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ORIGINAL RESEARCH ARTICLE

Sacubitril/valsartan decreases mortality in the rat model of the isoprenaline-induced takotsubo-like syndrome

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Abstract

Aims Takotsubo syndrome (TTS) is an acute potentially reversible cardiac syndrome characterized by variable regional myocardial akinesia that cannot be attributed to a culprit coronary artery occlusion. TTS is an important differential diagnosis of acute heart failure where brain natriuretic peptides are elevated. Sacubitril/valsartan is a novel and effective pharmacological agent for the treatment of patients with heart failure. Our aim was to explore whether treatment with sacubitril/valsartan could prevent isoprenaline-induced takotsubo-like phenotype in rats.

Methods and results A total number of 186 Sprague–Dawley male rats were randomized to receive pretreatment with water (CONTROL, *n* = 62), valsartan (VAL, *n* = 62), or sacubitril/valsartan (SAC/VAL, *n* = 62) before receiving isoprenaline for induction of TTS. We recorded heart rate and blood pressure invasively. Cardiac morphology and function were evaluated by high-resolution echocardiography 90 min after the administration of isoprenaline. We documented the survival rate at the time of echocardiography. Compared with the CONTROL group, the SAC/VAL group had less pronounced TTS-like cardiac dysfunction and lower mortality rate, while the VAL group did not differ. Heart rate and blood pressure were not significantly different between the groups. Analysis of cardiac lipids was performed with mass spectrometry. The VAL and SAC/VAL groups had significantly higher levels of lysophosphatidylcholine (LPC), in particular LPC 18:1 and LPC 16:0.

Conclusions Pretreatment with sacubitril/valsartan but not with valsartan reduces mortality and attenuates isoprenaline-induced apical akinesia in the TTS-like model in rats. Sacubitril/valsartan could be a potential treatment option in patients with TTS in humans.

Keywords Takotsubo syndrome; Isoprenaline; Sacubitril/valsartan; Valsartan; Lipidomics

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Introduction

Takotsubo syndrome (TTS), ever since been reported by Sato *et al*. in 1990,[1] is an increasingly recognized acute heart failure syndrome. The typical phenotype of TTS is characterized by the akinetic apex and hyperkinetic base of the left ventricle (LV).[2] In contrast, atypical phenotypes of TTS are characterized by variable LV regional akinesia that spares the apex.[3,4] TTS has a similar clinical presentation to acute myocardial infarction and acute heart failure. TTS is not as benign as initially thought because of the risk of serious complications.[5] Mortality was shown to be similar between TTS and acute myocardial infarction.[6] Treatment of patients with TTS is based on expert opinion, and we lack evidence from randomized clinical trials. Consequently, preclinical trials in relevant animal models are essential for understanding TTS pathophysiology and

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evaluating promising pharmacological strategies for the treatment of TTS in humans.

Takotsubo syndrome patients, like those with heart failure,[7,8] have elevated brain natriuretic peptide (BNP) levels.[9–11] Sacubitril/valsartan (Entresto®)—a drug that inhibits degradation of natriuretic peptides and increases BNP[12]—has been shown to substantially lower mortality in patients with heart failure with reduced ejection fraction.[13,14] The addition of sacubitril/valsartan to standard therapy in heart failure led to decreased cardiac sympathetic activity and increase survival.[15] While sacubitril/valsartan is approved for the treatment of heart failure,[16] its potential for the treatment of TTS has been explored neither in experimental nor in clinical settings.

In the rat model of TTS established by our group, isoprenaline induces TTS-like phenotype with typical apical akinesia in LV,[17–19] a phenotype that is exaggerated by cardio-stimulants and prevented by beta-blockers.[20] TTS phenotype in this rat model is also characterized by pronounced intracellular lipid accumulation in cardiomyocytes. Sacubitril/valsartan is a combination drug composed of neprilysin inhibitor (sacubitril) and valsartan in a 1.1 ratio.[21] Neprilysin inhibition decreases cardiac sympathetic activity[15] and attenuates myocardial hypoperfusion induced by isoprenaline.[22] Thus, it can be hypothesized that sacubitril could be beneficial in the setting of TTS.

We aimed, therefore, to explore whether treatment with sacubitril/valsartan could prevent isoprenaline-induced TTS-like phenotype and how sacubitril/valsartan affects myocardial lipid metabolism in rats.

Methods

Animals and animal handling

We followed NIH guidelines for the use of experimental animals, and the Animal Ethics committee at Gothenburg University approved the study protocol. In this study, a total number of 186 Sprague–Dawley male rats were used. The rats were kept in a temperature-controlled facility (25°C) with 12 h light/dark cycle and were given free access to both food and water. We used a combination of ketamine (50 mg/kg) and midazolam (5 mg/kg) for anaesthesia to allow echocardiographic studies and haemodynamic monitoring. The rats were anaesthetised, and hair was removed from the anterior surface of the neck and the chest with an electrical clipper and hair removal cream. The rats were then placed in a supine position on a heating pad to keep the body temperature at 38°C throughout the experimental protocol. Maintenance anaesthesia during the experimental period was ensured by administering additional doses of ketamine and midazolam.

Study protocol

The animals were randomized into three groups to receive daily treatment with sacubitril/valsartan 68 mg/kg (SAC/VAL, $n = 62$), valsartan 31 mg/kg (VAL, $n = 62$), or water (CONTROL, $n = 62$) for 3 days. Sacubitril/valsartan and valsartan were prepared according to 'Guidance to investigators for formulating and administering LCZ696-ABA and valsartan to rats' (Novartis).[23] Sacubitril/valsartan was formulated in water at a concentration required for administration. Valsartan was first dissolved in 1 N NaOH to generate a stock solution of 200 mg/mL after which water was added to generate a solution at a concentration required for administration. On the third day, all animals received isoprenaline 50 mg/kg as a single intraperitoneal dose. A subset of rats ($n = 8$) from each of the three groups were used for the invasive study of haemodynamics. We based this study on our rat model of isoprenaline-induced TTS-like cardiac dysfunction.[17] Hearts and blood were collected after echocardiography.

Invasive haemodynamic recording

Continuous invasive blood pressure and heart rate recording were obtained over 90 min while the rats were kept anaesthetized. The right common carotid artery was dissected free, cannulated, and connected via a pressure sensor (Pharmlab Astra Zeneca, Möndal, Sweden). We ensured that the rats were adequately anaesthetized for the whole duration of the experiment. After invasive haemodynamic recordings, the rats underwent echocardiographic evaluation. We harvested hearts and blood before euthanasia.

Echocardiography

We used VisualSonics 770 VEVO imaging station equipped with a 35 MHz linear transducer (RMV 707) and an integrated rail system for consistent positioning of the transducer to study cardiac function. An optimal parasternal long-axis view (i.e. visualization of both the mitral and aortic valves and maximum distance between the aortic valve and the cardiac apex) was achieved. A cine loop of >1000 frames was acquired using the electrocardiogram-gated kilohertz visualization technique. The extent of akinesia was traced in the long axis along the endocardial border and expressed as a percentage of total LV endocardial length. Fractional area change, an index of cardiac function, was calculated as FS = (EDA − ESA)/EDA, where EDA and ESA are end-diastolic and end-systolic areas, respectively. We defined the presence of apical ballooning when the extent of akinesia was $\geq 20\%$.

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Lipid analyses

In total, 30 lipid species and two derived lipid variables were analysed in N = 10 samples each of CONTROL, VAL, and SAC/VAL. Rat heart biopsies were homogenized in butanol:methanol (3:1 vol/vol) for 5 min at 25 Hz using a Mixer Mill instrument (Retch, Haan, Germany). Known amounts of heptadecanoyl-containing internal standards [phosphatidylcholine 17:0/17:0 and lysophosphatidylcholine (LPC) 17:0] were added to the samples before extraction, which was performed as described previously.[24] The lipid extracts were evaporated under a stream of nitrogen and reconstituted in chloroform–methanol (2:1 vol/vol) and stored at −20°C until further analysis. For mass spectrometry, a small volume of the total extracts were diluted 1:400 in chloroform:methanol (1:2) with 5 mM ammonium acetate and infused into a QTRAP 5500 mass spectrometer using the robotic nanoflow ion source TriVersa NanoMate (Advion BioSciences, Ithaca, NJ, USA). Phosphatidylcholines were detected using precursor ion scanning in positive mode using m/z 184.1 as fragment ion.[25,26]

Statistics

We used the Shapiro–Wilk test and histograms to test whether variables were normally distributed. Stata software (Version 16.1) was used for all statistical analyses. The Kruskal–Wallis or Mann–Whitney test was used for comparison of akinesia between groups. For comparison of blood pressure and heart rate over time between groups, we used a mixed linear model. The χ^2 test for trend was used for mortality comparison. Data were expressed as mean ± SEM and median with interquartile range (IQR). $P < 0.05$ was considered significant. For the lipid-data analysis, R Version 3.6.3 was used. Linear discriminant analysis was performed using package 'MASS'. Significance tests were performed using the Mann–Whitney U test.

Results

Left ventricular function and apical akinesia

The indices of LV function and morphology are presented in Table 1. The median degree of akinesia was 8.2% (IQR 0–19.4%), and apical akinesia (LV ballooning) was present in 23.3% of rats in the CONTROL group. In the VAL group, the median akinesia was 6.6% (IQR 0–11.5, P = 0.129 vs. CONTROL), while the incidence of LV ballooning was 5.7% (P = 0.041 vs. CONTROL). In the SAC/VAL group, the median of akinesia was 0% (IQR 0–9.0, P = 0.031 vs. CONTROL), and 5.6% of animals had LV ballooning (P = 0.037 vs. CONTROL) (*Figures 1* and *2*, Supporting Information, *Videos S1–S3*).

Blood pressure and heart rate

Systolic and diastolic blood pressure decreased in all groups at 60 min post-isoprenaline administration. There were no

Figure 1 Effect of pretreatment with sacubitril/valsartan and valsartan on the incidence of akinesia in isoprenaline-induced TTS-like model in rats in the left ventricle. CONTROL, isoprenaline; SAC/VAL, sacubitril/valsartan; VAL, valsartan. *$P < 0.001$ versus CONTROL.

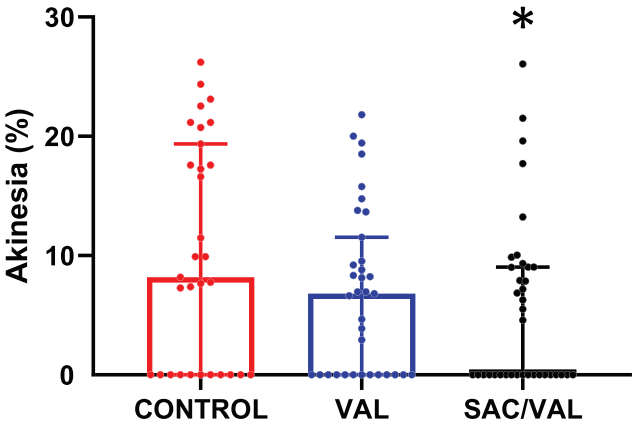
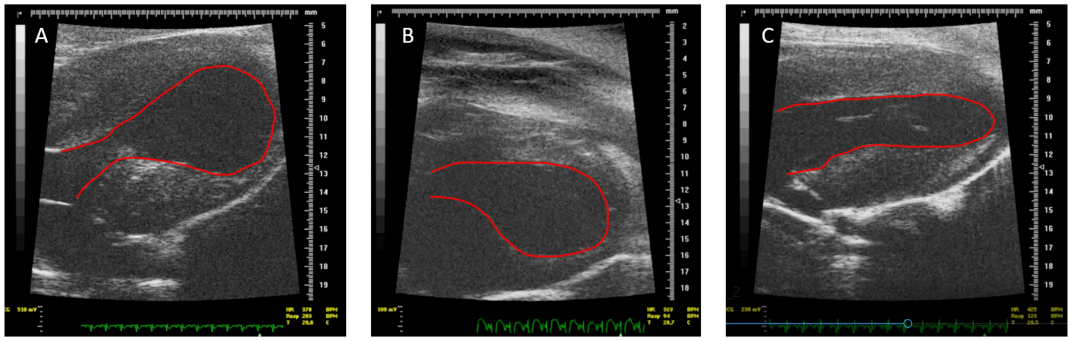


Table 1 Echocardiography

	Control	Valsartan	Sacubitril/valsartan
Akinesis (%)	10.2 (9.2)	6.6 (6.9)	5.4 (7.0)*
End-diastolic area (mm^2)	49.2 (8.8)	51.1 (7.47)	49.1 (6.9)
End-systolic area (mm^2)	33.6 (10.6)	32.3 (7.2)	31.4 (8.4)
Fractional area change (%)	31.5 (16.8)	36.9 (9.2)*	37.8 (16.8)
LV volume in diastole (mm^2)	206.8 (71.6)	216.1 (56.5)	198.3 (46.2)
LV volume in systole (mm^2)	103.0 (54.1)	91.6 (37.9)	88.4 (40.3)
Stroke volume (μL)	103.7 (69.7)	124.4 (37.5)*	109.9 (40.1)
Cardiac output (mL/min)	44.6 (29.7)	51.2 (25.9)*	48.0 (18.8)
Ejection fraction (%)	49.5 (18.9)	57.7 (11.0)*	56.0 (16.0)

LV, left ventricular.
*$P < 0.05$ versus CONTROL.

Figure 2 Effect of pretreatment with sacubitril/valsartan and valsartan on systolic blood pressure (A) and diastolic blood pressure (B) in isoprenaline-induced TTS-like model in rats. CONTROL, isoprenaline; SAC/VAL, sacubitril/valsartan; VAL, valsartan.



significant differences in systolic and diastolic blood pressure among the groups (time–treatment interaction $P > 0.05$) (*Figure 3A* and *3B*). Heart rate increased in all groups after isoprenaline administration. There were no significant differences in heart rate between the groups (*Figure 4*).

Mortality

Mortality was 24% in the CONTROL group, 23% in the VAL group ($P = 0.895$ vs. CONTROL), and 13% in the SAC/VAL group ($P = 0.0150$ vs. CONTROL) (*Figure 5*).

Lipid analyses

In the subset of samples ($N = 10$ each of CONTROL, VAL, and SAC/VAL) analysed for heart–lipid alterations, we found alterations in the myocardial content of LPC (*Figure 6*). Comparing the CONTROL group to the VAL and SAC/VAL groups, we found LPC 18:1, LPC 16:0, and the sum of all LPCs to be significantly higher (false discovery rate-adjusted *P*-values of 0.010, 0.037, and 0.041, respectively). No other statistically significant differences were found.

Discussion

The main finding in this study is that sacubitril/valsartan reduced mortality and apical akinesia in isoprenaline-induced TTS-like model in rats.

Sacubitril/valsartan—a novel approach to heart failure therapy with angiotensin receptor and neprilysin inhibition—has been shown to reduce cardiovascular mortality by 20% and overall mortality by 16% on top of modern treatment with angiotensin-converting enzyme inhibitors, beta-blockade aldosterone antagonists, cardiac resynchronization therapy, and implantable cardioverter–defibrillator.[13] Neprilysin is a neutral endopeptidase involved in the metabolism of several vasoactive peptides. Sacubitril blocks the action of neprilysin, resulting in higher levels of peptides, including natriuretic peptides, bradykinin, and adrenomedullin, which have vasodilator properties, facilitate sodium excretion, and counteract pathologic cardiac remodelling. Sacubitril/valsartan is approved for the treatment of heart failure[16] based on the exceptionally positive data from the PARADIGM-HF trial,[13] and this drug is currently widely used for the treatment of patients with chronic heart failure. While studies are ongoing to evaluate the potential of sacubitril/valsartan for therapy of acute post-infarction heart failure,[27] sacubitril/valsartan has not been tested either in experimental or in clinical settings for the treatment of TTS. Our aim in this study was not to address the intricate cellular and molecular mechanisms but rather to provide initial evidence that of sacubitril/valsartan may be effective for treating TTS with a simple and straightforward experimental design.

Pretreatment with valsartan improved LV function but did not reduce mortality nor apical akinesia. Indices of LV systolic function were numerically higher in the sacubitril/valsartan than in the control group. Still, these differences did not reach statistical significance due to the higher dispersion of measurements. Haemodynamic variables, heart rate, and blood pressure were similar between the groups suggesting that positive effects of sacubitril/valsartan are not primarily mediated by alteration in heart rate and blood pressure—two critical determinants of the development of apical akinesia in the rat model.[28] We have shown that interventions with adenosine, isoflurane, and beta-blockers have positive effects on cardiac function and morphology in small-animal models of the TTS-like phenotype. However, none of these treatments decreased mortality in such quantity as sacubitril/valsartan.

Treatment with sacubitril/valsartan as well as with valsartan alone has altered intracellular lipid metabolism. We are not aware of previous studies specifically addressing the effects of these two pharmacological agents on myocardial lipid metabolism, which is surprising because the lipid metabolism is profoundly altered in the failing heart.[29–31] Lipid metabolism is essential for maintenance of normal

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Figure 3 Effect of pretreatment with sacubitril/valsartan and valsartan on heart rate in isoprenaline-induced TTS-like model in rats.

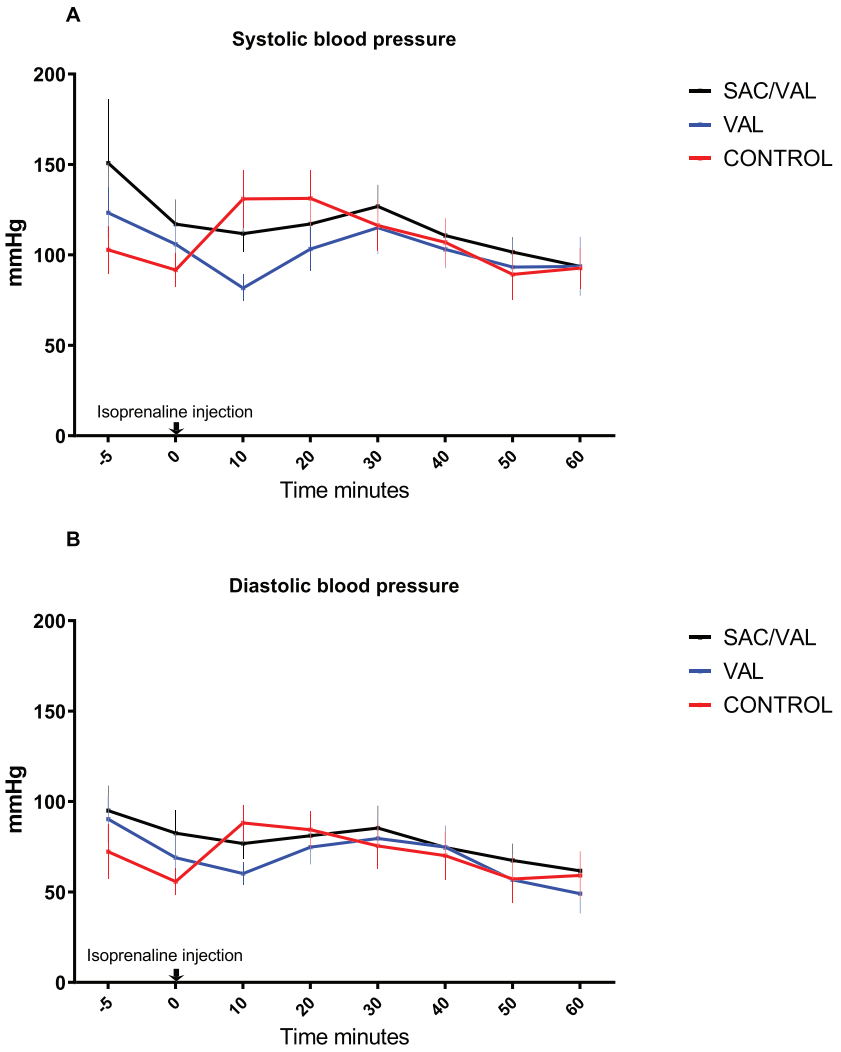

Figure 4 Effect of pretreatment with sacubitril/valsartan and valsartan on mortality in isoprenaline-induced TTS-like model in rats. CONTROL, isoprenaline; SAC/VAL, sacubitril/valsartan; VAL, valsartan. *P = 0.015 versus CONTROL.

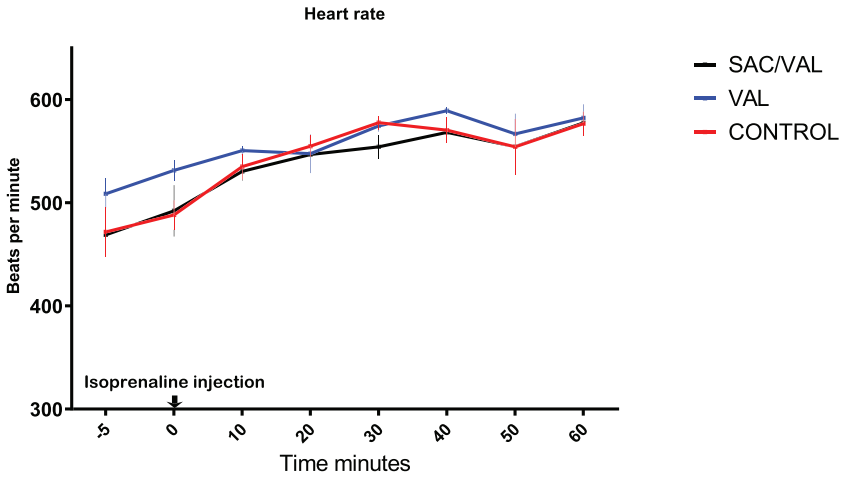

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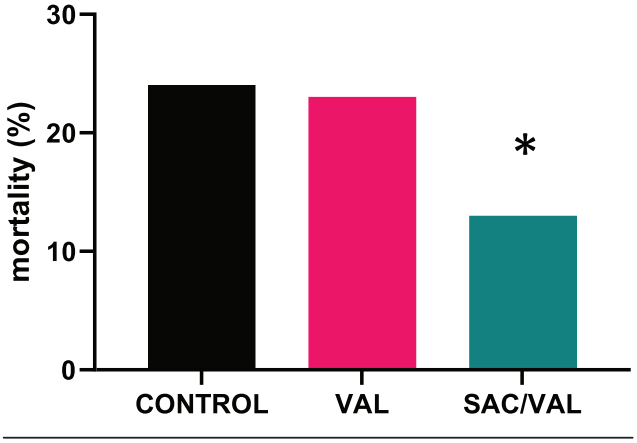
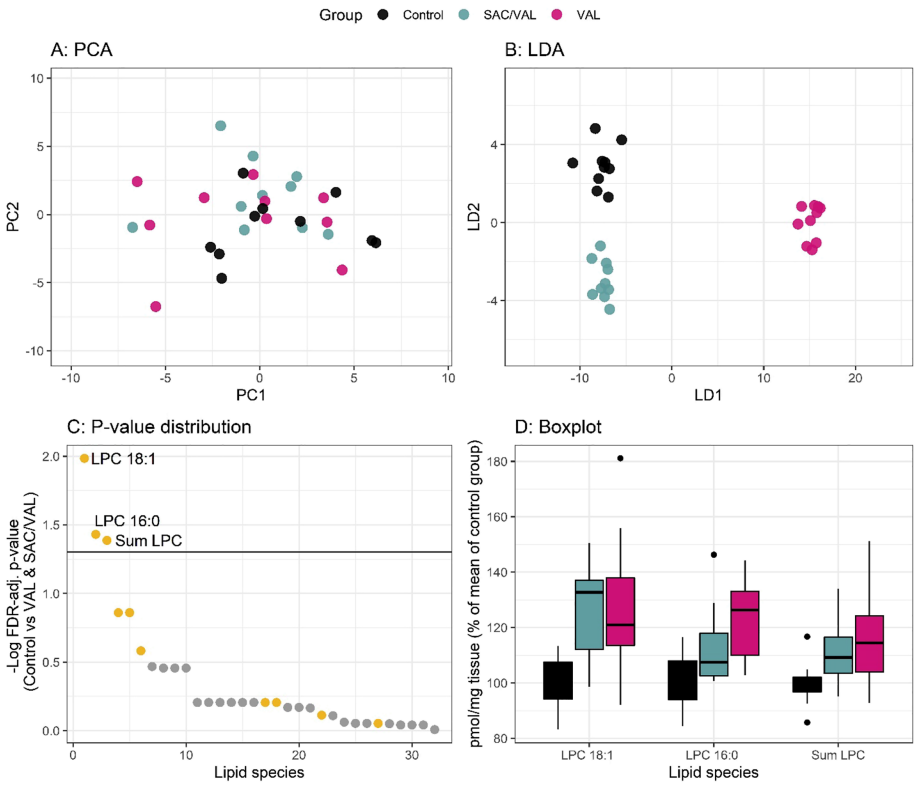
Figure 5 (A) Principal components analysis (PCA) showing that the first two principal components do not separate the groups. (B) Linear discriminant analysis (LDA) showing group separation, indicating possible alterations in certain lipid species between the groups. (C) Distribution of *P*-values (false discovery rate adjusted) between CONTROL (*N* = 10) and VAL + SAC/VAL (*N* = 20) showing a non-random pattern. Lysophosphatidylcholine (LPC) species are highlighted in yellow, and phosphatidylcholine (PC) species are highlighted in grey. The false discovery rate-adjusted *P*-value of 0.05 is marked as a horizontal line. (D) Boxplot of the three lipid species/variables (LPC 18:1, LPC 16:0, and the sum of all LPCs) that show a significant between-group difference. CONTROL, isoprenaline; SAC/VAL, sacubitril/valsartan; VAL, valsartan.



function and structure of the heart.[32] Indeed, the importance of cardiac lipid metabolism has been further emphasized by our recent publication in which we provide translational evidence that cardiac glycosphingolipids are required to maintain β-adrenergic signalling and contractile capacity in cardiomyocytes and to preserve normal heart function.[33] We found that both sacubitril/valsartan and valsartan affected the metabolism of LPC but not of other lipids, which is rather intriguing observation given the importance of LPC for normal function. LPC is a major phospholipid component in cell membranes accounting for 40% of total phospholipids in the heart tissue.[29,34,35] Because of its amphiphilic property, LPC is readily incorporated into the lipid bilayers of the cell membrane, changing the physicochemical property of the cell membrane and thereby affecting the receptors, enzymes, and ion channels embedded in the membrane.[36] LPC is also involved in the regulation of intracellular pH.[37] Given these effects, the observed higher survival rate in the rats treated with sacubitril/valsartan could be due to suppression of malignant ventricular arrhythmias rather than better cardiac function. This assumption is supported by Chang *et al.*,[38] who reported that sacubitril/valsartan reduces the inducibility of ventricular tachyarrhythmia in rats with heart failure and by the fact that cardiac function was not significantly

Figure 6 Effect of pretreatment with sacubitril/valsartan and valsartan on LV function and development of LV apical ballooning. Apical akinesia is apparent in the rats from the control group (A) and the valsartan group (B) but not in the rats from the sacubitril/valsartan group (C). The red line traces the endocardial border of the left ventricle.



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better in the sacubitril/valsartan group. Reduced pro-arrhythmic signal transduction in the heart through LPC-mediated desensitization of beta-adrenergic receptors could be one of the anti-arrhythmic mechanisms of sacubitril/valsartan.[39] Anti-arrhythmic effects of sacubitril/valsartan on malignant ventricular arrhythmias have also been demonstrated in humans.[40]

Based on available experimental evidence, we speculate that the protective effects of sacubitril/valsartan could be mediated by the anti-sympathetic effects of the drug.[15] Sympathetic overstimulation of the heart is essential in the pathogenesis of TTS[41] and arrhythmias,[42,43] and we have previously shown that beta-blockers reduce mortality in this TTS model.[20] Exogenous catecholamines can cause TTS in humans[44] and animals.[45] TTS patients often have high levels of catecholamines.[46] While isoprenaline augments internal catecholamines secretion,[47] sacubitril/valsartan decreases levels of catecholamines.[48,49] Our study is in line with Imran *et al*., who showed that sacubitril/valsartan protects the cellular antioxidant defence system against oxidative stress induced by isoproterenol by reducing lipid peroxidation activity,[50] which is another possible mechanism behind the positive effects. Sacubitril/valsartan could attenuate induction of TTS phenotype in this model via increasing BNP[51,52] that counters ventricular stretch and mediates cardioprotection.[53]

Significant limitations of this study include the absence of data about sympathetic tone, levels of plasma, and tissue (cardiac) catecholamines and BNP. In addition, we were unable to distinguish between arrhythmia and progressive heart failure as specific cause of death. We have not performed invasive evaluation of LV haemodynamics with pressure–volume loops. The strength of the study is the use of a well-established and reproducible rat model of isoprenaline-induced TTS that replicates the essential phenotype of TTS in humans. However, the model is artificial as it is based on administering a high dose of a synthetic catecholamine.

In conclusion, pretreatment with sacubitril/valsartan prevents isoprenaline-induced TTS-like phenotype in rats. Sacubitril/valsartan could be a potential treatment option in patients with TTS in humans.

Conflict of interest

None declared.

Funding

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Video S1. Echocardiography from the control rat with apparent apical akinesia.

Video S2. *Echocardiography from the valsartan rat with apparent apical akinesia.*

Video S3. Echocardiography from the sacubitril/valsartan rat without apical akinesia.

References

1. Sato H, Tateishi H, Uchida T, Dote K, Ishihara M, Kodama K, Haze K, Hori M. Clinical aspect of myocardial injury: from ischemia to heart failure. *Kagaku Hyoronsha.* 1990: 55–64.
2. Dote K, Sato H, Tateishi H, Uchida T, Ishihara M. Myocardial stunning due to simultaneous multivessel coronary spasms: a review of 5 cases. *J Cardiol.* 1991; **21**: 203–214.
3. David-Cojocariu A, de Hemptinne Q, Vandenbossche J-L, Unger P. Assessment of variant forms of Takotsubo cardiomyopathy by two-dimensional speckle tracking echocardiography. *Eur Heart J Cardiovasc Imaging* 2014; **15**: 712–712.
4. Demirelli S, Ermis E, Hatem E, Uslu A, Askin L. Focal mid-ventricular anterior ballooning: an unusual pattern of Takotsubo cardiomyopathy. *Intractable Rare Dis Res* 2015; **4**: 108–110.
5. Zalewska-Adamiec M, Bachorzewska-Gajewska H, Tomaszuk-Kazberuk A, Nowak K, Drozdowski P, Bychowski J, Krynicki R, Musial WJ, Dobrzycki S. Takotsubo cardiomyopathy: serious early complications and two-year mortality – a 101 case study. *Neth Heart J* 2016; **24**: 511–519.
6. Redfors B, Vedad R, Angeras O, Ramunddal T, Petursson P, Haraldsson I, Ali A, Dworeck C, Odenstedt J, Ioaness D, Libungan B, Shao Y, Albertsson P, Stone GW, Omerovic E. Mortality in takotsubo syndrome is similar to mortality in myocardial infarction - A report from the SWEDEHEART registry. *Int J Cardiol* 2015; **185**: 282–289.
7. Fonarow GC, Hernandez AF, Solomon SD, Yancy CW. Potential mortality reduction with optimal implementation of angiotensin receptor neprilysin inhibitor therapy in heart failure. *JAMA Cardiol* 2016; **1**: 714–717.
8. Doust J, Lehman R, Glasziou P. The role of BNP testing in heart failure. *Am Fam Physician* 2006; **74**: 1893–1898.
9. Akashi YJ, Goldstein DS, Barbaro G, Ueyama T. Takotsubo cardiomyopathy: a new form of acute, reversible heart failure. *Circulation* 2008; **118**: 2754–2762.
10. Nef HM, Möllmann H, Weber M, Deetjen A, Brandt R, Hamm CW, Elsässer A. Release pattern of cardiac biomarkers in left ventricular apical ballooning. *Int J Cardiol* 2007; **115**: 128–129.

11. Frohlich GM, Schoch B, Schmid F, Keller P, Sudano I, Luscher TF, Noll G, Ruschitzka F, Enseleit F. Takotsubo cardiomyopathy has a unique cardiac biomarker profile: NT-proBNP/myoglobin and NT-proBNP/troponin T ratios for the differential diagnosis of acute coronary syndromes and stress induced cardiomyopathy. *Int J Cardiol* 2012; **154**: 328–332.
12. Myhre PL, Vaduganathan M, Claggett B, Packer M, Desai AS, Rouleau JL, Zile MR, Swedberg K, Lefkowitz M, Shi V, McMurray JJV, Solomon SD. B-type natriuretic peptide during treatment with sacubitril/valsartan: the PARADIGM-HF trial. *J Am Coll Cardiol* 2019; **73**: 1264–1272.
13. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile MR. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N Engl J Med* 2014; **371**: 993–1004.
14. Chaplin S. Sacubitril/valsartan for chronic heart failure: its future potential. *Prescriber* 2016; **27**: 26–34.
15. Sayer G, Kalantari S, Tayazime S, Medvedofsky D, Kim G, Sarswat N, Raikhelkar J, Beiser D, Liao J, Ward P, Lang R, Uriel N. Reduction of sympathetic innervation following transition to sacubitril/valsartan in the remodel (Reverse Remodeling Effects of Entresto) study. *J Card Fail* 2018; **24**: S32.
16. Sauer AJ, Cole R, Jensen BC, Pal J, Sharma N, Yehya A, Vader J. Practical guidance on the use of sacubitril/valsartan for heart failure. *Heart Fail Rev* 2019; **24**: 167–176.
17. Shao Y, Redfors B, Scharin Tang M, Mollmann H, Troidl C, Szardien S, Hamm C, Nef H, Boren J, Omerovic E. Novel rat model reveals important roles of beta-adrenoreceptors in stress-induced cardiomyopathy. *Int J Cardiol* 2013; **168**: 1943–1950.
18. Willis BC, Salazar-Cantú A, Silva-Platas C, Fernández-Sada E, Villegas CA, Rios-Argaiz E, González-Serrano P, Sánchez LA, Guerrero-Beltrán CE, García N, Torre-Amione G, García-Rivas GJ, Altamirano J. Impaired oxidative metabolism and calcium mishandling underlie cardiac dysfunction in a rat model of post-acute isoproterenol-induced cardiomyopathy. *Am J Physiol Heart Circ Physiol* 2015; **308**: H467–H477.
19. Sachdeva J, Dai W, Kloner RA. Functional and histological assessment of an experimental model of Takotsubo's cardiomyopathy. *J Am Heart Assoc* 2014; **3**: e000921.
20. Ali A, Redfors B, Lundgren J, Alkhoury J, Oras J, Gan L-M, Omerovic E. Effects of pretreatment with cardiostimulants and beta-blockers on isoprenaline-induced takotsubo-like cardiac dysfunction in rats. *Int J Cardiol* 2019; **281**: 99–104.
21. Khalil P, Kabbach G, Said S, Mukherjee D. Entresto, a new Panacea for heart failure? *Cardiovasc Hematol Agents Med Chem* 2018; **16**: 5–11.
22. Sengenes C, Berlan M, De Glisezinski I, Lafontan M, Galitzky J. Natriuretic peptides: a new lipolytic pathway in human adipocytes. *FASEB J* 2000; **14**: 1345–1351.
23. Gu J, Noe A, Chandra P, Al-Fayoumi S, Ligueros-Saylan M, Sarangapani R, Maahs S, Ksander G, Rigel DF, Jeng AY, Lin TH, Zheng W, Dole WP. Pharmacokinetics and pharmacodynamics of LCZ696, a novel dual-acting angiotensin receptor-neprilysin inhibitor (ARNi). *J Clin Pharmacol* 2010; **50**: 401–414.
24. Lofgren L, Forsberg GB, Stahlman M. The BUME method: a new rapid and simple chloroform-free method for total lipid extraction of animal tissue. *Sci Rep* 2016; **6**: 27688.
25. Ejsing CS, Sampaio JL, Surendranath V, Duchoslav E, Ekroos K, Klemm RW, Simons K, Shevchenko A. Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. *Proc Natl Acad Sci U S A* 2009; **106**: 2136–2141.
26. Brugger B, Erben G, Sandhoff R, Wieland FT, Lehmann WD. Quantitative analysis of biological membrane lipids at the low picomole level by nano-electrospray ionization tandem mass spectrometry. *Proc Natl Acad Sci U S A* 1997; **94**: 2339–2344.
27. Novartis. Prospective ARNI vs ACE Inhibitor Trial to DetermIne Superiority in Reducing Heart Failure Events After MI (PARADISE-MI). NLM identifier: NCT02924727. 2020.
28. Anwar Ali BR, Alkhoury J, Oras J, Gan L-M, Omerovic E. The importance of heart rate in isoprenaline-induced takotsubo-like cardiac dysfunction in rats. *ESC Heart Fail* 2020 In print.
29. Tomczyk MM, Dolinsky VW. The cardiac lipidome in models of cardiovascular disease. *Metabolites.* 2020; **10**: 254.
30. Goldberg IJ, Trent CM, Schulze PC. Lipid metabolism and toxicity in the heart. *Cell Metab* 2012; **15**: 805–812.
31. Murashige D, Jang C, Neinast M, Edwards JJ, Cowan A, Hyman MC, Rabinowitz JD, Frankel DS, Arany Z. Comprehensive quantification of fuel use by the failing and nonfailing human heart. *Science* 2020; **370**: 364–368.
32. Schulze PC, Drosatos K, Goldberg IJ. Lipid Use and Misuse by the Heart. *Circ Res.* 2016; **118**: 1736–1751.
33. Andersson L, Cinato M, Mardani I, Miljanovic A, Arif M, Koh A, Lindbom M, Laudette M, Bollano E, Omerovic E, Klevstig M, Henricsson M, Fogelstrand P, Swärd K, Ekstrand M, Levin M, Wikström J, Doran S, Hyötyläinen T, Sinisalu L, Orešic M, Tivesten Å, Adiels M, Bergo MO, Proia R, Mardinoglu A, Jeppsson A, Borén J, Levin MC. Glucosylceramide synthase deficiency in the heart compromises ß1-adrenergic receptor trafficking. *Eur Heart J* 2021.
34. Law SH, Chan ML, Marathe GK, Parveen F, Chen CH, Ke LY. An updated review of lysophosphatidylcholine metabolism in human diseases. *Int J Mol Sci* 2019; **20**: 1149.
35. Pradas I, Huynh K, Cabre R, Ayala V, Meikle PJ, Jove M, Pamplona R. Lipidomics reveals a tissue-specific fingerprint. *Front Physiol* 2018; **9**: 1165.
36. Hashizume H, Chen M, Ma H, Hoque N, Hara A, Yazawa K, Akahira M, Xiao C-Y and Abiko Y. Lysophosphatidylcholine: A Possible Modulator of Ischemic Injury in the Heart. In: Y. Abiko and M. Karmazyn, eds. *Protection Against Ischemia/Reperfusion Damage of the Heart*. Tokyo: Springer Japan; 1998: 75-87.
37. Yamaguchi S, Tamagawa M, Nakajima N, Nakaya H. Selective impairment of HCO3(-)-dependent pHi regulation by lysophosphatidylcholine in guinea pig ventricular myocardium. *Cardiovasc Res* 1998; **37**: 179–186.
38. Chang P-C, Lin S-F, Chu Y, Wo H-T, Lee H-L, Huang Y-C, Wen M-S, Chou C-C. LCZ696 therapy reduces ventricular tachyarrhythmia inducibility in a myocardial infarction-induced heart failure rat model. *Cardiovasc Ther* 2019; **2019**: 6032631.
39. Kume H, Ito S, Ito Y, Yamaki K. Role of lysophosphatidylcholine in the desensitization of beta-adrenergic receptors by Ca(2) sensitization in tracheal smooth muscle. *Am J Respir Cell Mol Biol* 2001; **25**: 291–298.
40. Martens P, Nuyens D, Rivero-Ayerza M, Van Herendael H, Vercammen J, Ceyssens W, Luwel E, Dupont M, Mullens W. Sacubitril/valsartan reduces ventricular arrhythmias in parallel with left ventricular reverse remodeling in heart failure with reduced ejection fraction. *Clin Res Cardiol* 2019; **108**: 1074–1082.
41. Wittstein IS. The sympathetic nervous system in the pathogenesis of Takotsubo syndrome. *Heart Fail Clin* 2016; **12**: 485–498.
42. Manolis AA, Manolis TA, Apostolopoulos EJ, Apostolaki NE, Melita H, Manolis AS. The role of the autonomic nervous system in cardiac arrhythmias: the neuro-cardiac axis, more foe than friend? *Trends Cardiovasc Med.* 2021; **31**: 290–302.
43. Shen MJ, Zipes DP. Role of the autonomic nervous system in modulating cardiac arrhythmias. *Circ Res* 2014; **114**: 1004–1021.
44. Maes S, Dhooghe NSJJ, Schotte H, Cattoir S, Jacobs TF, Van Landuyt K. Takotsubo cardiomyopathy induced by epinephrine infiltration for liposuction: broken heart syndrome. *Aesthet Surg J* 2019; **39**: NP431–NP436.
45. Redfors B, Ali A, Shao Y, Lundgren J, Gan L-M, Omerovic E. Different catecholamines induce different patterns of takotsubo-like cardiac dysfunction in an apparently afterload dependent

manner. *Int J Cardiol* 2014; **174**: 330–336.
46. Wittstein IS, Thiemann DR, Lima JA, Baughman KL, Schulman SP, Gerstenblith G, Wu KC, Rade JJ, Bivalacqua TJ, Champion HC. Neurohumoral features of myocardial stunning due to sudden emotional stress. *N Engl J Med* 2005; **352**: 539–548.
47. Feuerstein G, Krausz M, Cohen S, Gutman Y, Khosla MC, Bumpus MF. Angiotensin II mediation of adrenal catecholamine secretion induced by intrarenal isoprenaline infusion. *Eur J Pharmacol* 1978; **52**: 375–377.
48. Jordan J, Stinkens R, Jax T, Engeli S, Blaak EE, May M, Havekes B, Schindler C, Albrecht D, Pal P, Heise T, Goossens GH, Langenickel TH. Improved insulin sensitivity with angiotensin receptor neprilysin inhibition in individuals with obesity and hypertension. *Clin Pharmacol Therapeut* 2017; **101**: 254–263.
49. Engeli S, Stinkens R, Heise T, May M, Goossens Gijs H, Blaak Ellen E, Havekes B, Jax T, Albrecht D, Pal P, Tegtbur U, Haufe S, Langenickel Thomas H, Jordan J. Effect of sacubitril/valsartan on exercise-induced lipid metabolism in patients with obesity and hypertension. *Hypertension* 2018; **71**: 70–77.
50. Imran M, Hassan MQ, Akhtar MS, Rahman O, Akhtar M, Najmi AK. Sacubitril and valsartan protect from experimental myocardial infarction by ameliorating oxidative damage in Wistar rats. *Clin Exp Hypertens* 2019; **41**: 62–69.
51. Packer M, McMurray JJ, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile M, Andersen K, Arango JL, Arnold JM, Belohlávek J, Böhm M, Boytsov S, Burgess LJ, Cabrera W, Calvo C, Chen CH, Dukat A, Duarte YC, Erglis A, Fu M, Gomez E, Gonzàlez-Medina A, Hagège AA, Huang J, Katova T, Kiatchoosakun S, Kim KS, Kozan Ö, Llamas EB, Martinez F, Merkely B, Mendoza I, Mosterd A, Negrusz-Kawecka M, Peuhkurinen K, Ramires FJ, Refsgaard J, Rosenthal A, Senni M, Sibulo AS Jr, Silva-Cardoso J, Squire IB, Starling RC, Teerlink JR, Vanhaecke J, Vinereanu D, Wong RC. Angiotensin receptor neprilysin inhibition compared with enalapril on the risk of clinical progression in surviving patients with heart failure. *Circulation* 2015; **131**: 54–61.
52. Solomon SD, Zile M, Pieske B, Voors A, Shah A, Kraigher-Krainer E, Shi V, Bransford T, Takeuchi M, Gong J, Lefkowitz M, Packer M, McMurray JJ. The angiotensin receptor neprilysin inhibitor LCZ696 in heart failure with preserved ejection fraction: a phase 2 double-blind randomised controlled trial. *Lancet* 2012; **380**: 1387–1395.
53. Woods RL. Cardioprotective functions of atrial natriuretic peptide and B-type natriuretic peptide: a brief review. *Clin Exp Pharmacol Physiol* 2004; **31**: 791–794.