Interaction Between Sacubitril and Valsartan in Preventing Heart Failure Induced by Aortic Valve Insufficiency in Rats

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ABSTRACT

Background: Synergistic interactions between neprilysin inhibition (NEPi) with sacubitril and angiotensin receptor type1 blockade (ARB) with valsartan have been implicated in improvement of left ventricular (LV) contractility, relaxation, exercise tolerance, and fibrosis in preexisting heart failure (HF) induced by aortic valve insufficiency (AVI). It is not known whether this pharmacologic synergy can prevent cardiovascular pathology in a similar AVI model. Our aim was to investigate the pharmacology of sacubitril/valsartan in an experimental setting with therapy beginning immediately after creation of AVI.

Methods: HF was induced through partial disruption of the aortic valve in rats. Therapy began 3 hours after valve disruption and lasted 8 weeks. Sacubitril/valsartan (68 mg/kg), valsartan (31 mg/kg), sacubitril (31 mg/kg), or vehicle were administered daily via oral gavage (N=8 in each group). Hemodynamic assessments were conducted using Millar technology, and an exercise tolerance test was conducted using a rodent treadmill.

Results: Only sacubitril/valsartan increased total arterial compliance and ejection fraction (EF). Therapies with sacubitril/valsartan and valsartan similarly improved load-dependent (dP/dt_{max}) and load independent indices (Ees) of LV contractility, and exercise tolerance, whereas sacubitril did not. None of the therapies improved LV relaxation (dP/dt_{min}), whereas all reduced myocardial fibrosis.

Conclusions: 1) The synergistic interaction between NEPi and ARB in early therapy with sacubitril/valsartan leads to increased total arterial compliance and EF. 2) Improvement in indices of LV contractility, and exercise tolerance with sacubitril/valsartan is likely because of ARB effect of valsartan. 3) All three therapies provided antifibrotic effects, suggesting both ARB and NEPi are capable of reducing myocardial fibrosis. (*J Cardiac Fail 2019;25:921–931*)

Key Words: Sacubitril/valsartan, heart failure, aortic valve insufficiency, cardiac function, total arterial compliance.

Pharmacologic elevation of atrial natriuretic peptide (ANP) by inhibiting neprilysin (NEP), an enzyme that degrades this hormone, was proposed as a strategy to treat heart failure (HF) 3 decades ago.[20,72] However, subsequent clinical trials demonstrated limited efficacy of this approach,[12,51] which some suggested was because of elevation of angiotensin II (ANG II)[26] from NEP inhibition (NEPi).[11,78] Because ANP and ANG II have opposing effects on vascular tone, natriuresis, and blood volume,[29] the rationale behind the combination of NEPi and angiotensin receptor blockade (ARB) was to promote the therapeutic effects of ANP and prevent the negative effects of ANG II elevation.[47] The benefits of simultaneous NEPi with sacubitril and ARB with valsartan on patients with HF have been widely recognized. The PARADIGM-HF study demonstrated that sacubitril/valsartan is superior to enalapril not only in reducing mortality but also in improving health related quality of life and exercise tolerance in patients with HF.[6,39,48,63] Thus far, the PARADIGM-HF study has been the largest clinical trial for HF, which included patients with history of myocardial ischemia, hypertension, or diabetes, but only 0.07% of patients had a history of aortic valve insufficiency (AVI),[7,13,79] even though the prevalence of this condition is 4.9% in the population.[46]

Although the health benefits of sacubitril/valsartan are striking, the efficacy may vary between cardiovascular diseases of different etiologies. Pharmacology of sacubitril/valsartan has been studied using animal models of myocardial infarction,[25,37,69] hypertension,[38,45,62] diabetes,[14,66] and renal dysfunction.[27,65,71] However, there is only 1 study where the

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Manuscript received May 3, 2019; revised manuscript received August 3, 2019; revised manuscript accepted September 12, 2019.

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Funding: This project was funded by the Novartis Pharmaceuticals Corp (LCZ696BUSNC05T).

1071-9164/$ - see front matter

https://doi.org/10.1016/j.cardfail.2019.09.008

pharmacologic effects of sacubitril/valsartan were investigated using a HF model induced by AVI.[44] This recent investigation suggested synergistic interactions between NEPi with sacubitril and ARB with valsartan in improving some indices of left ventricular (LV) contractility, exercise tolerance, and reduction of myocardial collagen content in *preexisting* HF. Although these findings are intriguing, it is not known if early *preemptive* therapy with sacubitril/valsartan leads to similar synergistic interactions between NEPi and ARB. In this study, we investigated the pharmacologic effects of preemptive therapy with sacubitril/valsartan and potential interactions between sacubitril and valsartan in the HF model induced by acute AVI in rats.

Methods

The animal study protocol was approved by the Institutional Animal Care and Use Committee at Concord Biomedical Science and Emerging Technologies, Inc (Lexington, MA). Eighty Sprague-Dawley male rats (weight 375−425 g) purchased from Charles Rivers Laboratories (Wilmington, MA) were randomly assigned into 5 groups: 1) Sham control (N=16), 2) AVI control + therapy vehicle (N=16), 3) AVI + therapy with sacubitril/valsartan (N=16), 4) AVI + therapy with valsartan (N=16), and 5) AVI + therapy with sacubitril (N=16; Table 1). Each group was randomly divided into 2 equal subgroups for a) Hemodynamic assessment (N=8 in each group), and b) exercise tolerance testing (N=8 in each group). The parallel group design of the study was necessary to prevent the potential interference of exercise tolerance testing on hemodynamic indices. Experimental groups are summarized in Table 1.

Table 1. Hemodynamic, Morphologic, Metabolic, and Functional Assessments in Rats With Heart Failure 8 Weeks after Inducing Aortic Valve Insufficiency

Assessments	Sham Control N=8	HF + Vehicle (Control) N=8
Steady-state hemodynamic data		
HR, bpm	292±29	274±37
ESV, mcl	168±19	250±29*
EDV, mcl	261±26	362±28*
SV, mcl	85±12	92±21
EF, %	35±4	27±5*
CO, mL/min	23845±4729	24331±5188
dP/dtmax, mmHg/s	6197±356	5262±693*
−dP/dtmin, mmHg/s	4632±260	3527±518*
MAP, mmHg	89±4	76±11*
SVR, mmHg*min/L	4137±709	3272±617*
Total arterial compliance, mcl/mmHg	2.7±0.5	2.9±0.2
Regression hemodynamic data		
Ees	1.37±0.24	0.46±0.13*
LV Morphologic data		
LVW/BW	1.98±0.20	2.51±0.30*
Myocardial collagen content, %	2.28±0.77	5.84±1.75*
Biochemical data		
ANP, pg/mL	79±14	197±25*
ANG II, pg/mL	101±18	173±24*
Exercise tolerance data		
Running distance, m	1464±239	1074±182*

Data expressed as means ± SD. A *t* test was used to evaluate heart failure induced by aortic valve insufficiency.

ANG II=angiotensin II, ANP=atrial natriuretic peptide, CO=cardiac output, dP/dtmax=maximal rate of rise of left ventricular pressure during systole, dP/dtmin=maximal rate of decline of left ventricular pressure during diastole, EDV=end diastolic volume, Ees=the slope of the end-systolic pressure volume relationship, EF=ejection fraction, ESV=end systolic volume, HR=heart rate, LVW/BW=left ventricular weight to body weight ratio, MAP=mean arterial pressure, SV=stroke volume, SVR=systemic vascular resistance.

*$P \leq .05$ compared with Sham controls.

Presurgical Preparation

Animals were sedated with inhaled isoflurane 1%−2% in 100% oxygen (1 L/min) in a sealed anesthetizing chamber. The neck was shaved and prepped with consecutive applications of iodine and alcohol. Each rat was secured to a heating pad, which was adjusted to maintain rectal temperature at 37°C. The duration of each survival surgical procedure was 30−40 minutes.

AVI Model Creation by Disruption of the Aortic Valve

The surgical procedure is described in detail elsewhere.[44] General anesthesia was maintained with nasal administration of inhaled isoflurane 1%−2% in 100% oxygen (1 L/min) delivered by a nasal mask using animal veterinary anesthesia system (VetEquip IMPAC6, Pleasanton, CA). The AVI model was created via mechanical disruption of the aortic valve using a Portex epidural catheter advanced retrograde from a right carotid arteriotomy.[2,58,70] In a pilot study of 10 animals the decline in diastolic blood pressure was 30±4% (Supplementary Fig. S1). Immediately after AVI creation animals were randomly assigned to either AVI control or 1 of the 3 therapy groups.

Pharmacologic Therapy

Each therapy was administered daily by oral gavage for 8 weeks starting 3 hours after aortic valve disruption. This 3-hour postsurgical interval was sufficient for the animals to recover from anesthesia to allow for safe oral gavaging. This regimen of drug administration was chosen to investigate whether early therapy can mitigate HF development due to AVI. Sacubitril/valsartan (68 mg/kg), valsartan (31 mg/kg), and sacubitril (31 mg/kg) were dissolved in starch (1%) aqueous solution (1 mL) as recommended by the Division of the Cardiovascular and Metabolic Diseases at Novartis Institute for Biomedical Research (Cambridge, MA). Sacubitril/valsartan is a salt complex of sacubitril, valsartan, sodium, and H_2O and their ratio is 1:1:3:2.5. Thus, for 68 mg of sacubitril/valsartan there is essentially 31 mg sacubitril, 31 mg valsartan, and 6 mg sodium/water.[21] The sham control and AVI control groups were gavaged with starch solution as a placebo to account for the impact of animal handling stress related changes on cardiovascular function, and to establish the extent of the resulting HF.

Hemodynamic Data Acquisition

Hemodynamic indices were assessed 8 weeks after AVI induction as described elsewhere.[43−45] Briefly, the animals were re-anesthetized with inhaled isoflurane and connected to

a rodent ventilator (#683, Harvard Apparatus, Holliston, MA) through a tracheotomy. The tidal volume and respiration rate were calculated using the following formulas: a) tidal volume (mL) = $6.2 \times M^{1.01}$ (M = animal mass, kg); b) respiration rate (min^{-1}) = $53.5 \times M^{-0.26}$.[53] A heating lamp and pad were adjusted to maintain rectal temperature at approximately 37°C. The right femoral artery was cannulated for mean arterial blood pressure (MAP) and pulse pressure (PP) measurements (transducer: #TRN050, amplifier #TRN005; Kent Scientific, Torrington, CT). The heart was exposed through bilateral anterolateral thoracotomies for transapical placement of the Millar conductance catheter (#SPR-869, 6mm , Millar catheter, MPVS-300 system; Houston, TX) into the LV chamber. Hemodynamic data were acquired at steady state. LV pressure and volume signals from the Millar catheter were acquired using a Powerlab A/D converter (AD Instruments, Colorado Springs, CO) and recorded with Chart 5.0 (AD Instruments). The data were analyzed using LabChart 7 software (AD Instruments). Indices of LV function included heart rate (HR), end systolic and end diastolic volumes (ESV, EDV). Stroke volume (SV) was calculated as the difference in EDV and ESV. Cardiac output (CO) was calculated as the product of the HR and the SV. The first derivative of left ventricular pressure signal was processed for maxima and minima (dP/dt_{max}[maximal rate of rise of left ventricular pressure during systole] and dP/dt_{min} [maximal rate of decline of left ventricular pressure during diastole]) and used as indices of load-dependent myocardial contraction and relaxation, respectively. Indices of afterload included systemic vascular resistance (SVR) and total arterial compliance. SVR was calculated as follows: SVR = (MAP) / CO; (central venous pressure was assumed to be 0). Total arterial compliance, an index of the elasticity of arteries, which impacts the load on the heart, was calculated as SV/PP.[75] The end-systolic pressure volume relationship (ESPVR) describes the end-systolic pressure that can be developed by the LV at any given volume. A load independent index of LV contractility was characterized by the slope (Ees [the slope of the end-systolic pressure volume relationship]) of the ESPVR acquired during a brief ligature occlusion of the inferior vena cava.[64]

Tissue and Blood Harvesting and Processing, and Euthanasia

At the end of hemodynamic data acquisition a blood sample was taken from the femoral artery and the heart quickly excised. Heparinized blood samples were centrifuged at 1100 g for 15 min and the supernatant was stored at −20°C for measurement of ANP and ANG II levels. Harvested myocardial tissue samples were rinsed with saline at 4°C, blotted, trimmed off the right ventricle and atria, and LV weighed (Mettler AJ100, Columbus, OH), and fixed in 10% formalin for morphometric and histologic analysis. At the end of the exercise tolerance experiments animals were euthanized with inhaled CO_2.

Fixated LV samples were dehydrated in graded alcohols, cleared in xylenes, and embedded in paraffin. Embedded blocks were sectioned into 5-μm-thick slices and stained with Masson's Trichrome. Digital light microscope (Olympus BX50, Center Valley, PA; Digital camera, DSC-717, Tokyo, Japan) was used to generate digital images of the LV sections (field-of-view ~200 μm). ImageJ software (ImageJ 1.45S, NIH) was used for assessment of the extent of fibrosis. Two transmural areas, anterior and inferior, from each circumferential LV section were used in quantitative assessments of myocardial collagen content as marker of myocardial fibrosis and the average value was used in the analysis. The ratio of collagen stained area to the entire area of the imaged LV section was used to evaluate the extent of the myocardial fibrosis. Plasma ANP and ANG II levels were evaluated using colorimetric enzyme-linked immunosorbent assays (ELISA #LS-F14046 and ELISA #LS-F23256-1; LifeSpan Biosciences Inc, Seattle, WA, respectively).

Exercise Tolerance Testing

A rodent treadmill (Panlab/Harvard Apparatus 3-Lane Rat Treadmill, Holliston, MA) was used to evaluate exercise tolerance. Animals were familiarized to the treadmill on two occasions (5 days and 3 days prior to the terminal experiment) for 15 minutes at the lowest speed (5 m/min). During the exercise tolerance testing, animals underwent forced exercise (15 m/min) until exhaustion. When the animal refused to run it fell behind the running belt and received escapable aversive stimuli in the form of mild electric shocks (1 mA) from an electrified wire mesh grid. The electric shock was intended to provoke the animal to resume running. The experiment was immediately terminated when the animal refused to run and remained on the electric grid longer than 3 seconds. The running distance wherein the shock no longer provoked the animal to continue running was used as a measure of the exercise tolerance.

Sample Size Calculation

The number of the animals required was calculated based on data from a pilot investigation conducted in our laboratory. This pilot study had demonstrated that the disruption of the aortic valve reduced ejection fraction (EF) by 30% after 8 weeks. The requirement of our study was to detect at least 15% improvement in EF with sacubitril/valsartan. Assuming a 95% chance of rejection the null hypothesis with a 5% acceptance of a type I error, the sample size calculation showed that N=8 in each group would be sufficient to detect 15% improvement in EF with studied therapies.

Statistical Analysis

Hemodynamic, exercise tolerance, and biochemical data are represented as percentage change from intact sham controls to validate HF due to AVI; values of the intact sham controls were considered as 100% (Fig. 1). The data from the treatment groups are represented as percentage change from AVI controls to evaluate each therapy; for these data the AVI controls were considered as 100% (Figs. 2 and 3). All datasets were analyzed for normality using a

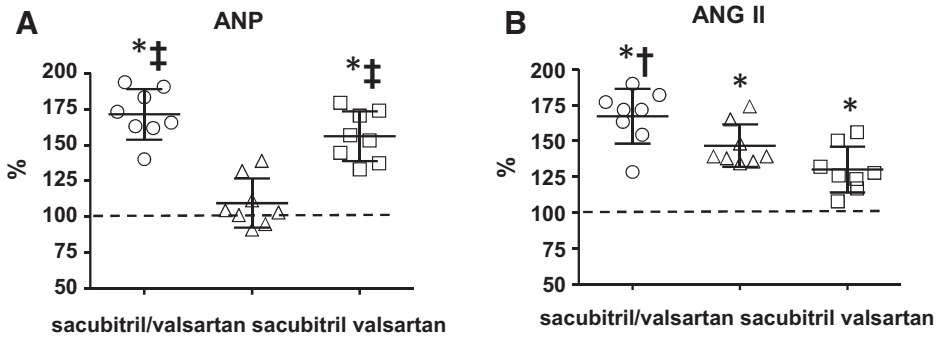

Fig. 1. Effects of sacubitril/valsartan, valsartan, or sacubitril on plasma levels of ANP (**A**), and ANG II (**B**) in the model of AVI (N=8 in each group). Therapy groups were compared with HF control group (rats with aortic insufficiency without treatment) and to each other to evaluate the effects of treatments. Values of AVI controls (dashed line) are presented as 100%. Values of therapy groups are presented as percentage change from AVI controls. The figure displays individual data from each animal (circles, therapy sacubitril/valsartan; triangles, therapy with valsartan; squares, therapy with sacubitril), and means (horizontal lines) and standard deviations (vertical bars). A one-way ANOVA test with Tukey's correction for multiple comparisons was used in the analysis to compare therapies with AVI controls and between each other. *$P \leq .05$ compared with AVI controls. ‡$P \leq .05$ compared with therapy with valsartan; †$P \leq .05$ compared with therapy with sacubitril.

D'Agostino-Pearson normality test. A *t* test was used to evaluate AVI model. A one-way ANOVA test with Tukey's correction for multiple comparisons was used to investigate the pharmacology of sacubitril/valsartan by comparing them to treatment with its constitutive components: valsartan and sacubitril (GraphPad Prism, 5.0). A Pearson correlation analysis was used to evaluate the relationship between the actual values of EF and indices of afterload (ESV and total arterial elastance; GraphPad Prism, 5.0). Data were considered statistically distinct if the *P*value was less than .05.

Results

Validation of the HF Model Due to AVI Induced by the Aortic Valve Disruption: Cardiovascular, Biochemical, and Functional Changes

AVI induced by disruption of the aortic valve led to cardiovascular dysfunction by 8 weeks. There was an increase in left ventricular weight to body weight ratio (LVW/BW) by 27%, ESV by 49%, and EDV by 39%. There was also a decline in LV systolic function as shown by a reduction in dP/dt_{max} by 15%, EF by 23%, and Ees by 67%, and diastolic function as shown by a decline in $-dP/dt_{min}$ by 24% (Table 1). Vascular tone (SVR) and MAP were reduced by 15% and 21%, respectively. Total arterial compliance was not different from sham controls. Cardiovascular dysfunction resulted in significant exercise intolerance as shown by reduction in the running distance by 27%. There was an increase in plasma concentrations of vasoactive peptides (ANP and ANG II), and myocardial collagen content by 149%, 71%, and 156%, respectively.

Pharmacologic Effects of Sacubitril/Valsartan, Valsartan, and Sacubitril

Effects on Concentrations of Plasma Vasoactive Peptides. Combined NEPi and ARB with sacubitril/valsartan elevated concentrations of ANP by 72%, and ANG II by 67% compared with HF controls (Fig. 1A, B). Isolated ARB with valsartan did not change ANP, but increased ANG II concentrations by 47% compared with HF controls. Isolated NEPi with sacubitril increased concentrations of ANP by 56% and ANG II by 30% compared with HF controls. Concentrations of ANP after therapy with sacubitril/valsartan were 57% higher than with valsartan, but not different from therapy with sacubitril. Concentrations of ANG II following therapy with sacubitril/valsartan were 14% and 29% higher than with valsartan and sacubitril, respectively.

Cardiovascular Effects. Combined NEPi and ARB with sacubitril/valsartan improved LV function: EF by 27%, dP/dt_{max} by 15%, and Ees by 67% compared with HF controls (Fig. 2A–C). However, there was no improvement in LV relaxation (dP/dt_{min}; Fig. 2D). LV mass (LVW/BW; data not shown), ESV, and EDV (Fig. 2E,F) were not different from HF controls. However, ESV was 22% and 34% lower than with valsartan and sacubitril, respectively (Fig. 2E). SVR (Fig. 2G) and MAP (Fig. 2H) remained reduced and were not different from AVI controls or other therapies. Total arterial compliance was 44%, 43%, and 67% higher than in HF controls, valsartan and sacubitril treatment groups (Fig. 2I). Myocardial collagen content was reduced by 40% compared with HF controls (Fig. 2J).

Isolated ARB with valsartan had therapeutic effects on LV contractility as shown by an increase in dP/dt_{max} and Ees by 15% and 65%, respectively, compared with HF controls (Fig. 2B, C). However, there was no improvement in EF (Fig. 2A). LV relaxation (dP/dt_{min}) also remained depressed (Fig. 2D). Therapy with ARB neither mitigated the increase in LV mass (LVW/BW; data not shown), ESV, and EDV (Fig. 2E, F), nor improved SVR (Fig. 2G) and MAP (Fig. 2H). Total arterial compliance was not different from HF controls (Fig. 2I). ARB lowered myocardial collagen content by 36% compared with HF controls (Fig. 2J).

Isolated NEPi with sacubitril did not improve LV function as EF, dP/dt_{max}, Ees, and dP/dt_{min} remained depressed

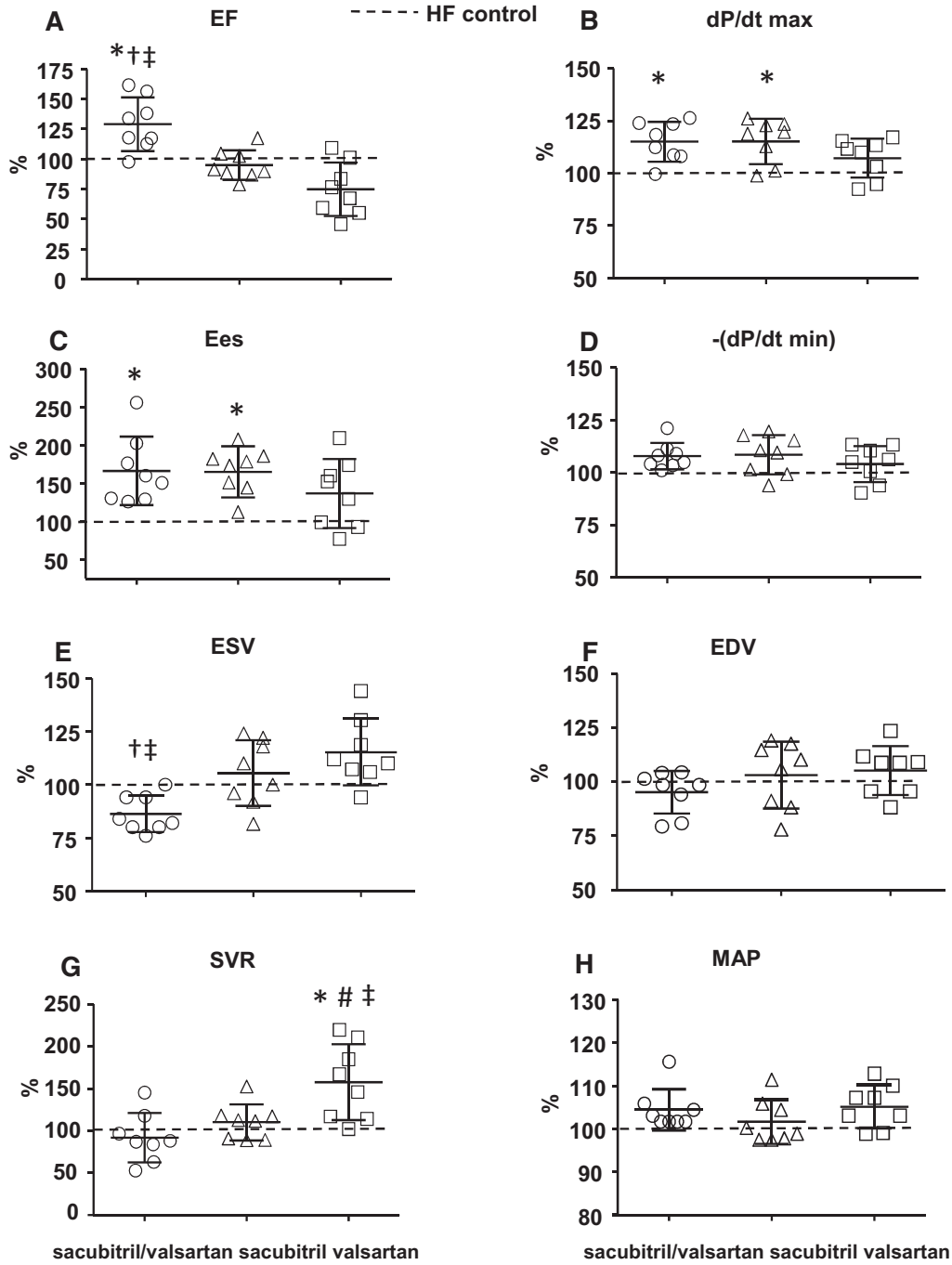

Fig. 2. Effects of sacubitril/valsartan, valsartan, or sacubitril on EF (**A**), load-dependent index of contractility (dP/dt max; **B**), load independent index of contractility (Ees; **C**), and relaxation (dP/dt min; **D**), ESV (**E**), EDV (**F**), SVR (**G**), MAP (**H**), total arterial compliance (**I**), myocardial collagen content (**J**), and exercise tolerance (running distance; **K**) in rats with AVI (N=8 in each group). Therapy groups were compared with HF control group (rats with AVI without treatment) and to each other to evaluate the effects of treatments. Values of AVI controls (dashed line) are presented as 100%. Values of therapy groups are presented as percentage change from AVI controls. The figure displays individual data from each animal (circles, therapy sacubitril/valsartan; triangles, therapy with valsartan; squares, therapy with sacubitril), and means (horizontal lines) and standard deviations (vertical bars). A one-way ANOVA test with Tukey's correction for multiple comparisons was used in the analysis to compare therapies with AVI controls and between each other. *****$P \leq .05$ compared with AVI controls. #$P \leq .05$ compared with therapy with sacubitril/valsartan treatment group. ‡$P \leq .05$ compared with therapy with valsartan. †$P \leq .05$ compared with therapy with sacubitril.

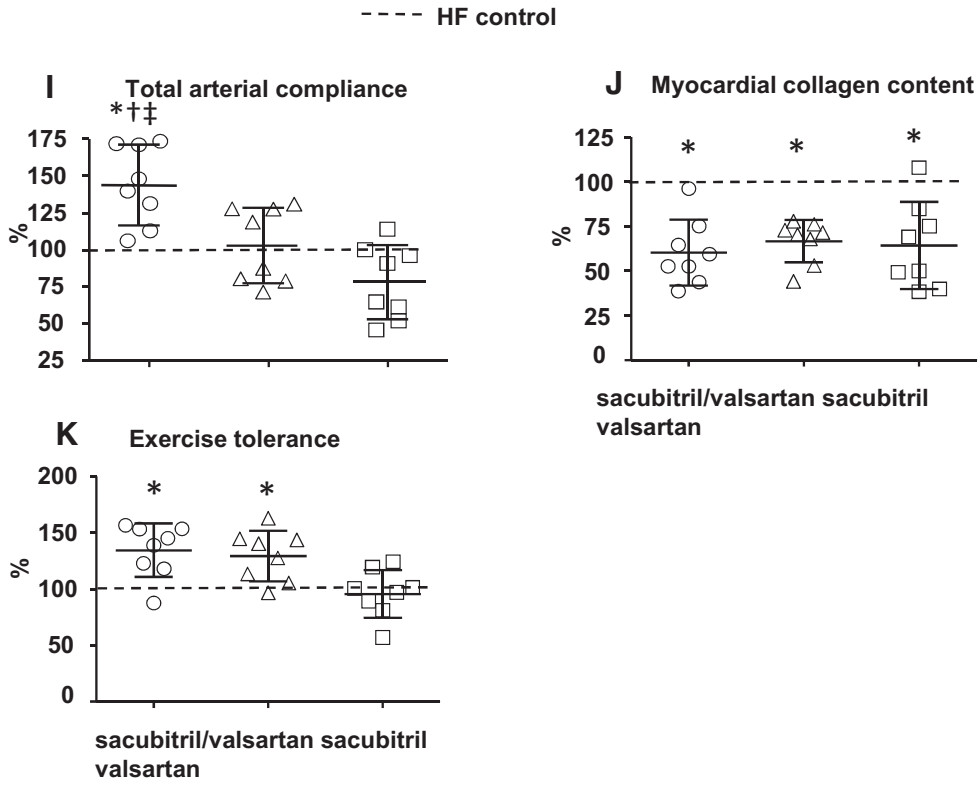

Fig. 2 Continued.

(Fig. 2A–D). LV mass (LVW/BW; data not shown), ESV and EDV (Fig. 2E, F) were also not different form HF controls. Vascular tone was significantly increased as SVR was 58%, 71%, and 43% higher than HF controls, sacubitril/valsartan, and valsartan, respectively (Fig. 2G). However, MAP was not different from HF controls (Fig. 2H). Total arterial compliance remained the same as HF controls (Fig. 2I). NEPi mitigated myocardial fibrosis as myocardial collagen content was 30% lower than in HF controls (Fig. 2J).

A Pearson correlation analysis of the combined dataset of all groups revealed a significant correlation between EF and ESV ($r = -0.71$, $P < .0001$; Fig. 3A), and EF and total arterial compliance ($r = 0.67$, $P < .0001$; Fig. 3B).

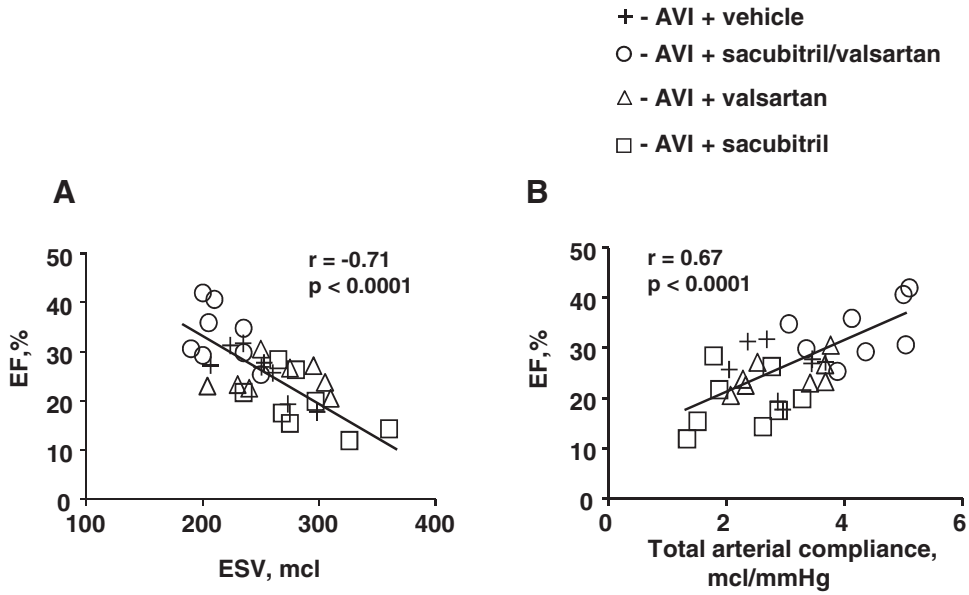

Fig. 3. A Pearson correlation analysis evaluating the relationship between the actual values of EF and ESV (**A**), and EF and total arterial elastance (**B**). All groups with AVI (including controls and therapies) are combined in one dataset.

Effects on Exercise Tolerance. Combined NEPi and ARB with sacubitril/valsartan and isolated ARB with valsartan improved exercise tolerance as shown by an increase in running distance by 34% and 28%, respectively (Fig. 2K). Isolated NEPi with sacubitril did not improve exercise tolerance.

Discussion

In this study, we investigated the pharmacologic effects of early preemptive treatment with combined NEPi (sacubitril) and ARB (valsartan) in rats with acute AVI. We analyzed potential interactions between NEPi and ARB by comparing combination therapy with isolated treatments of each. The data suggested that early treatment with sacubitril/valsartan prevented the development of HF as demonstrated by maintained LV contractile function and exercise tolerance. In addition, synergistic interactions between sacubitril and valsartan prevented the decline in EF seen in AVI. Last, though there was improvement in indices of contractility (dP/dt_{max} and Ees) and exercise tolerance by sacubitril/valsartan, this effect was likely a result of ARB by the valsartan constituent.

Validation of the Rodent Model of HF Induced by Aortic Valve Disruption Leading to LV Volume-Overload

AVI led to LV dysfunction (EF, dP/dt_{max}, dP/dt_{min}, and Ees), and exercise intolerance suggesting the development of HF. In addition, there were other morphologic abnormalities such as an increase in LV mass (LVW/BW), LV dilatation (EDV), and myocardial fibrosis (myocardial collagen content). Pathologic changes in circulation manifested as reductions in vascular tone (SVR) and MAP. These observations are consistent with other animal studies,[1–3,16,58,60,70] and are analogous to pathologic changes seen in patients with HF due to aortic valve insufficiency.[8,9,15,17,22,28,33,59,74] Therefore, the rat HF model induced by AVI was appropriate to study the pharmacologic effects of NEPi and ARB by sacubitril/valsartan.

Pharmacologic Effects of the Early Treatment with Sacubitril/Valsartan: Analysis of Interaction Between Sacubitril and Valsartan

The objective of studying the pharmacologic effects of sacubitril/valsartan in parallel with monotherapies with valsartan and sacubitril was to reveal potential interactions between NEPi and ARB. In a recent study using a model of *preexisting* HF induced by AVI[44] we concluded that therapy with sacubitril/valsartan had distinct synergistic interactions between NEPi and ARB leading to an increase in load-dependent indices of LV contractility (dP/dt_{max}) and relaxation (dP/dt_{min}), reduction of myocardial fibrosis, and improvement of exercise tolerance. In the present study of *preemptive* treatment for HF, we showed that sacubitril/valsartan has a different pharmacologic profile.

Effects on Plasma Level of Vasoactive Peptides. Early preemptive treatment with combined NEPi and ARB with sacubitril/valsartan, and isolated NEPi with sacubitril significantly increased both plasma ANP and ANG II concentrations as a result of diminished degradation of these peptides with neprilysin.[10] The increase in plasma ANG II with monotherapy with valsartan was likely a result of the interrupted positive feedback to the activation of the renin-angiotensin-aldosterone system (RAAS). Note that ANG II increases glomerular filtration rate (GFR) in patients with HF.[54] Valsartan blocks the angiotensin II receptors in the kidney, diminishing GFR. The reduction in GFR further activates RAAS resulting in elevated ANG II level. These observations are consistent with prior studies of sacubitril/valsartan in AVI models of preexisting HF.[44]

Effects on LV Function. Comparison of the LV effects of sacubitril/valsartan to the isolated therapies allowed assessing the role of each constituent of the combination therapy. Even though valsartan[77] and ANP elevation[32] improved EF in patients with HF, in our AVI model early preemptive treatment with valsartan or ANP elevation with NEPi did not have beneficial effects on this index. However, combination therapy with sacubitril/valsartan significantly improved EF suggesting synergistic relationship between NEPi and ARB. The likely cause for this improvement is reduction in afterload (see the following section). Both sacubitril/valsartan and valsartan similarly improved load independent and load depended indices of LV contractility (Ees and dP/dt_{max}), whereas sacubitril did not. NEPi with sacubitril elevated not only ANP but also ANG II. ANG II is known to directly cause mechanical myocardial dysfunction even in healthy animals because of alterations in Ca^{2+} handling,[24] and increase afterload which may also limit EF.[73] Therefore, it is likely that this deleterious effect of ANG II outweighed the therapeutic effect of ANP in animals treated with sacubitril. Importantly, the effects of sacubitril/valsartan and valsartan on Ees and dP/dt_{max} were comparable and not statistically different, suggesting the likely role of the ARB constituent, rather than ANP elevation with NEPi. The role of valsartan in improving these indices is supported by several experimental studies.[30,40,44,67] Other studies reported that ANP elevation improves dP/dt_{max} and Ees.[50,52] Perhaps because of the severity of our model, ANP elevation with isolated NEPi apparently was not sufficient to lead to improvements in dP/dt_{max} and Ees (Fig. 2B, C). In particular, it has been shown that ANP elevation improves dP/dt_{max} only in NYHA class I HF, but not in patients with class II and III HF.[50] In addition, although several studies have shown that ANP elevation and valsartan improve a load-dependent index of LV relaxation (dP/dt_{min}),[30,40,50] the severity of our model was also probably the reason why neither monotherapy, nor even their combination, were sufficient to improve this index.

Synergistic Interaction Between Sacubitril and Valsartan Leading to Improvement of EF. Comparison of the effects of sacubitril/valsartan and valsartan on the factors determining EF suggested that sacubitril/valsartan improved this index by reducing LV afterload via increasing total arterial compliance. Specifically, although SV was not significantly higher

with sacubitril/valsartan compared with valsartan, ESV was 22% lower suggesting a lower afterload[56] with the combination therapy. Interestingly, such constituents of the afterload as systemic vascular resistance, end diastolic pressure, and arterial elastance (data not shown) were not different between sacubitril/valsartan and valsartan. However, another important index of afterload, the total arterial compliance[55] was 33% higher with sacubitril/valsartan compared with AVI controls, whereas other therapies did not increase this index. Significant correlation between ESV and EF suggested that improvement in this index of LV function occurred due to reduced afterload (Fig. 3A). Moreover, correlation between EF and total arterial compliance indicated that the reduction in afterload likely resulted from the increased ability of the arteries to distend in response to changes in blood pressure (Fig. 3B). Even though our data do not directly suggest a potential mechanism for an increase in total arterial compliance, others have found in ex vivo setting that maximal vasorelaxation in response to acetylcholine was better with sacubitril/valsartan than with valsartan.[69] An increased vasorelaxation, in turn, is speculated to improve total arterial compliance.[19] The increase in total arterial compliance may also slow the rise in arterial pressure during systole resulting in lower wall stress and lower oxygen consumption by LV myocytes,[49] and hence lower energetic cost of LV systole.[36] Although this study does not provide the mechanism of the synergistic interaction between sacubitril and valsartan in increasing total arterial compliance, several studies suggested the role of nitric oxide (NO) and endothelium-derived hyperpolarizing factor.[4,5,35,61,76] Because sacubitril/valsartan increases NO bioavailability[69] this could be one of the potential causes for increased total arterial compliance.

Effects of Sacubitril/Valsartan on LV Morphology, Myocardial Fibrosis, and SVR. Even though sacubitril/valsartan and valsartan improved LV function there was no mitigation of the increase in LV mass (LVW/BW) or EDV. The lack of the effect on these indices was somewhat expected in aortic valve insufficiency and LV overload, which were present throughout the entire experiment.

All three therapies reduced myocardial collagen content. Because ANG II directly causes myocardial fibrosis,[18,23,42,68] it had been expected that combined NEPi and ARB, and isolated ARB would mitigate this pathologic process. In addition, ANP has also been reported to directly inhibit myocardial fibrosis.[18,41] Interestingly, valsartan and ANP are thought to have distinct and differential mechanisms leading to inhibiting myocardial fibrosis: valsartan by reducing expression of transforming growth factor-β/Smad and hypoxia-inducible factor-1α,[67] and ANP by blocking endothelin-1 gene expression.[34] However, the combination of these two suggested mechanisms with sacubitril/valsartan did not lead to an apparent advantage over monotherapies with sacubitril and valsartan. Therefore, it is not clear which one of these underlying mechanisms played the dominant role in the combination therapy.

Effects of Sacubitril/Valsartan on Exercise Tolerance. Both sacubitril/valsartan and valsartan similarly improved exercise tolerance, whereas sacubitril did not (Fig. 2J). These data suggest the absence of synergistic interaction between NEPi and ARB in the combination therapy and that the improvement was largely because of ARB constituent. Indeed, there are no published data indicating the potential role of ANP on exercise tolerance. The supporting evidence that ANG II directly reduces functional capacity[31] indicates the likely role of ARB in improving exercise tolerance in our study.

In contrast to these data, ARB with valsartan did not improve exercise tolerance in animals and patients with preexisting HF,[44,57] suggesting that valsartan may be more effective as prophylaxis for degenerating exercise intolerance in early stage in patients with HF.

Preemptive Treatment Compared With Treatment of Preexisting HF

This study suggests a shift in pharmacologic effects of early preemptive treatment with sacubitril/valsartan compared with treatment of *preexisting* HF. Previously we demonstrated in a similar AVI model of preexisting HF a distinct synergistic interaction between sacubitril and valsartan in improving indices of LV contractility (dP/dt_{max} and Ees), LV relaxation (dP/dt_{min}), reducing myocardial fibrosis, and improving exercise tolerance.[44] In this study we show that *preemptive* administration of sacubitril/valsartan does not offer these particular synergistic benefits and that improvements in LV function, morphology, and exercise tolerance are solely due to valsartan. However, preemptive therapy demonstrated synergistic interaction between sacubitril and valsartan in reducing afterload by increasing total arterial compliance. This synergistic effect was the likely cause for improved EF compared with AVI controls and other therapies in this preemptive dosing study.

Limitations and Further Directions

Although this study demonstrates that *preemptive* therapy with sacubitril/valsartan offers the benefit of synergistic interaction between NEPi and ARB in increasing total arterial compliance and hence lower afterload and better EF, the data do not suggest the actual mechanism for this improvement. Several studies have suggested the role of NO[4,5,35,61,76] in maintaining total arterial compliance. Because sacubitril/valsartan was shown to increase NO bioavailability[69] this could be the likely mechanism underlying increased total arterial compliance. Further investigation is warranted to confirm these mechanisms. In addition, several reports indicated that increased total arterial compliance may lower myocyte oxygen consumption[49] and lower energetic cost of LV systole.[36] Therefore, confirmation that sacubitril/valsartan leads to these improvements is also important. Even though the rat model used in this study does not directly translate to clinical HF caused by AVI and therefore, the results cannot be directly extrapolated to clinical practice, these data provide basis for investigating the

potential synergistic effect between NEPi and ARB on total arterial compliance in patients.

Conclusions

Early therapy with sacubitril/valsartan of the HF associated with AVI offers synergistic interactions between NEPi and ARB in improving EF. EF improvement likely resulted from the reduction of the afterload (ESV) due to increased total arterial compliance. There was no synergistic interaction between NEPi and ARB in improving load-dependent and load-independent indices of LV contractility (dP/dt_{max}, Ees), myocardial fibrosis, and exercise tolerance as these improvements likely resulted from ARB constituent of the therapy.

Acknowledgments

We thank Brett Zani, MS, PhD (CBSET: Director, Applied Sciences) for directing the subcontracted work (logistics, scheduling), and Sara Woldemariam, BS (CBSET: Research Technician) for animal care and drug administration.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.cardfail.2019.09.008.

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