Sacubitril/Valsartan Reverses Cardiac Structure and Function in Experimental Model of Hypertension-Induced Hypertrophic Cardiomyopathy

Jovana Jeremic
University of Kragujevac
Jovana Bradic
University of Kragujevac
Isidora Milosavljevic
University of Kragujevac
Ivan Srejovic
University of Kragujevac
Vladimir Zivkovic Srejovic
University of Kragujevac
Nevena Jeremic
University of Kragujevac
Tamara Nikolic Turnic
University of Kragujevac
Irena Tanaskovic
University of Kragujevac
Stefani Bolevich
I.M. Sechenov First Moscow State Medical University
Vladimir Jakovljevic (drvladakgbg@yahoo.com)
University of Kragujevac
Sergey Bolevich
I.M. Sechenov First Moscow State Medical University
Marko N Zivanovic
University of Kragujevac
Nduka Okwose
Newcastle upon Tyne Hospitals NHS Foundation Trust
Dragana Seklic
University of Kragujevac
Nevena Milivojevic
University of Kragujevac
Jelena Grujic

University of Kragujevac
Lazar Velicki
University of Novi Sad
Guy MacGowan
Newcastle upon Tyne Hospitals NHS Foundation Trust
Djordje G Jakovljevic
Coventry University
Nenad Filipovic
University of Kragujevac

Research Article

Keywords: animal model, spontaneously hypertensive rats, neprilysin inhibition, Entresto

Posted Date: August 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1956344/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Molecular and Cellular Biochemistry on March 30th, 2023. See the published version at https://doi.org/10.1007/s11010-023-04690-7.

Abstract

This study evaluated the effect of sacubtril/valsartan on cardiac remodeling, molecular and cellular adaptations in experimental (rat) model of hypertension-induced hypertrophic cardiomyopathy. Thirty *Wistar Kyoto* rats; 10 normal (control) and 20 rats with confirmed hypertension-induced hypertrophic cardiomyopathy (HpCM) were used for this study. The HpCM group was further subdivided into untreated, and sacubitril/valsartan treated group. Myocardial structure and function were assessed using echocardiography, Langendorff's isolated heart experiment, blood sampling and qualitative polymerase chain reaction. Left ventricular internal diameter in systole (0.5 vs 0.22 cm, $P < 0.01$) and diastole (0.82 vs 0.50 cm, $P < 0.01$) were greater in treated compared to untreated HpCM rats. Interventricular septal wall thickness in end-systole was higher in untreated and treated HpCM rats compared to controls (0.22 vs 0.20 vs 0.16 cm, $P < 0.05$ respectively). Fractional shortening was lower in treated compared to untreated rats (34% vs 43%, $P < 0.01$). Systolic and diastolic blood pressures decreased by 58 and 37 mmHg ($P < 0.01$) respectively in treated compared to untreated rats. Similarly, sacubitril/valsartan treatment reduced oxidative stress and apoptosis (reduced expression of Bax and Cas3 genes) compared to untreated rats. There was a regular histomorphology of cardiomyocytes, interstitium, and blood vessels in treated rats compared to untreated HpCM rats which expressed hypertrophic cardiomyocytes, with polymorphic nuclei, prominent nucleoli and moderately dilated interstitium. In experimental model of hypertension-induced hypertrophic cardiomyopathy, sacubitril/valsartan treatment led to improved cardiac structure, haemodynamic performance, and reduced oxidative stress and apoptosis. Sacubitril/valsartan thus presents as a potential therapeutic strategy resulted in hypertension-induced hypertrophic cardiomyopathy.

Introduction

Hypertension represents one of the leading causes of cardiovascular morbidity and mortality worldwide, with its increasing prevalence warranting public health concern [1, 2]. Untreated chronic systemic hypertension leads to cardiac structural and functional abnormalities which may result in hypertensive hypertrophic cardiomyopathy (HpCM), manifested through left ventricular (LV) hypertrophy, diastolic or systolic dysfunction [3]. Initially, an increase in the LV wall thickness is a compensatory response to increased afterload to maintain cardiac output and reduce wall stress [4]. However, persistently increased pressure in aorta and increased afterload result in transition from physiological to pathophysiological hypertrophy and potentially to heart failure [5]. Crucial approach in the prevention of cardiac dysfunction is timely management of hypertension [3].

There is strong evidence linking abnormalities in the renin-angiotensin-aldosterone system (RAAS) to cardiac hypertensive complications such as the development of HpCM [6]. The RAAS is associated with the myocardial hypertrophic response and angiotensin II levels affect myocardial fibrosis [3, 7]. Therefore, RAAS-suppressing agents, such as angiotensin-converting–enzyme (ACE) inhibitors and angiotensin-receptor blockers (ARBs), constitute a cornerstone in the management of hypertension and hypertension-related heart damage. Significant effort has been invested in finding efficient therapeutic agents with the

potential to simultaneously control blood pressure and prevent cardiac complications. A potential therapeutic strategy involves the inhibition of neprilysin, a neutral endopeptidase, which degrades natriuretic peptides, bradykinin, and angiotensin II [8]. Neprilysin inhibition might exert significant protective effects in hypertensive conditions. Considering these facts, a dual-acting drug, containing sacubitril and valsartan in a 1:1 molar ratio has been shown to be effective in effective in neprilysin inhibition due to its diuretic and natriuretic effect, as well as inhibition of RAAS, which significantly contribute to the improvement of cardiac function.

The cardio-protective effects of this dual combination were confirmed in several clinical trials, with the main benefits observed in patients with heart failure with reduced ejection fraction [8]. Moreover, sacubitril/valsartan appeared to be superior in lowering blood pressure in hypertensive patients compared to ARB monotherapy [9]. Nevertheless, the effect of sacubitril/valsartan and the underlying molecular and cellular mechanisms have not been investigated in hypertrophic cardiomyopathy.

Therefore, the aim of the present study was to evaluate the effects of sacubitril/valsartan on cardiac functional, structural, molecular and cellular adaptations in experimental model of hypertension-induced hypertrophic cardiomyopathy.

Methods

This study was conducted on 10 male normotensive *Wistar Kyoto* rats (control; CTRL group) and 20 male spontaneously hypertensive (SHR) *Wistar Kyoto* rats with hypertrophic cardiomyopathy. Hypertension (systolic blood pressure up to 200 mmHg) developed in the SHR group during 12 to 14 weeks of age [4]. Hypertrophic cardiomyopathy was confirmed by echocardiography, and SHR rats were randomly divided into 10 untreated rats (HpCM group) and 10 rats treated with a fixed combination of sacubitril/valsartan (HpCM + sac/val group). A fixed combination of sacubitril/valsartan 103/97 mg in the HpCM + sac/val group was applied *per os* (by gavage) at a dose of 2.6 mg/kg twice a day for 4 weeks

All rats were sourced from the institute for medical research, university of Belgrade and housed under controlled environmental conditions throughout the experimental period: temperature (22 ± 2°C), humidity and illumination (12/12 hours light/darkness cycle). Food and water were provided *ad libitum*.

The preset study complies with the Declaration of Helsinki, and the protocol was approved by the ethical committee for experimental animals' well-being of the Faculty of Medical Sciences, University of Kragujevac, Serbia. All experiments were performed according to EU Directive for welfare of laboratory animals (86/609/EEC) and principles of Good Laboratory Practice (GLP). The study was also carried out in compliance with the ARRIVE guidelines

Cardiac functional and structural measurements

In vivo examinations in HpCM rats - Echocardiographic examination and evaluation of blood pressure and heart rate

A special ultrasound, the Hewlett-Packard Sonos 5500 ultrasound (Andover, MA, USA), equipped with a 15.0 MHz phased-array transducer for small animals, was used for *in vivo* investigation of rat hearts [10]. Prior to measurements, rats were anaesthetized with a mixture of ketamine and xylazine (75:5 mg/kg), shaved from the neckline to the middle chest and placed on the heating pad. Ultrasound measurements were taken from the parasternal long axis view in two-dimensional mode. Images were obtained in the M-mode and used to measure interventricular septal wall thickness at end-diastole (IVSd), LV internal dimension at end-diastole (LVIDd), LV posterior wall thickness at end-diastole (LVPWd), interventricular septal wall thickness end-systole (IVSs), LV internal diameter end-systole (LVIDs) and LV posterior wall thickness at end-systole (LVPWs). The percentage of fractional shortening (FS%) was also calculated from the M-mode.

Blood pressure and heart rate (HR) were measured using a non-invasive tail-cuff (Mouse and Rat Tail Cuff Blood Pressure Systems IITC Life Science Inc., Los Angeles, CA, USA). Eight to ten measurements were made over a period of five minutes and the mean values calculated [11].

Ex vivo examinations on isolated hearts of HpCM rats - Cardiodynamic parameters

Langendorff model of an isolated retrogradely perfused heart was used for the *ex vivo* examination of rat heart function. After short-term anesthesia caused by intraperitoneal application of ketamine (10 mg/kg) and xylazine (5 mg/kg) and premedication with heparin as an anticoagulant, animals were sacrificed by decapitation. The thoracic cavity was opened via midline thoracotomy, and the hearts were immediately removed and immersed in cold saline. Afterwards, the aortas were cannulated and retrogradely perfused according to the Langendorff technique, under gradually increasing coronary perfusion pressure (CPP) from 40 to 120 cm H_2O. The composition of Krebs-Henseleit buffer used for retrograde perfusion was as follows: NaCl 118 mmol/L, KCl 4.7 mmol/L, $MgSO_4 \times 7H_2O$ 1.7 mmol/L, $NaHCO_3$ 25 mmol/L, KH_2PO_4 1.2 mmol/L, $CaCl_2 \times 2H_2O$ 2.5 mmol/L, glucose 11 mmol/L, pyruvate 2 mmol/L, equilibrated with 95% O_2 and 5% CO_2 and warmed to 37°C (pH 7.4).

After placing the sensor (transducer BS473-0184, Experimetria Ltd., Budapest, Hungary) in the left ventricle, the following parameters of myocardial function were measured: maximum and minimum rate of pressure development (dp/dt max and dp/dt min respectively) in the left ventricle, systolic and diastolic left ventricular pressure (SLVP and DLVP respectively), and heart rate (HR). Coronary flow (CF) was measured flowmetrically. Following the establishment of heart perfusion, the hearts were stabilized within 30 minutes with a basal coronary perfusion pressure of 70 cm H_2O. To examine the effects of sacubitril/valsartan chronic treatment on cardiac function, the CPP was gradually decreased to 60 cm H_2O, and then increased to 80, 100 and 120 cm H_2O and reduced to 40 cm H_2O (pressure changing protocol 1; PCP 1). To examine cardiac autoregulation, CPP was again gradually increased from 40 to 120 cm H_2O (pressure changing protocol 2, PCP 2) and later compared with the values recorded in PCP1 [12].

Assessment of metabolic and lipid profile

After treatment with sacubitril/valsartan (the day before sacrificing animals), an oral glucose tolerance test (OGTT) was performed on both groups of animals. Following an overnight fast, (12–14h), a baseline blood sample was taken by tail bleeding to determine the fasting blood glucose and insulin levels. Subsequently, glucose was administered orally at a dose of 2 g/kg body weight, and blood glucose levels were assessed using a glucometer (Accu-Chek, Roche Diagnostics, Indianapolis, USA) at 30, 60, 120 and 180 minutes thereafter. Insulin levels were also assessed at baseline and 180 minutes using enzyme-linked immunosorbent assay (ELISA) method, as previously described [13]. Total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels were analyzed in serum, using spectrophotometry on a biochemical analyzer (Dimension Xpand, Siemens, IL, USA)

Mechanistic investigations of sacubitril/valsartan treatment

Biochemical analysis - Oxidative stress parameters

At the time of animal sacrifice, blood samples were collected from the jugular vein to assess systemic oxidative stress response. Pro-oxidants evaluated in plasma include index of lipid peroxidation (2-thiobarbituric acid reactive substances; TBARS), nitrites (NO_2^-), superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2). Conversely, markers of anti-oxidation ((superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH)) were determined using lysed erythrocytes [12].

Relative gene expression - (anti)oxidative, (anti)inflammatory, (anti)apoptotic and cardiac-specific parameters

Quantitative polymerase chain reaction (qPCR) assays of relative gene expression were performed in the Bioengineering Laboratory, Institute of Information Technologies Kragujevac according to laboratory protocols following the Minimum Information for the Publication of Quantitative Real-Time PCR Experiments (MIQE) Guidelines [14] and the GLP standards for PCR detection. All methods and consumables used were applied according to manufacturer instructions and qPCR reactions were performed in triplicates. The heart samples were firstly homogenized using the IKA® ULTRA-TURRAX® tube disperser workstation system (IKA Werke GmbH & Co. KG, Staufen im Breisgau, Deutschland). Samples immersed in 5 mL of pH-neutral pure PBS solution were placed in disperser tubes with glass balls and homogenized 120 seconds at 5000 rpm. Fresh samples (5 mg) were used for RNA extractions using a spin-column kit for purification of total RNA (Quick-RNA™ Miniprep Kit, Zymo research, R1054, Irvine, CA, US). The RNA concentration was estimated on an Eppendorf BioPhotometer® D30 (Hamburg, Germany) at 260 nm using an Eppendorf® UVette® cuvette (ref. nr. Z605050). The yield was optimal with an A_{260}/A_{280} ratio between 1.75 and 2.0. For each group of isolated RNA, reverse transcription was performed immediately by using FastGene Scriptase Basic cDNA Kit (Cat. No. LS62, NIPPON Genetics

EUROPE, Düren, Germany). cDNA was stored at -20°C until all samples were prepared and ready for qPCR relative gene expression detection. Primers were DSL purified and C18 desalinated, no-end modified. Relative gene expression was determined by FastGene 2x IC Green Universal with Fluorescein kit (Cat. No. LS41, NIPPON Genetics EUROPE, Düren, Germany) based on FastGene® IC Green as an intercalating dye MIC qPCR Cycler is controlled by intuitive software package micPCR v2.10.3 for obtaining Ct values and subsequent melting analyses. Since there is no single gene constitutively expressed in all cell types [15], we chose Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as reference genes.

Pathohistological analysis

Cardiac tissue samples were fixed in 4% paraformaldehyde buffer solution and immersed in paraffin. Sections, four micrometres thick were then placed on slides and stained with hematoxylin/eosin (HE) and Mason's trichrome [16].

Statistical Analysis

The data were expressed as mean ± standard error (SE), and statistical analysis performed using the IBM SPSS Statistics 20.0. Data distribution was checked by Shapiro–Wilk test and in the normal distribution, statistical comparisons were performed using the one-way analysis of variance (ANOVA) tests with a Tukey's post hoc test for multiple comparisons. The Kruskal–Wallis test was used when data was not normally distributed. Values of $p < 0.05$ were statistically significant. All qPCR reactions were performed in triplicates. Relative gene expression was calculated using the delta-delta Ct formula according to Livak and Schmittgen [17].

Results

Cardiac effects of sacubitril/valsartan

In HpCM rats, IVSd and IVSs were increased, while LVIDd and LVIDs were decreased compared to healthy rats (CTRL group). On the other hand, sacubitril/valsartan treatment in rats with HpCM increased LVIDs and LVIDd and decreased IVSs and FS compared with untreated rats (HpCM + sac/val vs. HpCM group) (Fig. 1A).

Systolic and diastolic blood pressures (SBP and DBP) increased, while heart rate was decreased in untreated HpCM compared to healthy rats. In contrast, the addition of sacubitril/valsartan treatment decreased both SBP and DBP (Fig. 1B).

Ex vivo examinations on isolated hearts of rats with HpCM - *Cardiodynamic parameters*

All *ex vivo* examined cardiodynamic parameters, except HR, were significantly reduced in untreated HpCM compared to healthy rats. However, in the treated HpCM rats, the values of dp / dt min (at CPP 120), as well as SLVP in all CPPs and DLVP in higher CPPs (80–120) were more like control values and significantly higher than values observed in untreated rats HpCM. Although other monitored cardio dynamic parameters did not change significantly, they were more like controls compared to untreated rats (Fig. 2A).

Similar results were observed during the autoregulation protocol on the Langendorff apparatus. The percentage changes in the values of the monitored cardio dynamic parameters in PCP1 and PCP2 were highest in the HpCM group (around 20%). Chronic sacubitril/valsartan administration reversed these changes thus reducing dp/dt max (at 60 CPP), dp/dt min (at 80 and 120 CPPs), SLVP (at 80–100 CPPs), DLVP (at 60 and 100 CPPs), HR (at 60, 80 and 120 CPPs), CF (at 60–120 CPPs) compared to the HpCM group (Fig. 2B).

Effects of sacubitril/valsartan on metabolic and lipid profile

Sacubitril/valsartan treatment did not affect glucose levels during OGTT compared to untreated SHR rats (Fig. 3A). However, TC, HDL and LDL were significantly reduced in both treated and untreated HpCM rats compared to healthy rats (Fig. 3B).

Effects of sacubitril/valsartan treatment on oxidative stress parameters

The level of NO_2^- was significantly decreased, and H_2O_2 increased in SHR group compared to CTRL, while TBARS was significantly decreased only in HpCM compared to the CTRL group of rats. Sacubitril/valsartan treated rats showed reduced H_2O_2 levels compared to untreated rats (Fig. 4A). There was no change in SOD among all groups. CAT was significantly reduced in HpCM compared to CTRL.

However, treatment with sacubitril/valsartan led to significant increase in CAT beyond levels found in the CTRL group. Similarly, the level of GSH was decreased in untreated HpCM rats compared to CTRL, while treatment significantly elevated GSH levels compared to HpCM (Fig. 4B).

The relative expression of SOD1 and SOD2 genes were increased in SHR group compared to the CTRL group. However, treatment with sacubitril/valsartan reduced the relative gene expression of these genes compared to HpCM rats (Fig. 4C).

Effects of sacubitril/valsartan treatment on apoptosis and pathological changes in the myocardium

The relative gene expression of Bax and Cas3 were increased in HpCM compaired to CTRL group, while sacubitril/valsartan treatment decreased the relative gene expression of these parameters. The relative gene expression of anti-apoptotic Bcl2 was increased in HpCM + sac/val compared to both HpCM and CTRL groups (Fig. 5A).

In the CTRL group, mitochondria are properly distributed in cardiac myofibers, nuclei are normochromic and polymorphic. Masson's trichrome staining revealed a non-fibrosed tissue with mildly dilated interstitium, and focal lymphocytic infiltration. Additionally, in the HpCM group, there were hypertrophic cardiomyocytes, with polymorphic nuclei, prominent nucleoli and moderately dilated interstitium. On the other hand, in HpCM + sac/val, there was a regular arrangement of cardiomyocytes, regular interstitium, blood vessels of normal histomorphology, and no hypertensive changes of the vascular wall (Fig. 5B).

Discussion

Considering that hypertension is the most frequent risk factor for hypertrophic cardiomyopathy and heart failure, there is an urgent need for finding an optimal therapeutic modality that will control blood pressure and prevent cardiac complications [18]. Since the approval of sacubitril/valsartan in 2014 for the treatment of chronic heart failure, several researchers have highlighted its benefits in the reduction of cardiovascular morbidity and mortality [19]. The present study explored the therapeutic influence of this pharmacological agent on cardio- protection in hypertension-induced cardiomyopathy. The primary focus was to evaluate sacubitril/valsartan effects on functional, structural and biochemical markers of this cardiac pathology. The principal findings indicates that sacubitril/valsartan combination has a great potential in triggering cardio-protection. To the best of our knowledge, this is the first study to utilize *in vivo* and *ex vivo* techniques to assess sacubitril/valsartan in a hypertension-induced hypertrophic cardiomyopathy rat model.

In vivo measurements indicate that sacubitril/valsartan treatment was associated with a reduction in both, SBP and DBP as well as improvement in heart function, confirmed by increased LVIDs and LVIDd and decreased IVSs and FS compared with untreated rats. These findings were expected since previous studies revealed that sacubitril/valsartan as neprilysin and angiotensin receptor inhibitor leads to improvement of cardiac reverse remodeling and left ventricular ejection fraction [20]. The positive effects

of sacubitril/valsartan on LV diameters, volumes and systolic-diastolic performance were reported previously [20, 21] however, due to conflicting results, it is still unclear which echocardiographic parameter is the most likely to change after sacubitril/valsartan treatment. Sacubitril/valsartan-induced alterations in echocardiographic markers in our study are of great importance since they reflect the heart's ability to contract and relax effectively and represent the main prognostic markers of heart failure in hypertensive individuals [19].

Ex vivo measurements on HpCM rats showed decreased dp/dt min, SLVP and DLVP during PCP 2 at different coronary perfusion pressures compared to PCP 1, reflecting abnormal cardiac contractility and relaxation of the isolated rat heart. Depression of inotropic and lusitropic response can lead to systolic and diastolic dysfunction, thus confirming deleterious effects of long-lasting hypertension on the heart. In contrast, markers of cardiac function remained unchanged in rats treated with sacubitril/valsartan, exposure to various perfusion pressures. Based on these observations, it is reasonable to conclude that sacubitril/valsartan treatment reduces the inotropic and lusitropic heart response to the pressure stimulants. Previous data also suggest the capacity of sacubitril to restore impaired myocyte contractility through phosphatase and tensin homolog inhibition. Additionally, it has been reported that activation of the serine/threonine-protein kinases; AKT1 and AKT3, which suppress the endothelial nitric oxide synthase are responsible for benefits of this drug on cardiac contractility force [22]. It is important to note that the protective action of valsartan on cardiac remodeling might be partially mediated by inhibiting guanine nucleotide-binding proteins, which is synergized by the addition of sacubitril [22].

Our findings also showed that sacubitril/valsartan treatment did not affect glucose or lipid profile. Animals in both groups showed normal fasting glucose levels and response to an OGTT although the SHR group showed better glucose control.

Given the fact that oxidative stress stands out as one of the fundamental factors contributing to hypertension and development of hypertensive complications, our goal was to assess the influence of sacubitril/valsartan treatment on systemic redox balance [23]. Systemic redox homeostasis was assessed by monitoring levels of pro-oxidants as well as capacity of antioxidant defense system. Elevated H_2O_2 and decreased NO_2^- level, were noticed in hypertensive rats compared to control, thus confirming that hypertensive state is associated with disturbed redox balance. However, sacubitril/valsartan treatment led to a reduction in H_2O_2 and increase in GSH value, while other observed systemic pro-oxidant and antioxidant markers remained unchanged. To provide insight into the cardiac redox status, we determined gene expression of SOD1 and SOD2 which are significance in mitigating the deleterious effects of pro-oxidants [24]. SOD1 and SOD2 gene expression was reduced in treated rats compared to hypertensive control. Considering that elevated SOD expression is expected during cardiomyocytes response to oxidative stress exposure [25], our findings indicate that the drug combination prevents cardiac oxidative damage via reduced expression of SOD1 and SOD2. This is in line with previous studies which reported that sacubitril/valsartan therapy exert cardio-protection via reducing oxidative stress [26, 27].

Furthermore, sacubitril/valsartan treatment reduced gene expression of the pro-apoptotic markers Bax and Cas9 and elevated expression of anti-apoptotic Bcl2 in comparison to untreated hypertensive rats. The anti-apoptotic potential of sacubitril/valsartan has also been previously demonstrated in other cardiac pathologies [28].

HE and Masson's trichrome tissue staining revealed that hypertension was linked to the presence of hypertrophic cardiomyocytes, with polymorphic nuclei and moderately dilated interstitium. Nevertheless, chronic use of sacubitril/valsartan alleviated hypertension-induced structural alterations of cardiomyocytes and the vascular wall. Our results are compatible with the previous studies that identified structural pathological changes in HpCM [29], which were markedly attenuated after receiving sacubitril/valsartan treatment. Several data support the fact that dual drug combination exerts synergistic effects in prevention and attenuation of cardiomyocyte cell death, hypertrophy and left ventricular remodeling [22].

Limitations

The presented study reports critical observations in the physiology of the heart muscle in response to Sacubitril/valsartan administration. However, the present study is limited as it does not provide adequate mechanistic rational for the findings of the study.

Conclusion

Based on our findings, we might conclude that molecular-cellular perturbations in HpCM were greatly reversed in rats treated with sacubitril/valsartan. Cardio-protection was achieved through improvement in cardiac structure and function, attenuation of oxidative stress and apoptosis, thus suggesting significant role of sacubitril/valsartan in HpCM. Such integrative results, encompassing morphological, functional, and biochemical analysis of sacubitril/valsartan therapy, may indicate rationale for further investigation and potentially translation of the use of sacubitril/valsartan in treatment of patients with hypertension-induced hypertrophic cardiomyopathy.

Declarations

Funding

This work was supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 777204. The authors gratefully acknowledge financial support from the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement No. Agreement No. 451-03-9/2021-14/200378). The design of the study, data collection, analyses, interpretation of data, and drafting of the manuscript do not reflect the views and opinions of the funders.

Acknowledgments

The authors are grateful to Katarina Virijević and Mihajlo Kokanović for sample handling and mRNA isolation.

Competing Interests

The authors declare no competing interests

Author Contributions

DGJ, JJ, JB, JG, NM, MNZ, NF: Study concept and design:

JJ, IM, JG, DS, NF: Study Supervision

JJ, JB, IM, IV, VZ, NJ, TNT, SB, DS, NM: Acquisition of data

JJ, NCO, IT, DGJ, SB, VJ, MNZ, DS, JG, VZ: Data analysis and interpretation of data

JJ, MNZ, NCO, DGJ, JB: Drafting of the manuscript

DGJ, JJ, NCO, GAM, LV, MNZ, NF: Critical revision of the manuscript

All authors approved the final version. DGJ and NF acts as the guarantor and take responsibility for the content of the manuscript, including the data and analysis.

Ethics approval and consent to participate

The protocol for this study was approved by the ethical committee for experimental animals' well-being of the Faculty of Medical Sciences, University of Kragujevac, Serbia. All animals used in this study were sourced from the Institute for Medical Research, University of Belgrade and written informed consent was obtained prior to use of the animals.

Consent for publication

Not applicable. No human data was used in this study

Availability of data and material

All data generated or analysed during this study are included in this published article. Additional information could be obtained from the corresponding author

References

1. Bijani M, Parvizi S, Dehghan A, Sedigh-Rahimabadi M, Rostami-chijan M, Kazemi M, et al (2020) Investigating the prevalence of hypertension and its associated risk factors in a population-based study: Fasa PERSIAN COHORT data. BMC Cardiovasc Disord 20. DOI: 10.1186/s12872-020-01797-3
2. Mills KT, Stefanescu A, He J (2020) The global epidemiology of hypertension. Nature Reviews Nephrology 16:223–37. DOI: 10.1038/s41581-019-0244-2
3. 3.. Kuroda K, Kato TS, Amano A (2015) Hypertensive cardiomyopathy: A clinical approach and literature review. World J Hypertens 5(2): 41–52. DOI: 10.5494/wjh.v5.i2.41
4. Drazner MH (2011) The progression of hypertensive heart disease. Circulation 123:327–34. DOI: 10.1161/CIRCULATIONAHA.108.845792
5. Burchfield JS, Xie M, Hill JA. Pathological ventricular remodeling: Mechanisms: Part 1 of 2. Circulation. 2013;128:388–400. DOI: 10.1161/CIRCULATIONAHA.113.001878
6. Strait JB, Lakatta EG (2012) Aging-Associated Cardiovascular Changes and Their Relationship to Heart Failure. Heart Failure Clinics 8:143–64. DOI: 10.1016/j.hfc.2011.08.011
7. Cuspidi C, Ciulla M, Zanchetti A (2006) Hypertensive myocardial fibrosis. Nephrol Dial Transplant 21:20–3. DOI: 10.1093/ndt/gfi237
8. Packer M, McMurray JJV, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, et al (2015) Angiotensin receptor neprilysin inhibition compared with enalapril on the risk of clinical progression in surviving patients with heart failure. Circulation 131:54–61. DOI: 10.1161/CIRCULATIONAHA.114.013748.
9. Yandrapalli S, Khan MH, Rochlani Y, Aronow WS (2018) Sacubitril/valsartan in cardiovascular disease: evidence to date and place in therapy. Therapeutic Advances in Cardiovascular Disease 12:217–31. DOI: 10.1177/1753944718784536
10. Joffe II, Travers KE, Perreault-Micale CL, Hampton T, Katz SE, Morgan JP, et al (1999) Abnormal cardiac function in the streptozotocin-induced, non-insulin- dependent diabetic rat: Noninvasive assessment with Doppler echocardiography and contribution of the nitric oxide pathway. J Am Coll Cardiol 34:2111–9. DOI: 10.1016/s0735-1097(99)00436-2
11. Feng M, Whitesall S, Zhang Y, Beibel M, D'Alecy L, DiPetrillo K (2008) Validation of volume-pressure recording tail-cuff blood pressure measurements. Am J Hypertens 21:1288–91. DOI: 10.1038/ajh.2008.301
12. Jakoviljevic V, Milic P, Bradic J, Jeremic J, Zivkovic V, Srejovic I, et al (2019) Standardized aronia melanocarpa extract as novel supplement against metabolic syndrome: A rat model. Int J Mol Sci 20. DOI: 10.3390/ijms20010006
13. Leng SH, Lu FE, Xu LJ (2004) Therapeutic effects of berberine in impaired glucose tolerance rats and its influence on insulin secretion. Acta Pharmacol Sin 25:496–502.
14. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al (2009) The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clin Chem 55:611–22. DOI: 10.1373/clinchem.2008.112797
15. Andersen CL, Jensen JL, Ørntoft TF (2004) Normalization of real-time quantitative reverse transcription-PCR data: A model-based variance estimation approach to identify genes suited for

normalization, applied to bladder and colon cancer data sets. Cancer Res 64:5245–50. DOI: 10.1158/0008-5472.CAN-04-0496

16. Stojic IM, Jakovljevic VL, Zivkovic VI, Srejovic IM, Nikolic TR, Jeremic JN, et al (2018) The perfusion of cisplatin and cisplatin structural analogues through the isolated rat heart: The effects on coronary flow and cardiodynamic parameters. Gen Physiol Biophys 37:515–25. DOI: 10.4149/gpb_2018004
17. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–8. DOI: 10.1006/meth.2001.1262
18. Di Palo KE, Barone NJ (2020) Hypertension and Heart Failure: Prevention, Targets, and Treatment. Heart Failure Clinics 16:99–106. DOI: 10.1016/j.hfc.2019.09.001
19. Bindra A, Hall SA (2017) New drugs for the treatment of heart failure. US Cardiol Rev 11:62–6.
20. Martens P, Beliën H, Dupont M, Vandervoort P, Mullens W (2018) The reverse remodeling response to sacubitril/valsartan therapy in heart failure with reduced ejection fraction. Cardiovasc Ther 36. DOI: 10.1111/1755-5922.12435
21. Landolfo M, Piani F, Esposti DD, Cosentino E, Bacchelli S, Dormi A, et al (2020) Effects of sacubitril valsartan on clinical and echocardiographic parameters of outpatients with heart failure and reduced ejection fraction. IJC Hear Vasc 31. DOI: 10.1016/j.ijcha.2020.100656
22. Iborra-Egea O, Gálvez-Montón C, Roura S, Perea-Gil I, Prat-Vidal C, Soler-Botija C, et al (2017) Mechanisms of action of sacubitril/valsartan on cardiac remodeling: a systems biology approach. npj Syst Biol Appl 3. DOI: 10.1038/s41540-017-0013-4
23. Rodrigo R, González J, Paoletto F (2011) The role of oxidative stress in the pathophysiology of hypertension. Hypertension Research 34:431–40. DOI: 10.1038/hr.2010.264
24. Nojima Y, Ito K, Ono H, Nakazato T, Bono H, Yokoyama T, et al (2015) Superoxide dismutases, SOD1 and SOD2, play a distinct role in the fat body during pupation in silkworm Bombyx mori. PLoS One 10. DOI: 10.1371/journal.pone.0116007
25. Ściskalska M, Ołdakowska M, Marek G, Milnerowicz H (2020) Changes in the activity and concentration of superoxide dismutase isoenzymes (Cu/zn sod, mnsod) in the blood of healthy subjects and patients with acute pancreatitis. Antioxidants 9:1–16. DOI: 10.3390/antiox9100948
26. Tseng WL, Chou SJ, Chiang HC, Wang ML, Chien CS, Chen KH, et al (2017) Imbalanced production of reactive oxygen species and mitochondrial antioxidant SOD2 in fabry disease-specific human induced pluripotent stem cell-differentiated vascular endothelial cells. Cell Transplant 26:513–27. DOI: 10.3727/096368916X694265
27. Yang CC, Chen YT, Chen CH, Li YC, Shao PL, Huang TH, et al (2019) The therapeutic impact of entresto on protecting against cardiorenal syndrome-associated renal damage in rats on high protein diet. Biomed Pharmacother 116. DOI: 10.1016/j.biopha.2019.108954
28. Lu HI, Tong MS, Chen KH, Lee FY, Chiang JY, Chung SY, et al (2018) Entresto therapy effectively protects heart and lung against transverse aortic constriction induced cardiopulmonary syndrome injury in rat. Am J Transl Res 10:2290–305.

29. McLeod CJ, Bos JM, Theis JL, Edwards WD, Gersh BJ, Ommen SR, et al (2009) Histologic characterization of hypertrophic cardiomyopathy with and without myofilament mutations. Am Heart J 158:799–805. DOI: 10.1016/j.ahj.2009.09.006

Figures

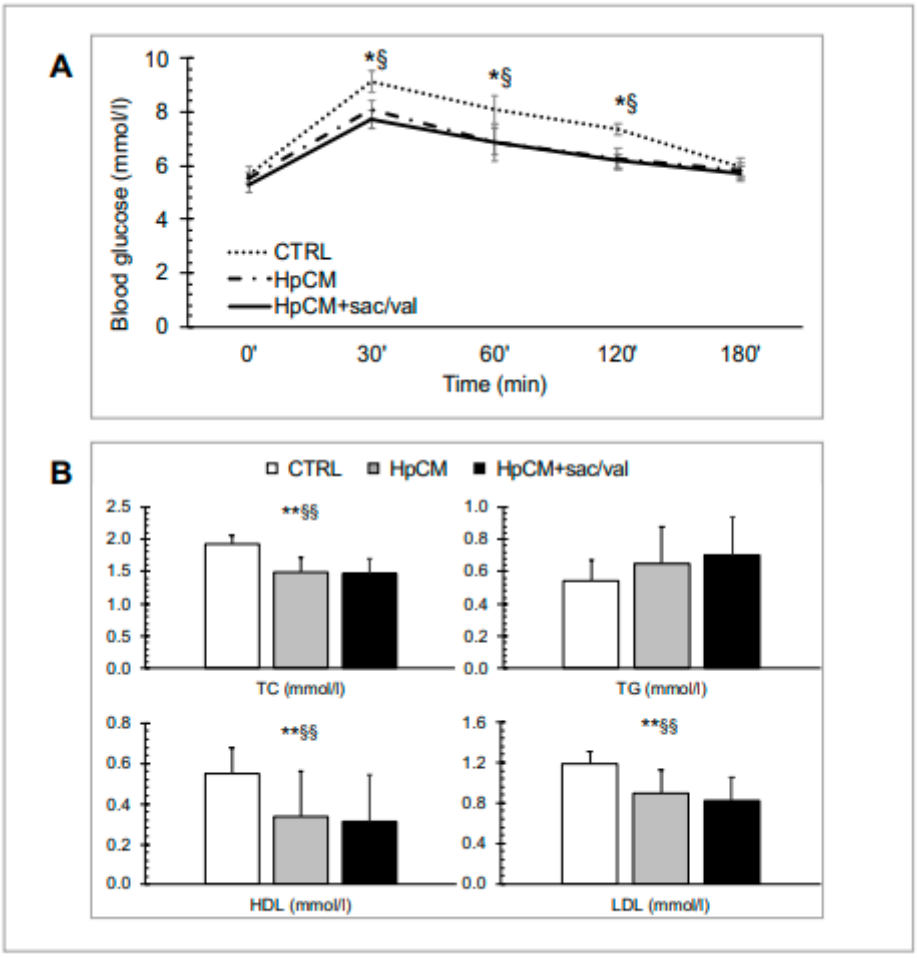

Figure 1

Effects of sacubitril/valsartan treatment on A) *in vivo* cardiac function; B) blood pressure and heart rate. Values are expressed as mean ± standard deviation for 10 animals, for each group. FS%; percentage of fractional shortening, HR; heart rate, IVSd; interventricular septal wall thickness at end-diastole, IVSs; interventricular septal wall thickness end-systole, LVIDd; left ventricular internal dimension at end-diastole, LVIDs; LV internal diameter end-systole, LVPWd; left ventricular posterior wall thickness at end-diastole, LVPWs; LV posterior wall thickness at end-systole, DBP; diastolic blood pressure, SBP; systolic blood pressure. Statistical analyses were calculated using one-way ANOVA. $p<0.05$ *CTRL vs. HpCM, $p<0.01$ **CTRL vs. HpCM, $p<0.05$ §CTRL vs. HpCM+sac/val, $p<0.01$ §§CTRL vs. HpCM+sac/val, $p<0.01$ ##HpCM vs. HpCM+sac/val.

A

	CTRL	HpCM	HpCM+sac/val
IVSd (cm)	0,148 ± 0,025	0,187 ± 0,029*	0,160 ± 0,022
LVIDd (cm)	0,745 ± 0,118	0,495 ± 0,045**	0,823 ± 0,103##
LVPWd (cm)	0,192 ± 0,020	0,191 ± 0,033	0,180 ± 0,020
IVSs (cm)	0,159 ± 0,030	0,215 ± 0,032*	0,197 ± 0,017§
LVIDs (cm)	0,483 ± 0,092	0,215 ± 0,025**	0,594 ± 0,081##
LVPWs (cm)	0,202 ± 0,033	0,229 ± 0,044	0,198 ± 0,027
FS (%)	38,250 ± 4,408	43,200 ± 2,977	34,167 ± 1,713##

B

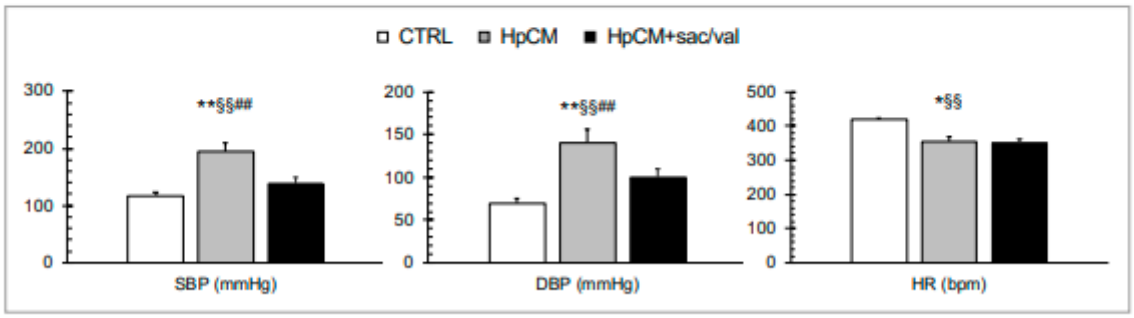

Figure 2

Effects of sacubitril/valsartan treatment on A) *ex vivo* cardiac function; B) autoregulation potential of isolated rat heart. PCP1 (full lines) vs PCP2 (dashed lines). CF; coronary flow, CPP; coronary perfusion pressure, DLVP; diastolic left ventricular pressure, SLVP; systolic left ventricular pressure, dp/dt max/min; maximum/minimum pressure development. Statistical analyses were calculated using two-way ANOVA for repeated measures. p<0.05 *CTRL vs. HpCM, p<0.01 **CTRL vs. HpCM, p<0.05 §CTRL vs. HpCM+sac/val, p<0.01 §§CTRL vs. HpCM+sac/val, p<0.05 #HpCM vs. HpCM+sac/val, p<0.01 ##HpCM vs. HpCM+sac/val.

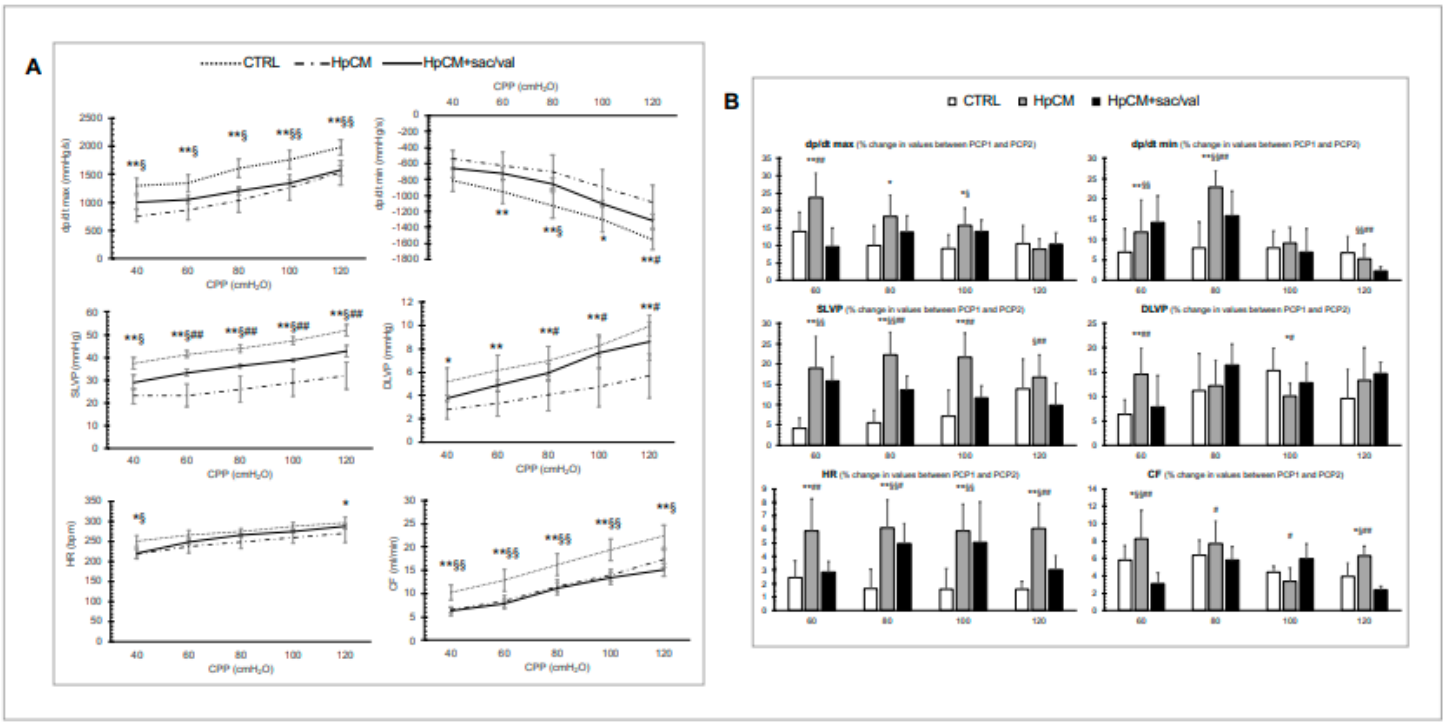

Figure 3

Effects of sacubitril/valsartan treatment on glucose and lipid profile in rats with HpCM: A) glucose levels during OGTT; B) lipid profile between rats with HpCM and control. HDL; high density lipoprotein, LDL; low density lipoprotein, TG; triglyceride, TC; total cholesterol. Statistical analyses were calculated using two-way analysis of variance (ANOVA) repeated measurements comparing for OGTT measurements and one-way ANOVA for lipid profile. $p<0.05$ *CTRL vs. HpCM; $p<0.01$ **CTRL vs. HpCM; $p<0.05$ §CTRL vs. HpCM+sac/val; $p<0.01$ §§CTRL vs. HpCM+sac/val.

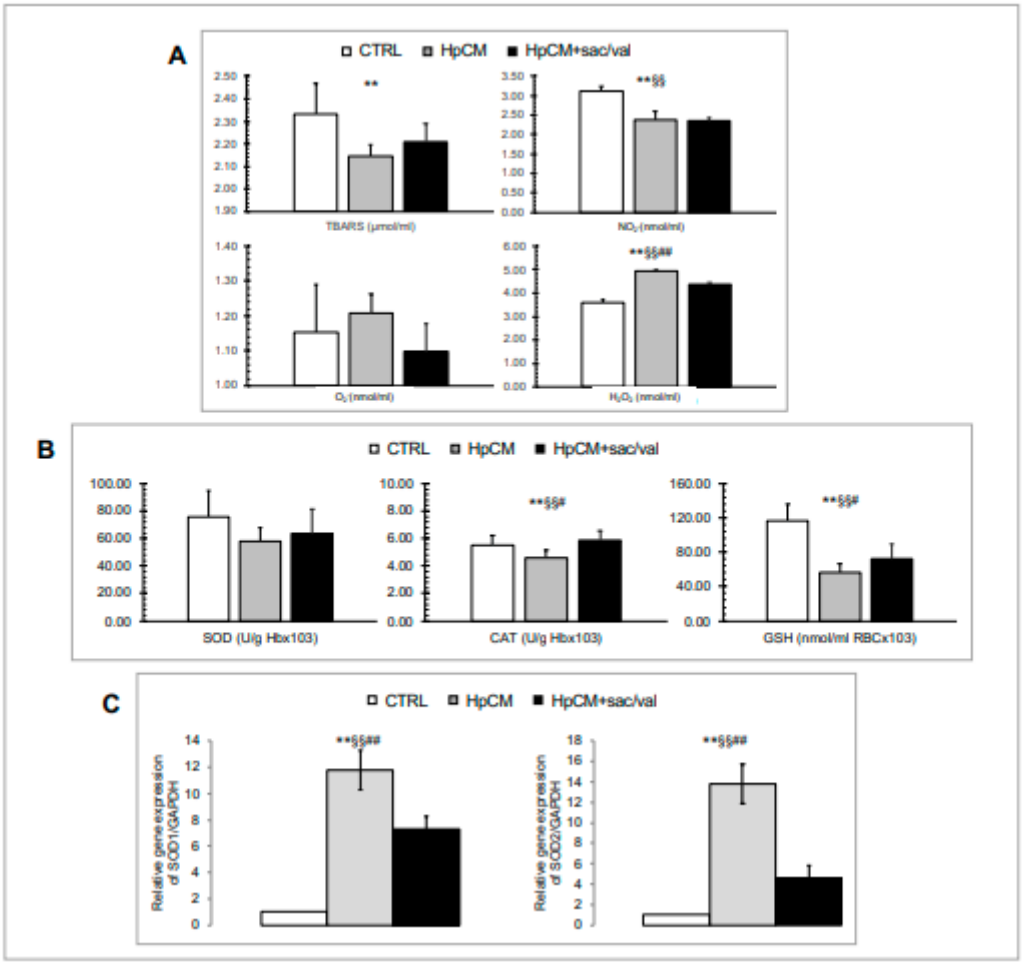

Figure 4

Effects of sacubitril/valsartan treatment on A) systemic pro-oxidant parameters, B) systemic antioxidant parameters, C) relative expression of SOD1 and SOD2 genes, measured in the LV of isolated rat heart. CAT; catalase, GADPH; Glyceraldehyde-3-phosphate dehydrogenase, GSH; reduced glutathione, H_2O_2; hydrogen peroxide, NO_2^-; nitrites, O_2^-; superoxide anion radical, SOD; superoxide dismutase, TBARS; thiobarbituric acid reactive substances. Statistical analyses were calculated using one-way ANOVA, $p<0.05$ *CTRL vs. HpCM, $p<0.01$ **CTRL vs. HpCM, $p<0.05$ §CTRL vs. HpCM+sac/val, $p<0.01$ §§CTRL vs. HpCM+sac/val, $p<0.05$ #HpCM vs. HpCM+sac/val, $p<0.01$ ##HpCM vs. HpCM+sac/val.

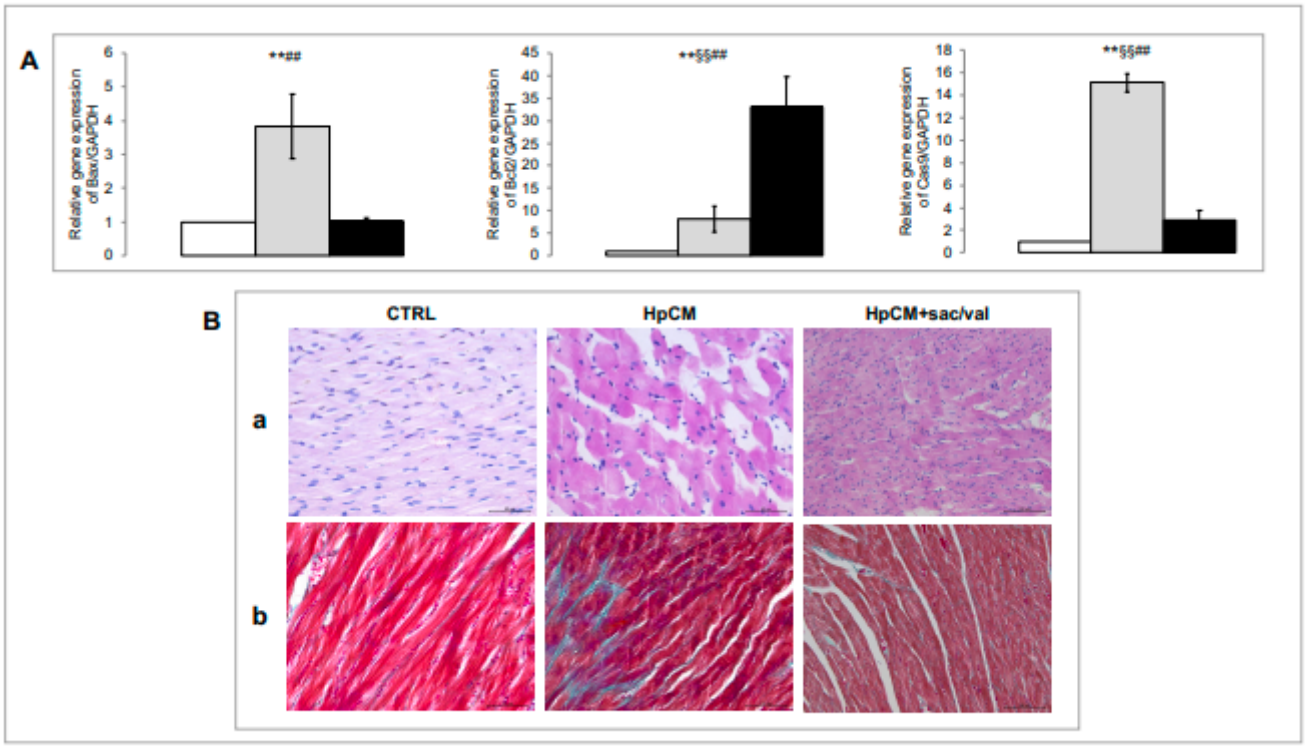

Figure 5

Effects of sacubitril/valsartan treatment on A) relative gene expression of Bax, Bcl2 and Cas3 measured in the left ventricle of isolated rat heart, B) Representative images of CTRL, HpCM and HpCM+sac/val hearts stained with (a) hematoxylin and eosin, and (b) Masson's trichrome. GADPH; Glyceraldehyde-3-phosphate dehydrogenase. Statistical analyses were calculated using one-way ANOVA, $p < 0.05$. *Ctrl vs. HpCM, #CTRL vs. HpCM/HpCM+sac/val.