Combination of tolvaptan and valsartan improves cardiac and renal functions in doxorubicin-induced heart failure in mice

Fengqin Yan, Hong Zhu, Yingxia He, Qinqin Wu, Xiaoyu Duan

Department of General Practice, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

ABSTRACT

Heart failure (HF) is often complicated by renal dysfunction. Tolvaptan and valsartan are two well-known agents for the treatment of HF. However, the role of tolvaptan/valsartan combination on HF with renal dysfunction remains unclear. To establish a mice model with HF with renal dysfunction, mice were intraperitoneally injected with doxorubicin (Dox). Echocardiogram was applied to assess the left ventricular function. Additionally, serum aldosterone (ALD) and angiotensin II (Ang II) level in mice were determined by ELISA. Meanwhile, Western blot assay was used to evaluate the expressions of B cell lymphoma-2 (Bcl-2), Bcl-2 associated X (Bax) and cleaved caspase 3 in the heart and kidney tissues of mice. In this study, we found that compared to tolvaptan or valsartan alone treatment group, tolvaptan/valsartan combination obviously improved the left ventricular ejection fraction (LVEF) and the left ventricular fractional shortening (LVFS), and reduced serum ALD and Ang II level in Dox-treated mice. Additionally, tolvaptan/valsartan combination significantly prevented the inflammation and fibrosis of heart and kidney tissues in Dox-treated mice. Meanwhile, tolvaptan/valsartan combination notably inhibited the myocardial and renal cell apoptosis in Dox-treated mice *via* upregulation of Bcl-2 and downregulation of Bax and cleaved caspase 3, compared to the single drug treatment. Collectively, tolvaptan/valsartan combination could improve cardiac and renal functions, as well as prevent the fibrosis, inflammation and apoptosis of heart and kidney tissues in Dox-treated mice. Taken together, combining tolvaptan with valsartan might be a promising approach to achieve enhanced therapeutic effect for treatment of HF with renal dysfunction.

Key words: Heart failure; renal dysfunction; tolvaptan; valsartan.

Correspondence: Hong Zhu, Department of General Practice, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, 26 Shengli Street, Jiangan District, Wuhan, Hubei 430014, China. E-mail: zhuhong_zh53@126.com

Contributions: FY, major contributions to the investigation, manuscript drafting; YH, QW, XD, responsible for data acquisition, analysis and interpretation; HZ, substantial contributions to conception and design of the study, manuscript revision. All the authors have read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: The authors declare no conflict of interest.

Ethics approval: This study was approved by the Animal Experimental Committee of The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology.

Funding: Scientific Research Project of Wuhan Health Commission in 2020 (No. WX20Q08).

Introduction

Heart failure (HF) is a heterogeneous clinical syndrome with a high risk of morbidity and mortality.[1,2] It is caused by the abnormalities in cardiac structure or function.[3] In addition, myocardial infarction, myocarditis, cardiomyopathy, *etc*. can impair cardiac function, leading to HF.[4-7] Unfortunately, HF is often complicated by renal dysfunction.[8] Evidence have shown that more that 40% of patients with HF have chronic kidney disease (CKD).[9,10] It has been shown that HF lead to a decrease in cardiac output, resulting in activation of renin-angiotensin-aldosterone system (RAAS).[11] Angiotensin II (Ang II) is the principal effector of RAAS, which could lead to salt and water retention *via* mediating aldosterone (ALD) production.[12-14] Moreover, HF also caused the increased secretion of arginine-vasopressin (AVP), resulting in salt and water retention and left ventricular remodeling.[15] Collectively, HF could cause renal retention of salt and water, which in return exacerbates the symptoms of HF.[16] Therefore, blocking RAAS system or AVP secretion are the promising choices for the treatment of HF.[17]

Tolvaptan is an orally selective vasopressin V2 receptor antagonist.[18,19] It has been shown that tolvaptan is an aquaretic agents, which could promote water excretion while retaining spontaneously the sodium.[20] Clinically, tolvaptan has unique curative effect in treating HF and CKD.[21,22] Valsartan, an angiotensin-receptor blocker, could prevent HF and attenuate CKD *via* blocking RAAS system.[23-26] Meanwhile, valsartan has been widely used in clinical practice to treat patients with HF.[27-29] Sacubitril/valsartan effectively improved cardiac function and echocardiographic parameters in patients with HF.[30] Li *et al*. found that combination of valsartan and perifosine effectively prevented mouse heart failure compared to single treatment.[3]. However, the role of tolvaptan/valsartan combination treatment on HF with renal dysfunction is still not studied. Therefore, we aimed to explore the effect of tolvaptan/valsartan combination on cardiac and renal structure and function in Dox-induced HF in mice.

Materials and Methods

Experimental animals and grouping

A total of 25 C57BL/6J mice (SPF grade) were purchased from Charles River Laboratories (Wilmington, MA, USA). The animal experimental protocols were approved by the Animal Experimental Committee of The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, and performed according to the ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments). Animals were randomized into 5 groups: control, model, model + tolvaptan, model + valsartan and model + tolvaptan + valsartan groups. To establish a mice model of HF with renal dysfunction, mice in the model groups were intraperitoneally injected with doxorubicin (5 mg/kg; Selleck Chemicals, Houston, TX, USA) once a week for 4 weeks, as previously described.[32] Meanwhile, mice in the tolvaptan or/and valsartan treatment groups were treated with tolvaptan (15 mg/kg/d, orally; Zhejiang Otsuka Pharmaceutical, Lingan, China), valsartan (40 mg/kg/d, orally; Tuoping, Tianda Pharmaceutical, Hong Kong, China) or tolvaptan (15 mg/kg/d) and valsartan (40 mg/kg/d) for 4 weeks. All mice were subjected to echocardiography after 4 weeks of treatment.

Assessment of cardiac functions

The Mylab X5 Vet Ultrasound imaging system (Esaote China Ltd., Hong Kong, China) was used to evaluate the cardiac functions of mice. Left ventricular end-systolic diameter (LVESD), left ventricular end-diastolic diameter (LVEDD), LVEF and LVFS were measured by echocardiography (left ventricular long axis). Evidence have shown that the LV long axis index could detect the LV diastolic dysfunction and is an important prognosticator in patients with HF.[33,34]

ELISA

Animals were sacrificed on days 28, and the serum, heart and kidney tissues were collected. The ALD, Ang II, IL-6, and TNF-a level in serum samples were assessed using the ALD ELISA Kit (catalog n. ab136933, Abcam, Cambridge, UK), mouse Ang II ELISA Kit (catalog n. ELK1401, ELK Biotechnology, Wuhan, China), mouse IL-6 ELISA Kit (catalog n. ELK1157, ELK Biotechnology) and mouse TNF-a ELISA Kit (catalog n. ELK1387, ELK Biotechnology).

RT-qPCR assay

Total RNA was extracted from heart and kidney tissues using the TRIpure Total RNA Extraction Reagent (catalog n. EP013, ELK Biotechnology). Next, total RNA was transcribed into cDNA using M-MLV Reverse Transcriptase (catalog n. EQ002, ELK Biotechnology). After that, to detect the IL-6 and TNF-a level in heart and kidney tissues, qPCR was carried out using the QuFast SYBR Green PCR Master Mix Kit (catalog n. EQ001, ELK Biotechnology). β-actin was used as an endogenous control for normalizing the IL-6 and TNF-a level. IL-6 forward, 5'-TTGCCTTCTTGGGACTGATG-3' and reverse, 5'-TCATTTCCACGATTTCCCAG-3'; TNF-a forward, 5'- CAGCCTCTTCTCATTCCTGCT-3' and reverse, 5'- GGGTCTGGGCCATAGAACTG-3'; β-actin forward, 5'- CTGAGAGGGAAATCGTGCGT-3' and reverse, 5'- CCACAGGATTCCATACCCAAGA-3'.

Histology analysis

The heart and kidney tissues were placed into 4% paraformaldehyde for 24 h. After that, the samples were embedded in paraffin, and sliced into 3 μm sections. Next, hematoxylin and eosin (HE), and Masson's trichrome staining was performed to observe the pathological changes and collagen deposition in heart and kidney tissues respectively. An Olympus microscope (objective: 20x; CX31; Tokyo, Japan) was used to photograph histological images. Masson's trichrome staining was used for assessing kidney fibrosis and the Image-Pro Plus (IPP) software was used for assessing the degree of kidney fibrosis within 3 random fields.

TUNEL staining assay

The heart and kidney samples were embedded in paraffin and sliced into 3 μm sections. Next, the sections were incubated with the TUNEL reaction solution (catalog n. 11684817910, Roche, Basel, Switzerland) for 1.5 h in darkness. Later on, the nuclear was stained with DAPI (10 μg/mL; D8417-1MG, Sigma-Aldrich, St. Louis, MI, USA) for 30 min in the dark. Subsequently, a fluorescence microscope (objective: 20x; light: mercury lamp; Eclipse Ci-L, Nikon, Tokyo, Japan) was used to observe cell apoptosis in heart and kidney tissues under green fluorescence (490 nm/530 nm) and blue fluorescence (364 nm/454 nm). The IPP software was used for evaluating the TUNEL-positive cells within 3 random fields.

Western blot assay

Protein (30 μg/lane) were subjected to 10% SDS-PAGE and then transferred onto PVDF membranes. The membranes were then incubated at 4°C overnight with primary antibodies: anti-Bax (catalog n. #2772, CST), anti-Bcl-2 (catalog n. ab196495, Abcam), anti-cleaved caspase 3 (catalog n. AF7022, Affinity Biosciences, Cincinnati, OH, USA) and anti-β-actin (catalog n. 66009-1-Ig,

Proteintech, Rosemont, IL, USA), followed by incubation with the corresponding secondary antibody for 1 h. Subsequently, blot signals were visualized by the ECL reagent (catalog n. AS1059, ASPEN, Wuhan, China) and quantified using the AlphaEaseFC software (Alpha Innotech, San Leandro, CA, USA).

Statistical analysis

The results were expressed as the mean ± SD. One-way analysis of variance (ANOVA) followed by Tukey's tests were used to determine the differences among five groups; p-values of <0.05 were considered statistically significant. All data were repeated at least three times independently.

Results

Combination of tolvaptan and valsartan improved cardiac function in Dox-treated mice

To assess the effects of tolvaptan/valsartan combination on cardiac function in mice with HF, echocardiography was performed. LVEF, LVFS, LVESD and LVEDD were notably reduced in the model group, as shown in Figure 1 A-E. However, tolvaptan or valsartan alone treatment increased LVEF, LVFS, LVESD and LVEDD in Dox-treated mice compared to the model group. As expected, tolvaptan/valsartan combination further restore the LVEF, LVFS, LVESD and LVEDD in Dox-treated mice compared to tolvaptan or valsartan alone treatment group (Figure 1 A-E). Collectively, tolvaptan/valsartan combination could exert cardioprotective effect in Dox-treated mice.

Combination of tolvaptan and valsartan improved heart failure and kidney damage in Dox-treated mice

ALD and Ang II are major risk factors of cardiovascular and renal damage.[35,36] To investigate the protective effect of tolvaptan/valsartan combination on heart failure and kidney damage in mice, ALD and Ang II level was evaluated. As shown in Figure 2 A,B, compared to the control group, the level of serum ALD and Ang II was markedly elevated in Dox-treated mice. However, tolvaptan/valsartan combination treatment obviously reduced the serum ALD and Ang II level in Dox-treated mice compared to the model group (Figure 2 A,B). Collectively, tolvaptan/valsartan combination could improve heart failure and kidney damage in Dox-treated mice.

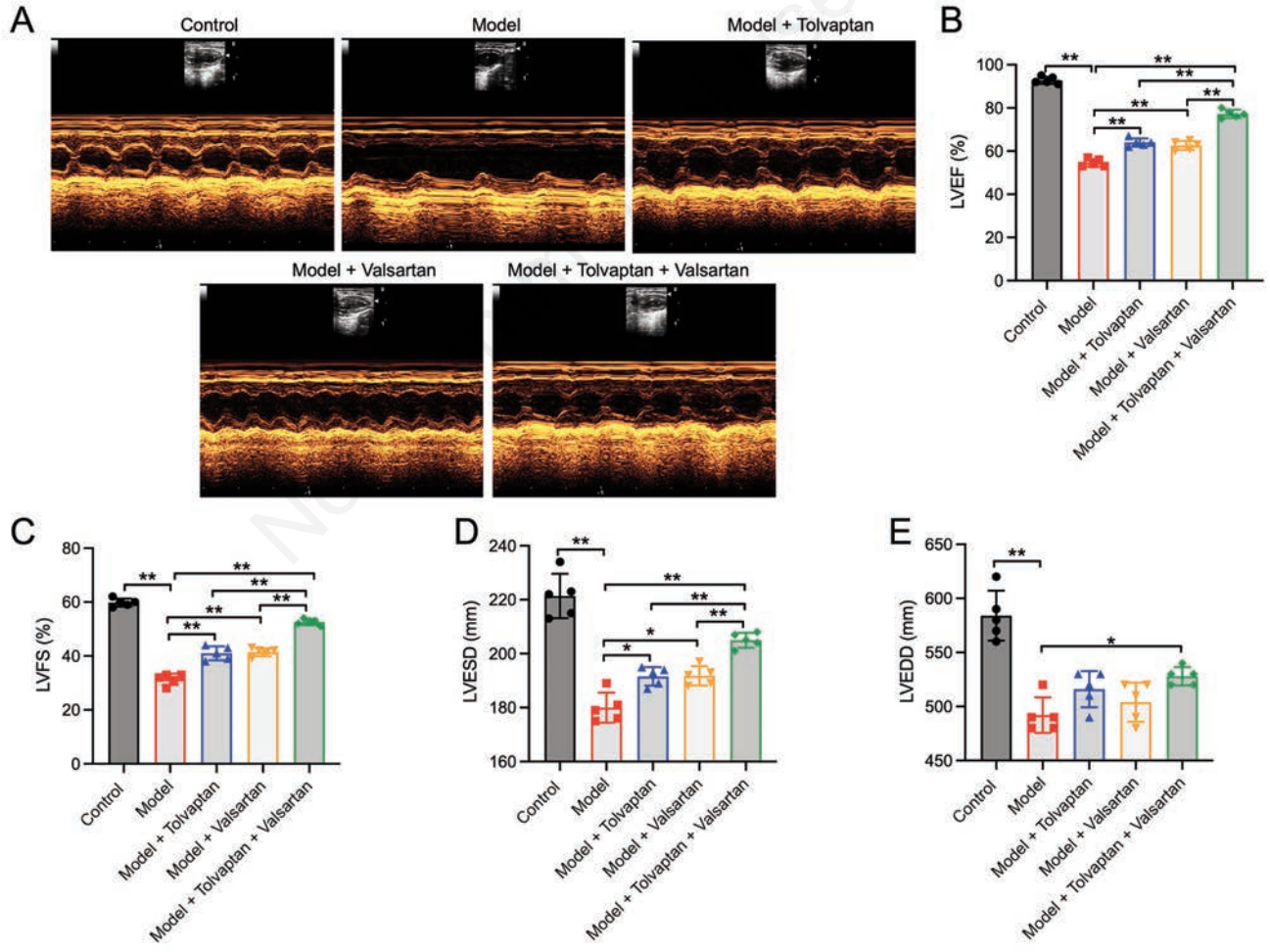

Figure 1. Combination of tolvaptan and valsartan improved cardiac function in Dox-treated mice. A) Representative image of echocardiography. B-E) The left ventricular parameters in each group including LVEF, LVFS, LVESD, LVEDD; *p<0.05, **p<0.01; n=5. The difference among five groups was performed by one-way ANOVA. LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end systolic diameter; LVFS, left ventricular fractional shortening.

Combination of tolvaptan and valsartan attenuated the inflammation and fibrosis in heart and kidney tissues in Dox-treated mice

Additionally, inflammation and fibrosis play important roles in HF.[37,38] As indicated in Figure 3 A-F, the level of pro-inflammatory factors IL-6 and TNF-a were remarkably increased in the serum samples, heart, and kidney tissues of Dox-treated mice compared to the control group; however, tolvaptan or valsartan treatment abolished those effects. As expected, tolvaptan/valsartan combination treatment further declined IL-6 and TNF-a level in Dox-treated mice compared to tolvaptan or valsartan alone treatment group (Figure 3 A-F). Furthermore, H&E staining assay showed that

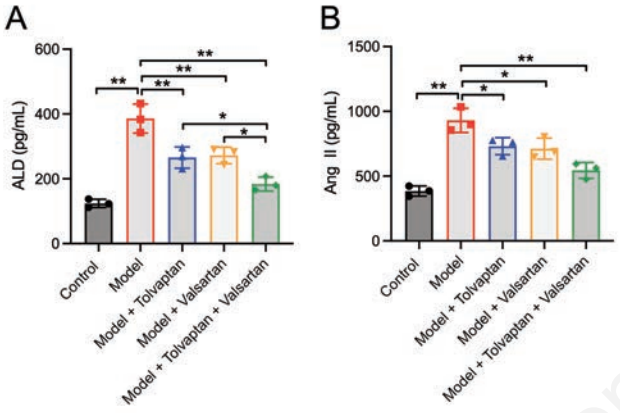

Figure 2. Combination of tolvaptan and valsartan improved heart failure and kidney damage in Dox-treated mice. A,B) ELISA was used to detect ALD and Ang II level in serum of mice; *p<0.05, **p<0.01; n=3. The difference among five groups was determined with one-way ANOVA. ALD, aldosterone; Ang II, angiotensin II.

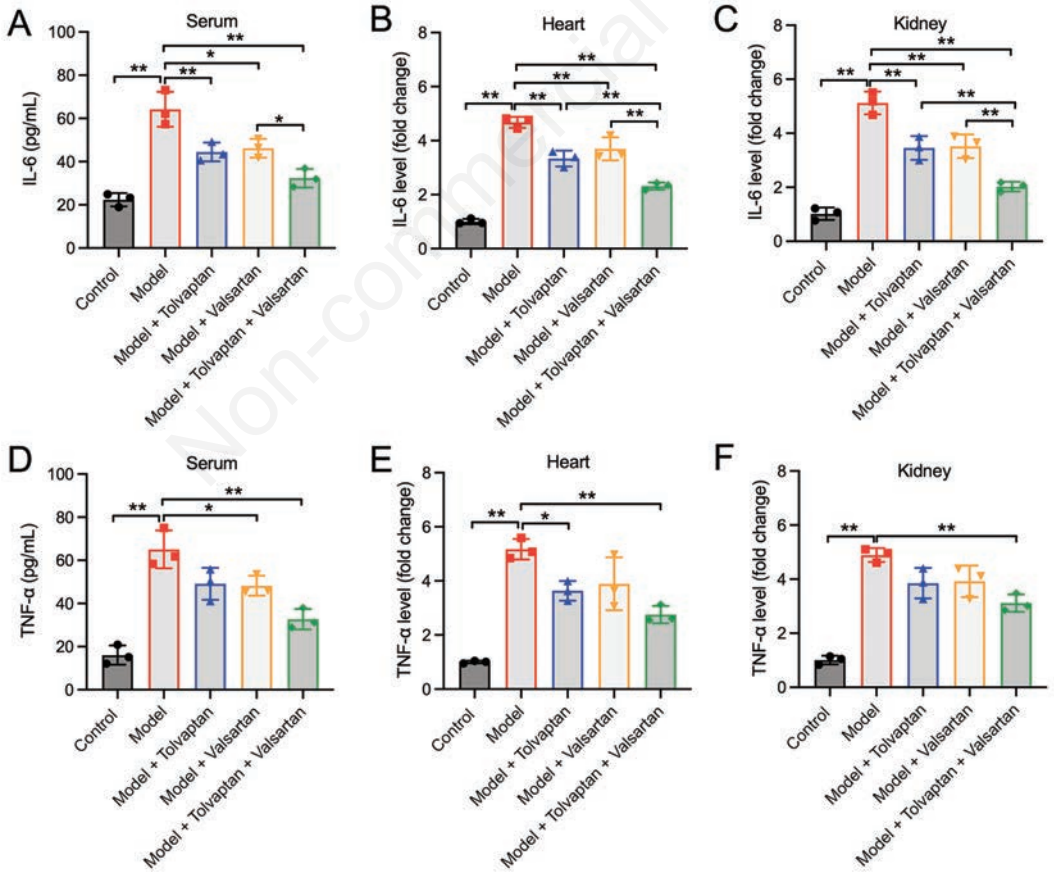

Figure 3. Combination of tolvaptan and valsartan attenuated the inflammation in heart and kidney tissues in Dox-treated mice. A-C) ELISA was used to assess IL-6 level in serum, heart, and kidney samples of mice. D-F) ELISA was used to assess TNF-a level in serum, heart, and kidney samples of mice; *p<0.05, **p<0.01; n=3. The difference among five groups was determined with one-way ANOVA. IL-6, interleukin 6; TNF-a, tumor necrosis factor-alpha.

inflammatory cell infiltration was observed in heart and kidney tissues from Dox-treated mice, whereas this phenomenon was reversed by tolvaptan/valsartan combination treatment (Figure 4 A,B; Supplementary Figures 1 A,B and 2 A,B). Moreover, Masson's staining assay showed that a widespread collagen deposition was observed in heart and kidney tissues from Dox-treated mice compared to the control group (Figure 4 A,B; Supplementary Figures 1 A,B and 2 A,B). However, tolvaptan/valsartan combination treatment obviously reversed these changes (Figure 4 A,B; Supplementary Figures 1 A,B and 2 A,B). Collectively, tolvaptan/valsartan combination could attenuate the inflammation and fibrosis in heart and kidney tissues from Dox-treated mice.

Combination of tolvaptan and valsartan reduced cell apoptosis in heart and kidney tissues in Dox-treated mice

Myocardial cell apoptosis is central to the pathogenesis of HF.[39] Therefore, the effect of tolvaptan/valsartan combination on the apoptosis of heart and kidney tissues in Dox-treated mice was evaluated by TUNEL assay. As indicated in Figure 5 A,B, increased number of TUNEL-positive cells were detected in heart and kidney tissues of mice in the model group. However, tolvaptan/valsartan combination sharply reduced cell apoptosis in heart and kidney tissues from Dox-treated mice (Figure 5 A,B). Moreover, compared to the control group, Bax and cleaved caspase 3 level were increased, and Bcl-2 level was decreased in heart and kidney tissues of mice in the model group; however, these protein levels were notably reversed by tolvaptan/valsartan combination (Figure 6 A,B). To sum up, combination of tolvaptan and valsartan could reduce cell apoptosis in heart and kidney tissues in Dox-treated mice.

Discussion

It is common to observe renal dysfunction in patients with HF with preserved ejection fraction (pEF).[40] Meanwhile, HF is also a major cause of morbidity and mortality among CKD patients.[41] Thus, the link between HF and renal dysfunction is tight. Tolvaptan (an arginine vasopressin V2 receptor antagonist) and valsartan (an Ang II type 1 receptor blocker) are two well-known agents administrated for HF.[42-44] However, the role of tolvaptan/valsartan combination treatment on HF with renal dysfunction remains largely unclear. It has been shown that tolvaptan could improve renal function in patients with HF with pEF.[45] Combination of tolvaptan and furosemide obviously increased the urine volume in patients with HF complicated by renal dysfunction compared to tolvaptan alone treatment.[46] Sacubitril-valsartan com-

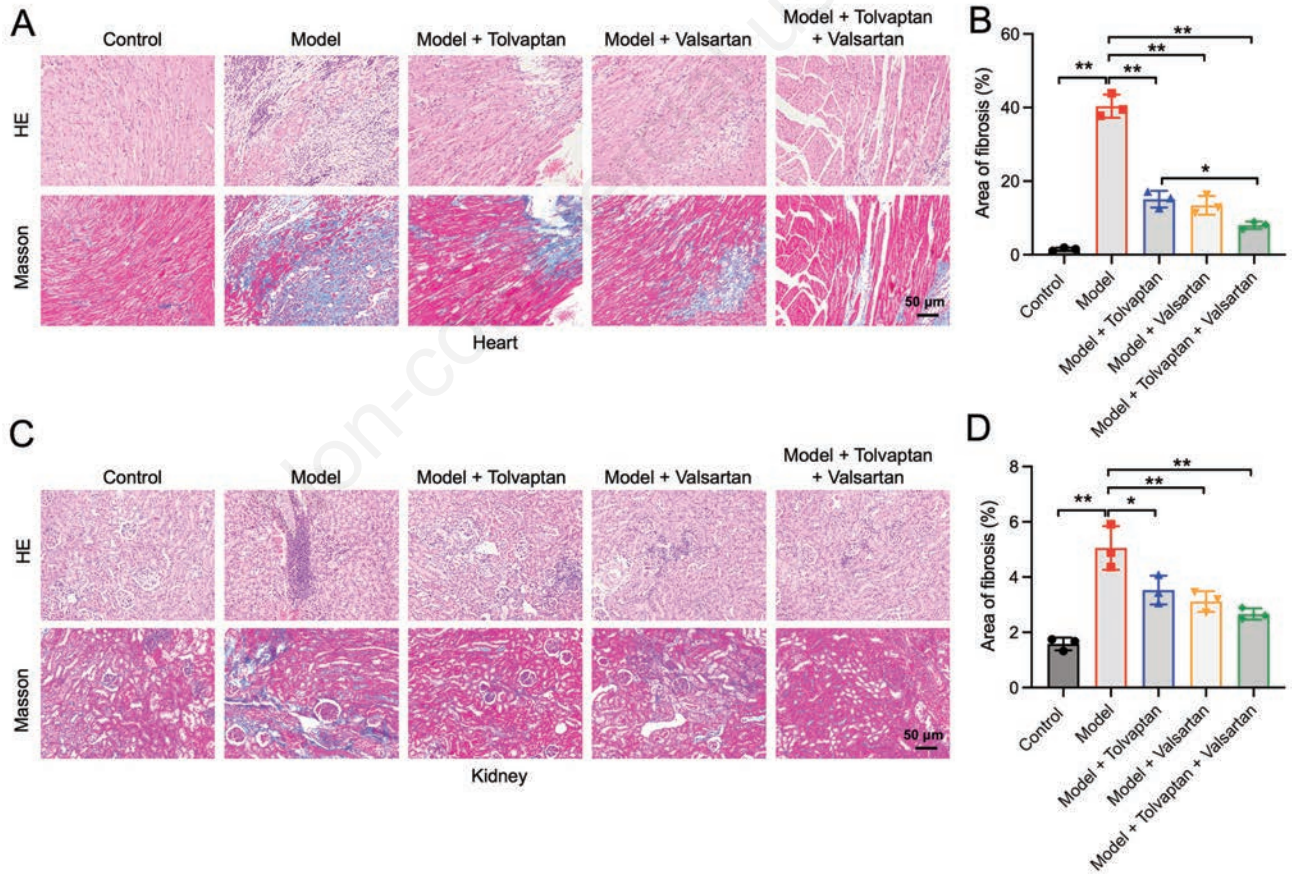

Figure 4. Combination of tolvaptan and valsartan attenuated the fibrosis in heart and kidney tissues in Dox-treated mice. A) HE staining analysis was used to evaluate the pathological changes in heart tissues of mice; the collagen deposition in heart tissues of mice were determined by Masson staining assay. B) Quantitative analysis of heart fibrosis. C) HE staining analysis was used to evaluate the pathological changes in kidney tissues of mice; the collagen deposition in kidney tissues of mice were determined by Masson staining assay. D) Quantitative analysis of kidney fibrosis; *p<0.05, **p<0.01; n=3. The difference among five groups was determined with one-way ANOVA. HE, hematoxylin and eosin.

bination obviously improved cardiac and renal function in patients with HF compared to valsartan alone treatment.[47,4]. In this study, we found that tolvaptan/valsartan combination significantly improved LVEF and LVFS and reduced serum ALD and Ang II level in Dox-treated mice compared to tolvaptan or valsartan alone treatment group. These results showed that compared to single treatment, tolvaptan/valsartan combination effectively improved cardiac functions in Dox-treated mice *via* blocking vasopressin V2 receptor and Ang II type 1 receptor. To the best of our knowledge, this study is the first to explore the effect of tolvaptan/valsartan combination on HF with renal dysfunction.

Cardiac fibrosis and inflammation are main drivers to the development of HF.[49,50] In addition, renal fibrosis is a major pathological feature of CKD.[38] Inhibition of fibrosis and inflammation could attenuate the progression of HF and CKD.[38,51] It has been shown that tolvaptan could improve cardiac function and ameliorate cardiac fibrosis and inflammation in rats with HF.[44] In addition, tolvaptan could ameliorate renal dysfunction and interstitial fibrosis in sensitive hypertensive rats with HF.[52] Moreover, valsartan treatment has been found to prevent renal inflammation and oxidative stress in mice with diabetes.[53] Meanwhile, Wang *et al*. showed that valsartan notably inhibited cardiac fibrosis in mice with diabetic nephropathy.[54] Consistent with the previous studies, our results showed that tolvaptan or valsartan alone treatment both attenuated the fibrosis and inflammation in heart and kidney tissues in Dox-treated mice. More importantly, tolvaptan/valsartan combination exhibited a better anti-fibrosis and anti-inflammation effects in Dox-treated mice compared to the single drug group.

Chen *et al*. found that Ang II could lead to the apoptosis of cardiomyocytes.[55] Wu *et al.* found that valsartan was able to prevent cardiac remodeling and myocardial apoptosis in rats with diabetic cardiomyopathy.[56] In this study, we found that compared to the single drug group, tolvaptan/valsartan combination remarkably inhibited the apoptosis in heart and kidney tissues in Dox-treated mice, as shown by the decreased Bax and cleaved caspase 3 level and increased Bcl-2 level. Thus, inhibition of cell apoptosis might be an effective strategy for the treatment of HF with renal dysfunction.

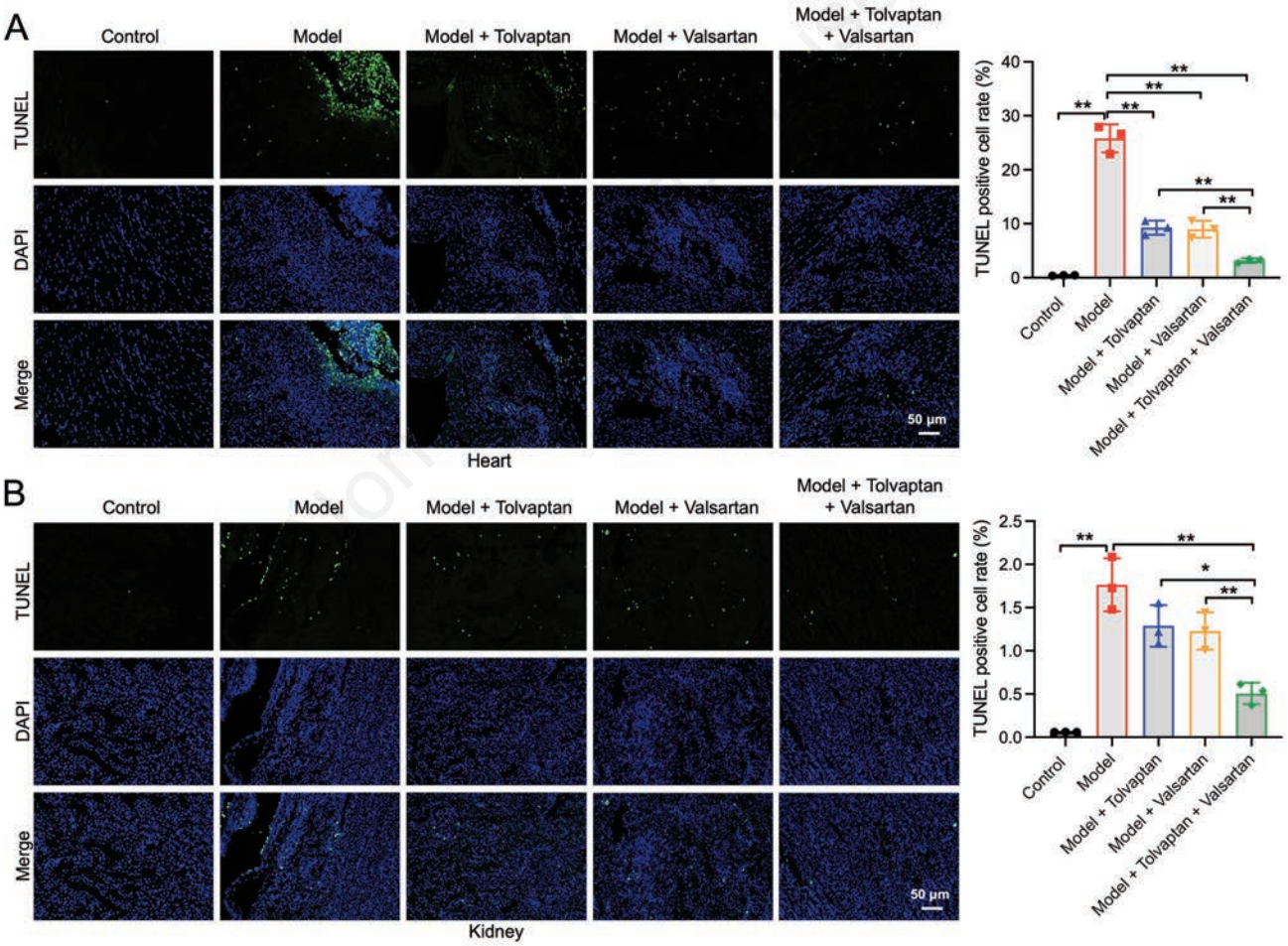

Figure 5. Combination of tolvaptan and valsartan reduced cell apoptosis in heart and kidney tissues in Dox-treated mice. A,B) TUNEL staining assay was performed to determine cell apoptosis (green color) in heart and kidney tissues of mice; *p<0.05, **p<0.01; n=3. The difference among five groups was determined with one-way ANOVA. DAPI, 4',6-diamidino-2-phenylindole; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling.

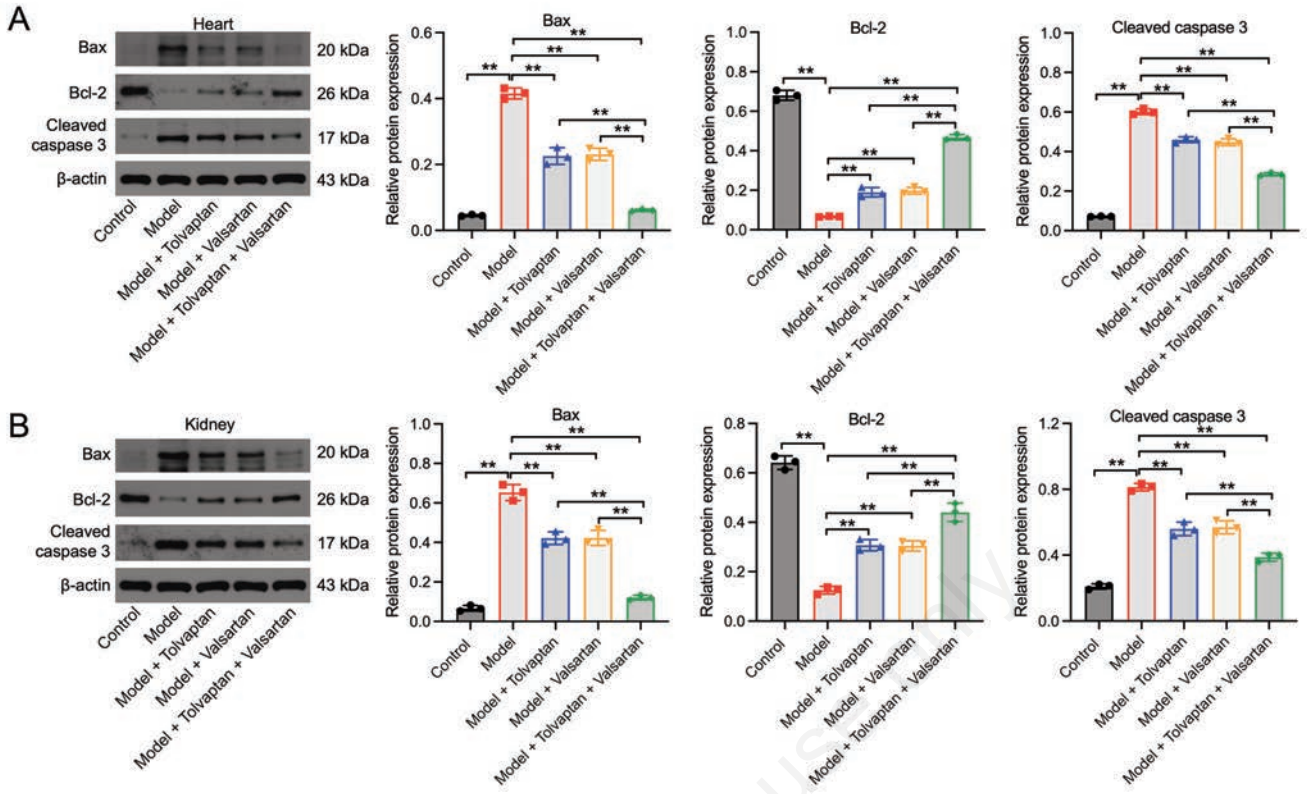

Figure 6. Combination of tolvaptan and valsartan reduced cell apoptosis in heart and kidney tissues in Dox-treated mice *via* Bcl-2/Bax/cleaved caspase-3 apoptotic pathway. A,B) Western blot assay was applied to detect Bax, Bcl-2 and cleaved caspase 3 level in heart and kidney tissues of mice; the Bax, Bcl-2 and cleaved caspase 3 protein expressions were quantified *via* normalization to β-actin; **p<0.01; n=3. The difference among five groups was determined with one-way ANOVA. Bax, Bcl-2 associated X; Bcl-2, B cell lymphoma-2.

Although tolvaptan/valsartan combination treatment exhibited cardiorenal protective effect in Dox-treated mice *in vivo*, the therapeutic effects of tolvaptan/valsartan combination on HF with renal dysfunction should be examined in both pre-clinical and clinical studies in the future.

Collectively, combination of tolvaptan and valsartan could improve cardiac functions, prevent cell fibrosis, inflammation and apoptosis in heart and kidney tissues in Dox-treated mice. These findings showed that combining tolvaptan with valsartan might be a promising approach to achieve enhanced therapeutic effect for treatment of HF with renal dysfunction.

References

1. Snipelisky D, Chaudhry SP, Stewart GC. The many faces of heart failure. Card Electrophysiol Clin 2019;11:11-20.
2. Smythe CM. Congestive heart failure. Tex Med 1993;89:52-7.
3. Wu T, Yao H, Zhang B, Zhou S, Hou P, Chen K. κ opioid receptor agonist inhibits myocardial injury in heart failure rats through activating Nrf2/HO-1 pathway and regulating Ca(2+)-SERCA2a. Oxid Med Cell Longev 2021;2021:7328437.
4. Scally C, Abbas H, Ahearn T, Srinivasan J, Mezincescu A, Rudd A, et al. Myocardial and systemic inflammation in acute stress-induced (Takotsubo) cardiomyopathy. Circulation 2019;1391581-92.
5. Yang W, Tu H, Tang K, Huang H, Ou S, Wu J. Reynoutrin Improves ischemic heart failure in rats via targeting S100A1. Front Pharmacol 2021;12:703962.
6. Post S, van den Broek AJ, Rensing BJ, Pasterkamp G, Goumans MJ, Doevendans PA. Reduced CD26 expression is associated with improved cardiac function after acute myocardial infarction: insights from the REPERATOR study. J Mol Cell Cardiol 2012;53:899-905.
7. Weckbach LT, Grabmaier U, Uhl A, Gess S, Boehm F, Zehrer A, et al. Midkine drives cardiac inflammation by promoting neutrophil trafficking and NETosis in myocarditis. J Exp Med 2019;216:350-68.
8. Farmakis D, Koeck T, Mullen W, Parissis J, Gogas BD, Nikolaou M, et al. Urine proteome analysis in heart failure with reduced ejection fraction complicated by chronic kidney disease: feasibility, and clinical and pathogenetic correlates. Eur J Heart Fail 2016;18:822-9.
9. Ahmed A, Rich MW, Sanders PW, Perry GJ, Bakris GL, Zile MR, et al. Chronic kidney disease associated mortality in diastolic versus systolic heart failure: a propensity matched study. Am J Cardiol 2007;99:393-8.
10. Shiba N, Shimokawa H. Chronic kidney disease and heart failure - Bidirectional close link and common therapeutic goal. J Cardiol 2011;57:8-17.
11. Vinod P, Krishnappa V, Chauvin AM, Khare A, Raina R. Cardiorenal syndrome: Role of arginine vasopressin and vaptans in heart failure. Cardiol Res 2017;8:87-95.
12. Giam B, Kaye DM, Rajapakse NW. Role of renal oxidative stress in the pathogenesis of the cardiorenal syndrome. Heart Lung Circ 2016;25:874-80.

13. Gasparini S, Melo MR, Nascimento PA, Andrade-Franzé GMF, Antunes-Rodrigues J, Yosten GLC, et al. Interaction of central angiotensin II and aldosterone on sodium intake and blood pressure. Brain Res 2019;1720:146299.
14. Itcho K, Oki K, Kobuke K, Ohno H, Yoneda M, Hattori N. Angiotensin 1-7 suppresses angiotensin II mediated aldosterone production via JAK/STAT signaling inhibition. J Steroid Biochem Mol Biol 2019;185:137-41.
15. Goldsmith SR. Vasopressin receptor antagonists: mechanisms of action and potential effects in heart failure. Cleve Clin J Med 2006;73:S20-3; discussion S30-3.
16. He FJ, Burnier M, Macgregor GA. Nutrition in cardiovascular disease: salt in hypertension and heart failure. Eur Heart J 2011;32:3073-80.
17. Felker GM, Ellison DH, Mullens W, Cox ZL, Testani JM. Diuretic Therapy for patients with heart failure: JACC state-of-the-art review. J Am Coll Cardiol 2020;75:1178-95.
18. Iida Y, Yoshitake A, Shimizu H. Safety and effectiveness of tolvaptan administration after total arch replacement. Ann Vasc Surg 2019;56:103-7.
19. Shigefuku R, Iwasa M, Eguchi A, Tempaku M, Tamai Y, Suzuki T, et al. Serum copeptin and zinc-α2-glycoprotein levels are novel biomarkers of tolvaptan treatment in decompensated cirrhotic patients with ascites. Intern Med 2021;60:3359-68.
20. Rangarajan B, Binoy V, Hingmire SS, Noronha V. Tolvaptan. South Asian J Cancer 2014;3:182-4.
21. Alskaf E, Tridente A, Al-Mohammad A. Tolvaptan for heart failure, systematic review and meta-analysis of trials. J Cardiovasc Pharmacol 2016;68:196-203.
22. 22 Futamura Y, Watanuki H, Okada M, Sugiyama K, Matsuyama K. The efficacy and renal protective effect of tolvaptan in chronic kidney disease patients after open-heart surgery. Ann Thorac Cardiovasc Surg 2021;27:317-21.
23. Cohn JN, Tognoni G. A randomized trial of the angiotensin-receptor blocker valsartan in chronic heart failure. N Engl J Med 2001;345:1667-75.
24. Maslov MY, Foianini S, Mayer D, Orlov MV, Lovich MA. Interaction between sacubitril and valsartan in preventing heart failure induced by aortic valve insufficiency in rats. J Card Fail 2019;25:921-31.
25. Lesogor A, Cohn JN, Latini R, Tognoni G, Krum H, Massie B, et al. Interaction between baseline and early worsening of renal function and efficacy of renin-angiotensin-aldosterone system blockade in patients with heart failure: insights from the Val-HeFT study. Eur J Heart Fail 2013;15:1236-44.
26. Kang H, Zhang J, Zhang X, Qin G, Wang K, Deng Z, et al. Effects of sacubitril/valsartan in patients with heart failure and chronic kidney disease: A meta-analysis. Eur J Pharmacol 2020;884:173444.
27. Chen L, Yan KP, Liu XC, Wang W, Li C, Li M, et al. Valsartan regulates TGF-β/Smads and TGF-β/p38 pathways through lncRNA CHRF to improve doxorubicin-induced heart failure. Arch Pharm Res 2018;41:101-9.
28. Mann DL, Givertz MM, Vader JM, Starling RC, Shah P, McNulty SE, et al. Effect of treatment with sacubitril/valsartan in patients with advanced heart failure and reduced ejection fraction: A randomized clinical trial. JAMA Cardiol 2022;7:17-25.
29. Vaduganathan M, Claggett BL, Desai AS, Anker SD, Perrone SV, Janssens S, et al. Prior heart failure hospitalization, clinical outcomes, and response to sacubitril/valsartan compared with valsartan in HFpEF. J Am Coll Cardiol 2020;75:245-54.
30. Cosentino ER, Degli Esposti D, Miceli R, Bentivenga C, Landolfo M, Fg Cicero A, et al. Sacubitril/valsartan improves both functional and echocardiographic parameters in patients with chronic heart failure with reduced ejection fraction. Curr Med Res Opin 2019;35:s9-12.
31. Li WJ, Liao HH, Feng H, Zhou ZY, Mou SQ, Zhang N, et al. Combination treatment of perifosine and valsartan showed more efficiency in protecting against pressure overload induced mouse heart failure. J Pharmacol Sci 2020;143:199-208.
32. Xu S, Wang Y, Yu M, Wang D, Liang Y, Chen Y, et al. LongShengZhi capsule inhibits doxorubicin-induced heart failure by anti-oxidative stress. Biomed Pharmacother 2020;123:109803.
33. Sugihara K, Fujimoto S, Motomiya Y, Hashimoto T, Nakamura S, Dohi K. Usefulness of long axis M-mode echocardiographic measurements for optimum dialysis in patients on maintenance hemodialysis: comparison with changes in plasma levels of atrial natriuretic peptide and brain natriuretic peptide. Clin Nephrol 2001;56:140-9.
34. Wang J, Fang F, Wai-Kwok Yip G, Sanderson JE, Feng W, Xie JM, et al. Left ventricular long-axis performance during exercise is an important prognosticator in patients with heart failure and preserved ejection fraction. Int J Cardiol 2015;178:131-5.
35. Struthers AD, MacDonald TM. Review of aldosterone- and angiotensin II-induced target organ damage and prevention. Cardiovasc Res 2004;61:663-70.
36. Frimodt-Møller M, Persson F, Rossing P. Mitigating risk of aldosterone in diabetic kidney disease. Curr Opin Nephrol Hypertens 2020;29:145-51.
37. Oppedisano F, Mollace R, Tavernese A, Gliozzi M, Musolino V, Macrì R, et al. PUFA supplementation and heart failure: Effects on fibrosis and cardiac remodeling. Nutrients 2021;13:2965.
38. Bai M, Lei J, Wang S, Ding D, Yu X, Guo Y, et al. BMP1 inhibitor UK383,367 attenuates renal fibrosis and inflammation in CKD. Am J Physiol Renal Physiol 2019;317:F1430-f8.
39. Chen C, Chen H, Zhou HJ, Ji W, Min W. Mechanistic role of thioredoxin 2 in heart failure. Adv Exp Med Biol 2017;982:265-76.
40. Ter Maaten JM, Damman K, Verhaar MC, Paulus WJ, Duncker DJ, Cheng C, et al. Connecting heart failure with preserved ejection fraction and renal dysfunction: the role of endothelial dysfunction and inflammation. Eur J Heart Fail 2016;18:588-98.
41. Tuegel C, Bansal N. Heart failure in patients with kidney disease. Heart 2017;103:1848-53.
42. Konstam MA, Gheorghiade M, Burnett JC, Jr., Grinfeld L, Maggioni AP, Swedberg K, et al. Effects of oral tolvaptan in patients hospitalized for worsening heart failure: the EVEREST Outcome Trial. JAMA 2007;297:1319-31.
43. Jackson AM, Jhund PS, Anand IS, Düngen HD, Lam CSP, Lefkowitz MP, et al. Sacubitril-valsartan as a treatment for apparent resistant hypertension in patients with heart failure and preserved ejection fraction. Eur Heart J 2021;42:3741-52.
44. de Ligt M, Hesselink MKC, Jorgensen J, Jocken JWE, Blaak EE, Goossens GH. The angiotensin II type 1 receptor blocker valsartan in the battle against COVID-19. Obesity (Silver Spring) 2021;29:1423-6.
45. Tamaki S, Sato Y, Yamada T, Morita T, Furukawa Y, Iwasaki Y, et al. Tolvaptan reduces the risk of worsening renal function in patients with acute decompensated heart failure and preserved left ventricular ejection fraction - Prospective randomized controlled study. Circ J 2017;81:740-7.
46. Kida K, Shibagaki Y, Tominaga N, Matsumoto N, Akashi YJ, Miyake F, et al. Efficacy of tolvaptan added to furosemide in heart failure patients with advanced kidney dysfunction: a

pharmacokinetic and pharmacodynamic study. Clin Pharmacokinet 2015;54:273-84.
47. McMurray JJV, Jackson AM, Lam CSP, Redfield MM, Anand IS, Ge J, et al. Effects of sacubitril-valsartan versus valsartan in women compared with men with heart failure and preserved ejection fraction: Insights from PARAGON-HF. Circulation 2020;141:338-51.
48. Peikert A, Vaduganathan M, Mc Causland F, Claggett BL, Chatur S, Packer M, et al. Effects of sacubitril/valsartan versus valsartan on renal function in patients with and without diabetes and heart failure with preserved ejection fraction: insights from PARAGON-HF. Eur J Heart Fail 2022;24:794-803.
49. Martínez-Martínez E, Brugnolaro C, Ibarrola J, Ravassa S, Buonafine M, López B, et al. CT-1 (cardiotrophin-1)-Gal-3 (Galectin-3) axis in cardiac fibrosis and inflammation. Hypertension 2019;73:602-11.
50. Rao M, Wang X, Guo G, Wang L, Chen S, Yin P, et al. Resolving the intertwining of inflammation and fibrosis in human heart failure at single-cell level. Basic Res Cardiol 2021;116:55.
51. Chang D, Xu TT, Zhang SJ, Cai Y, Min SD, Zhao Z, et al. Telmisartan ameliorates cardiac fibrosis and diastolic function in cardiorenal heart failure with preserved ejection fraction. Exp Biol Med (Maywood) 2021;246:2511-21.
52. Ishikawa M, Kobayashi N, Sugiyama F, Onoda S, Ishimitsu T. Renoprotective effect of vasopressin v2 receptor antagonist tolvaptan in Dahl rats with end-stage heart failure. Int Heart J 2013;54:98-106.
53. Zhou G, Cheung AK, Liu X, Huang Y. Valsartan slows the progression of diabetic nephropathy in db/db mice via a reduction in podocyte injury, and renal oxidative stress and inflammation. Clin Sci (Lond) 2014;126707-20.
54. Wang J, Duan L, Gao Y, Zhou S, Liu Y, Wei S, et al. Angiotensin II receptor blocker valsartan ameliorates cardiac fibrosis partly by inhibiting miR-21 expression in diabetic nephropathy mice. Mol Cell Endocrinol 2018;472:149-58.
55. Chen QM, Tu VC. Apoptosis and heart failure: mechanisms and therapeutic implications. Am J Cardiovasc Drugs 2002;2:43-57.
56. Wu T, Dong Z, Geng J, Sun Y, Liu G, Kang W, et al. Valsartan protects against ER stress-induced myocardial apoptosis via CHOP/Puma signaling pathway in streptozotocin-induced diabetic rats. Eur J Pharm Sci 2011;42:496-502.

Received for publication: 21 September 2022. Accepted for publication: 2 November 2022.

Licensee PAGEPress, Italy
European Journal of Histochemistry 2022; 66:3563
doi:10.4081/ejh.2022.3563