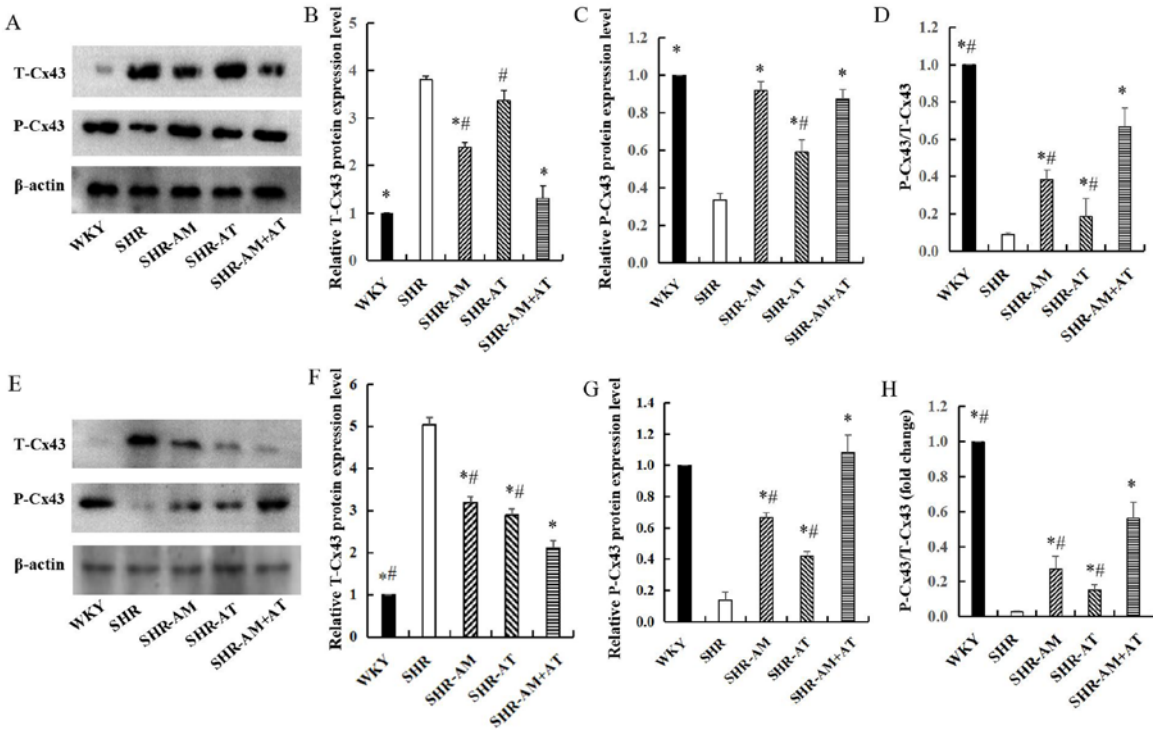

Figure 2. Comparison of T-Cx43 and P-Cx43 protein expression levels in left ventricular and thoracic aortic using western blotting. (A) Representative results of western blotting analysis on left ventricular tissue, and subsequent densitometric analysis of (B) T-Cx43 and (C) P-Cx43 protein expression levels; (D) P-Cx43/T-Cx43 ratio. (E) Results of western blotting analysis on thoracic aortic tissues, and subsequent densitometric analysis of (F) T-Cx43 and (G) P-Cx43; (H) P-Cx43/T-Cx43 ratio. Data are expressed as the mean ± SD; n=8; *P<0.05 vs. SHR; #P<0.05 vs. SHR-AM + AT. AM, amlodipine; AT, atorvastatin; Cx43, connexin 43; P-, phosphorylated; SHR, spontaneous hypertension model rat; T-, total; WKY, Wistar-Kyoto.

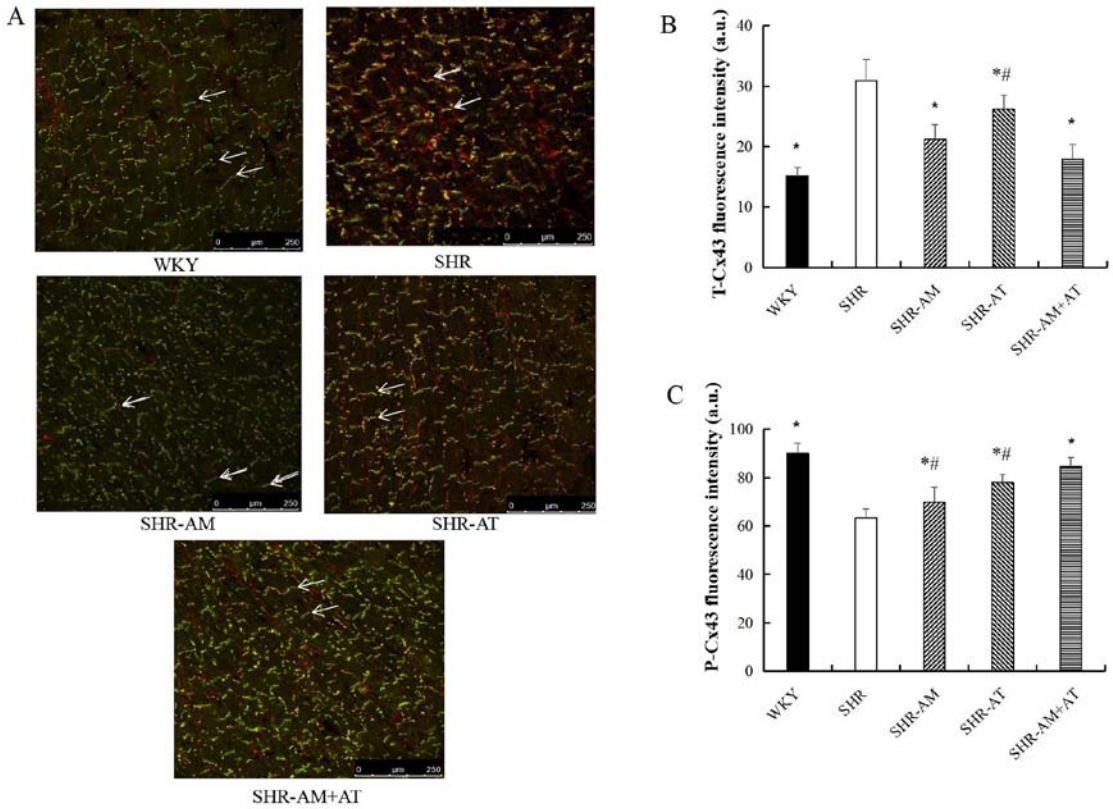

Figure 3. Comparison of T-Cx43 and P-Cx43 protein expression levels in left ventricular tissue using immunofluorescence double labeling. (A) Results of immunofluorescence double labeling analysis in left ventricular tissue. Green fluorescence, T-Cx43; red fluorescence, P-Cx43. Arrows indicate the intercalated disc. Magnification, x400. Fluorescence intensity for (B) T-Cx43 and (C) P-Cx43. Data are expressed as the mean ± SD; n=8; *P<0.05 vs. SHR; #P<0.05 vs. SHR-AM + AT. AM, amlodipine; AT, atorvastatin; a.u., arbitrary unit; Cx43, connexin 43; P-, phosphorylated; SHR, spontaneous hypertension model rat; T-, total; WKY, Wistar-Kyoto.

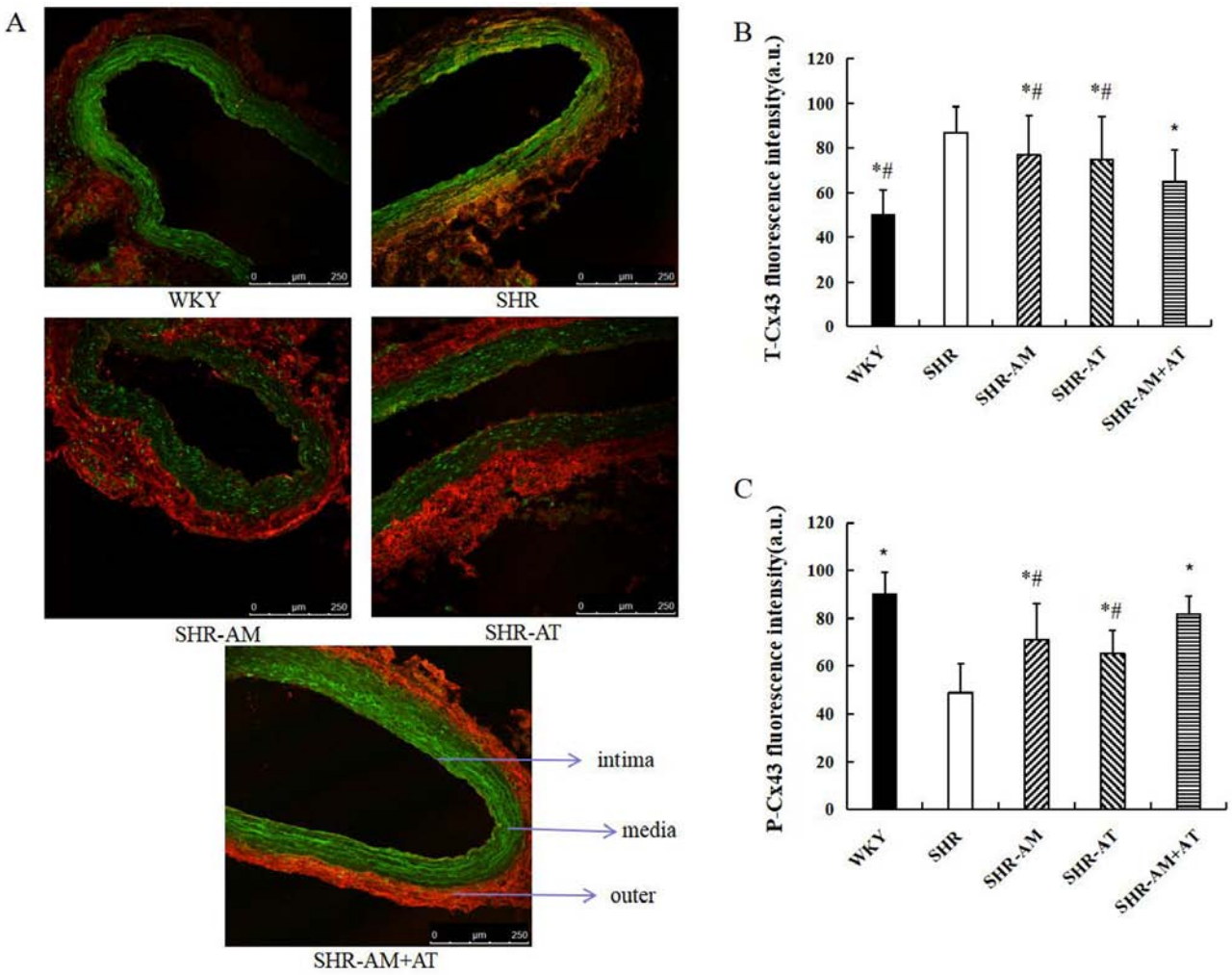

Figure 4. Comparison of T-Cx43 and P-Cx43 protein expression in the thoracic aorta using immunofluorescence double labeling. (A) Results of immunofluorescence double labeling analysis in thoracic aortic tissue. Green fluorescence, T-Cx43; red fluorescence, P-Cx43. Magnification, x400. Fluorescence intensity for (B) T-Cx43 and (C) P-Cx43 of thoracic aorta. Data are expressed as the mean ± SD; n=8; *P<0.05 vs. SHR; $^{\#}$P<0.05 vs. SHR-AM + AT. AM, amlodipine; AT, atorvastatin; a.u., arbitrary unit; Cx43, connexin 43; P-, phosphorylated; SHR, spontaneous hypertension model rat; T-, total; WKY, Wistar-Kyoto.

Furthermore, ischemic preconditioning may reduce the incidence of arrhythmias by increasing Cx43 expression and altering GJ channel remodeling (32). In the current study, it was suggested that the upregulation of T-Cx43 and downregulation of P-Cx43 in myocardial tissue of SHR may be an adaptive response for increasing blood pressure and cardiac load. Moreover, the expression of P-Cx43 was increased and T-Cx43 was decreased after AM + AT treatment, which could contribute to enhancing GJ communication among vascular smooth muscle cells in resistant arteries of SHR group, and causing an increased response to vasoconstrictors and hypertension (33). Thus, it was concluded that AM + AT may mitigate ventricular and vascular remodeling via increased phosphorylation of Cx43.

In summary, AM + AT could improve ventricular and vascular remodeling by inhibiting T-Cx43 expression and enhancing Cx43 phosphorylation in SHR, indicating that AM + AT is superior to AM and AT alone.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

XH, JY and GC conceived and designed the experiments; XH, JY, BS, MM, HW, SW and GC performed the experiments; XH, NW and GC analyzed the data; XH, SH and NW made data interpretation and critical manuscript revisions; XH and JY wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures and ethics associated with animals were reviewed and approved by Medical School of Xi'an Jiaotong University (approval no. 2018-898).

Patient consent for publication

Not applicable.

Competing interests

The authors declared that they have no competing interests.

References

1. Olsen MH, Angell SY, Asma S, Boutouyrie P, Burger D, Chirinos JA, Damasceno A, Delles C, Gimenez-Roqueplo AP, Hering D, *et al*: A call to action and a lifecourse strategy to address the global burden of raised blood pressure on current and future generations: The lancet commission on hypertension. Lancet 388: 2665-2712, 2016.
2. Ettehad D, Emdin CA, Kiran A, Anderson SG, Callender T, Emberson J, Chalmers J, Rodgers A and Rahimi K: Blood pressure lowering for prevention of cardiovascular disease and death: A systematic review and meta-analysis. Lancet 387: 957-967, 2016.
3. Vriz O, Magne J, Jarosh J, Bossone E, Aboyans V and Palatini P: Local carotid arterial stiffness is an independent determinant of left ventricular remodeling in never-treated hypertensive patients. Blood Press 28: 23-33, 2019.
4. Johnson RD and Camelliti P: Role of non-myocyte gap junctions and connexin hemichannels in cardiovascular health and disease: Novel therapeutic targets? Int J Mol Sci 19: 866, 2018.
5. Desai CS, Ning H and Lloyd-Jones DM: Competing cardiovascular outcomes associated with electrocardiographic left ventricular hypertrophy: The atherosclerosis risk in communities study. Heart 98: 330-334, 2012.
6. Opie LH, Commerford PJ, Gersh BJ and Pfeffer MA: Controversies in ventricular remodeling. Lancet 367: 356-367, 2006.
7. Camargo LL, Harvey AP, Rios FJ, Tsiropoulou S, Da Silva RNO, Cao Z, Graham D, McMaster C, Burchmore RJ, Hartley RC, *et al*: Vascular Nox (NADPH oxidase) compartmentalization, protein hyperoxidation, and endoplasmic reticulum stress response in hypertension. Hypertension 72: 235-246, 2018.
8. Schaffer AL, Buckley NA and Pearson SA: Who benefits from fixed-dose combinations? Two-year statin adherence trajectories in initiators of combined amlodipine/atorvastatin therapy. Pharmacoepidemiol Drug Saf 26: 1465-1473, 2017.
9. Chen HJ, Yao L, Chen TG, Yu M, Wang LH and Chen JZ: Atorvastatin prevents connexin43 remodeling in hypertrophied left ventricular myocardium of spontaneously hypertensive rats. Chin Med J (Engl) 120: 1902-1907, 2007.
10. Waters D, Higginson L, Gladstone P, Kimball B, Le May M, Boccuzzi SJ and Lespérance J: Effects of monotherapy with an HMG-CoA reductase inhibitor on the progression of coronary atherosclerosis as assessed by serial quantitative arteriography. The Canadian coronary atherosclerosis treatment trial. Circulation 3: 959-968, 1994.
11. Salameh A: Life cycle of connexins: Regulation of connexin synthesis and degradation. Adv Cardiol 42: 57-70, 2006.
12. Söhl G and Willecke K: Gap junctions and the connexin protein family. Cardiovasc Res 62: 228-232, 2004.
13. Rodriguez-Sinovas A: Cx43 phosphorylation and cardioprotection. Cardiovasc Res 83: 613-614, 2009.
14. Egan Benova T, Szeiffova Bacova B, Viczenczova C, Diez E, Barancik M and Tribulova N: Protection of cardiac cell-to-cell coupling attenuate myocardial remodeling and proarrhythmia induced by hypertension. Physiol Res 65 (Suppl 1): S29-S42, 2016.
15. Cheng G, Chen CY, Shou XL and Han XY: Effects of captopril on the expression of connexin 43 in thoracic aorta from spontaneous hypertensive rats. J Shaanxi Med 44: 1571-1573, 2015.
16. Martins-Marques T, Catarino S, Marques C, Matafome P, Ribeiro-Rodrigues T, Baptista R, Pereira P and Girão H: Heart ischemia results in connexin43 ubiquitination localized at the intercalated discs. Biochimie 112: 196-201, 2015.
17. Martins-Marques T, Catarino S, Zuzarte M, Marques C, Matafome P, Pereira P and Girão H: Ischaemia-induced autophagy leads to degradation of gap junction protein connexin43 in cardiomyocytes. Biochem J 467: 231-245, 2015.
18. Xue J, Yan X, Yang Y, Chen M, Wu L, Gou Z, Sun Z, Talabieke S, Zheng Y and Luo D: Connexin 43 dephosphorylation contributes to arrhythmias and cardiomyocyte apoptosis in ischemia/reperfusion hearts. Basic Res Cardiol 114: 40, 2019.
19. Hradec J, Zamorano J and Sutradhar S: Post hoc analysis of the cluster randomized usual care versus caduet investigation assessing long-term risk (CRUCIAL) trial. Curr Med Res Opin 29: 589-596, 2013.
20. Lu J, Liu F, Chen F, Jin Y, Chen H, Liu D and Cui W: Amlodipine and atorvastatin improve ventricular hypertrophy and diastolic function via inhibiting TNF-α, IL-1β and NF-κB inflammatory cytokine networks in elderly spontaneously hypertensive rats. Biomed Pharmacother 83: 330-339, 2016.
21. Zhang ZY, Li Y, Li R, Zhang AA, Shang B, Yu J and Xie XD: Tetrahydrobiopterin protects against radiation-induced growth inhibition in H9c2 cardiomyocytes. Chin Med J (Engl) 129: 2733-2740, 2016.
22. Jin Y, Jing M, Zhang L, Song S and Ma X: Internet access and hypertension management among the elderly population: A nationally representative cross-sectional survey in China. J Med Internet Res 31: e11280, 2019.
23. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK and He J: Global burden of hypertension: Analysis of worldwide data. Lancet 365: 217-223, 2005.
24. Delvaeye T, Vandenabeele P, Bultynck G, Leybaert L and Krysko DV: Therapeutic targeting of connexin channels: New views and challenges. Trends Mol Med 24: 1036-1053, 2018.
25. Nagasawa K, Takahashi K, Matsuura N, Takatsu M, Hattori T, Watanabe S, Harada E, Niinuma K, Murohara T and Nagata K: Comparative effects of valsartan combination with cilnidipine or amlodipine on cardiac remodeling and diastolic dysfunction in Dahl salt-sensitive rats. Hypertens Res 38: 39-47, 2015.
26. Chen Y, Chang Y, Zhang N, Guo X, Sun G and Sun Y: Atorvastatin attenuates myocardial hypertrophy in spontaneously hypertensive rats via the C/EBPβ/PGC-1α/UCP pathway. Cell Physiol Biochem 46: 1009-1018, 2018.
27. Naydenov Naydenov S, Margaritov Runev N, Ivanov Manov E and Georgieva Torbova-Gigova S: Efficacy and safety of a single-pill combination of atorvastatin/amlodipine in patients with arterial hypertension and dyslipidemia. Acta Clin Croat 57: 464-472, 2018.
28. Song D, Liu X, Liu R, Yang L, Zuo J and Liu W: Connexin 43 hemichannel regulates H9c2 cell proliferation by modulating intracellular ATP and [Ca2+]. Acta Biochim Biophys Sin (Shanghai) 42: 472-482, 2010.
29. Seki A, Nishii K and Hagiwara N: Gap junctional regulation of pressure, fluid force, and electrical fields in the epigenetics of cardiac morphogenesis and remodeling. Life Sci 129: 27-34, 2015.
30. Zhai H, Dai W and Wang Y: Metoprolol protects cardiomyocytes in rabbit model of heart failure by regulating Cx43. Exp Ther Med 15: 1902-1905, 2018.
31. Nao T, Ohkusa T, Hisamatsu Y, Inoue N, Matsumoto T, Yamada J, Shimizu A, Yoshiga Y, Yamagata T, Kobayashi S, *et al*: Comparison of expression of connexin in right atrial myocardium in patients with chronic atrial fibrillation versus those in sinus rhythm. Am J Cardiol 91: 678-683, 2003.
32. Xing D, Kjølbye AL, Nielsen MS, Petersen JS, Harlow KW, Holstein-Rathlou NH and Martins JB: ZP123 increases gap junctional conductance and prevents reentrant ventricular tachycardia during myocardial ischemia in open chest dogs. J Cardiovasc Electrophysiol 14: 510-520, 2003.
33. Wang LJ, Ma KT, Shi WY, Wang YZ, Zhao L, Chen XY, Li XZ, Jiang XW, Zhang ZS and Li L and Si JQ: Enhance gap junction channel activity between vascular smooth muscle cells in cerebral artery of spontaneously hypertensive rats. Clin Exp Hypertens 39: 295-305, 2017.