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Detection of soluble suppression of tumorigenicity 2 and N-terminal B-type natriuretic peptide in a rat model of aortic regurgitation: differential responses to omecamtiv mecarbil

Abstract

Objectives: Both N-terminal fragment of B-type natriuretic peptide (NT-proBNP) and soluble isoform of ST2 (sST2) have been identified as biomarkers of heart failure. We evaluated the plasma levels of NT-proBNP and sST2 in a rat model of severe aortic valve regurgitation (AR) and correlated these findings with echocardiographic measurements. We also examined the impact of omecamtiv mecarbil (OM) on these parameters.

Methods: The plasma levels of NT-proBNP and sST2 were measured in 18 rats both before and 2 months after surgical induction of AR, and at these same time points, in six rats assigned to a sham-procedure control group. Plasma biomarkers were then measured again after infusion of OM or placebo in rats with AR (n=8 and 10, respectively) and OM alone in the sham control rats (n=6). Echocardiographic measurements were collected before and 2 months after induction of AR.

Results: Our results revealed increased levels of plasma NT-proBNP (219 ± 34 pg/mL vs. 429 ± 374 pg/mL; p<0.001) in rats with AR at day 7 after infusion of placebo, whereas plasma levels of sST2 were higher in this cohort after infusion of either OM or placebo. We identified a significant positive correlation between plasma sST2 with posterior wall thickness in diastole (r=0.34, p<0.05) and total body weight (r=0.45, p<0.01) in rats with surgically induced AR.

Conclusions: Because sST2 increased markedly, whereas NT-proBNP remained unchanged, when OM was administered, we hypothesize that sST2 has a distinct capability to detect deleterious effects of passive muscle tension, not reliably assessed by NT-proBNP, in the setting of AR.

Keywords: aortic regurgitation; biomarkers; heart failure; omecamtiv mecarbil; overload.

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Introduction

First described in 1989 [1], the soluble isoform of suppression of tumorigenicity 2 (ST2) has been identified as a biomarker that can be used to monitor the progression and prognosis of acute and chronic heart failure (HF) [2]. ST2 is a member of the interleukin (IL)-1 receptor family and is the major receptor for IL-33, a proinflammatory cytokine that is secreted by numerous cells and cell types in response to damage [3] and is part of the cardioprotective signaling system. IL-33 binds to membrane-localized ST2 and exerts both antihypertrophic and antifibrotic effects. The soluble isoform of ST2 (sST2) can also interact with IL-33 and thereby, inhibit its cardioprotective effects [4]. Cardiac fibroblasts and cardiomyocytes release sST2 in response to stress and overload; the circulating levels of this protein reflect the degree of myocardial stress, ventricular remodeling, and fibrosis [5]. Interestingly, the main source of sST2 in HF may be the vascular endothelium rather than myocardial cells [6].

By contrast, a B-type natriuretic peptide (BNP) and its N-terminal fragment (NT-proBNP) are neurohormones synthesized by the ventricular myocardium. Increases in ventricular wall stress and myocardial hypertrophy in states of pressure and/or volume overload signal the release of these peptides [7]. Plasma BNP levels have been incorporated into clinical practice for prognostic stratification and management of HF [8]. Circulating levels of sST2

serve as an independent predictor of this condition that can be added to those determined for BNP [9]. Omecamtiv mecarbil (OM) is an inotropic drug targeting sacromeres [10], binds specifically to the catalytic S1 domain of cardiac myosin, and thus has no significant impact on the forms of myosin found in smooth or skeletal muscle [10]. Mechanistically, OM activates cardiac myosin and accelerates the hydrolysis of ATP, thereby increasing the binding to actin and enhancing force generation and the strength of cardiac contractions [10]. In an experimental model of canine HF, the administration of OM increased the systolic ejection time, cardiac myocyte fractional shortening (FS), stroke volume (SV), and cardiac output (CO) and reduced left ventricular (LV) end-diastolic pressure and peripheral vascular resistance [11].

Aortic valve regurgitation (AR) is a common heart disease; chronic severe AR imposes a combined volume and pressure overload; and myocardial remodeling in AR leads to an alteration of LV function and wall stress.

Effects of AR on sST2 and NT-proBNP are unknown in rodents, and those of OM-mediated cardiac contraction strength increases on these parameters. We tested the hypothesis that severe chronic AR increases NT-proBNP and sST2 in rats in relation to changes in LV morphology and function. In addition, we anticipated that OM administration in AR could exert differential effects on sST2 and NT-proBNP, given the importance of myocardial stress in sST2 release.

Methods

Animals

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the "Université Libre de Bruxelles". Studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85–23; revised 1996). Our study included 24 male adult Wistar rats (482 ± 57 g body weight). Six rats were assigned to the sham intervention group and 18 rats underwent the induction of AR. The 18 rats that were subjected to AR induction were divided at random into the OM (n=8) or placebo, a physiologic saline solution (0.9% NaCl), (n=10) treatment groups. AR was induced under general anesthesia (1.5% inhaled isoflurane) by retrograde puncture of the aortic valve leaflet, as previously described [12]. The six rats assigned to the sham (control) group underwent cannulation of the right carotid artery under general anesthesia without aortic valve puncture. Heart rate and rhythm were monitored via limb leads throughout the procedure. Invasive arterial pressures were measured with a micromanometer (rodent catheter 1.6 F, Transonic Systems BV, Elsloo, the Netherlands) inserted in the right common carotid artery. The clinical condition of each rat was monitored daily throughout the experiment with a particular focus on the respiratory function.

Echocardiography

All rats were assessed by echocardiography performed by experienced cardiologists who were blinded to the group assignments and treatments. Transthoracic 2D, M-mode, and Doppler echocardiography were performed under general anesthesia (1.5% isoflurane) using an ultrasound scanner (Vivid-E90, GE Healthcare, Wauwatosa, WI, USA) equipped with a 12-MHz phased-array transducer (GE 12S-D, GE Healthcare). Echocardiographic measurements were obtained according to the American Society of Echocardiography guidelines [13, 14]. Standard right parasternal (long and short axis) and left apical parasternal views were used for data acquisition. FS was calculated using the formula FS=(LV end-diastolic diameter [LVEDD] − LV end-systolic diameter [LVESD])/LVEDD × 100 in M-mode from a short-axis view of the LV at the level of the chordae tendineae using measured parameters including (a) diastolic (d) and systolic (s) septal wall thickness (SWTd and SWTs, respectively), (b) posterior wall thickness (PWTd and PWTs, respectively), and (c) LVEDD and LVESD, as defined above. Ejection fraction (EF) was derived using the Teicholz formula. LV mass was calculated using the formula recommended by the American Society of Echocardiography, i.e., LV mass = $0.8 \times \{1.04[(\text{LVEDD} + \text{PWTd} + \text{SWTd})^3 - (\text{LVEDD})^3]\} + 0.6$ g. The aortic diameter was measured from the right long-axis parasternal view. The aortic flow was measured from the left apical view to calculate the forward SV and CO. A pressure half-time (PHT) of <200 ms was considered to be indicative of severe AR. The relative wall thickness (RWT) was calculated using the formula RWT=2 × PWTd/LVEDD, with PWTd measured at end-diastole (in mm).

Experimental design

Doppler echocardiography was performed before the induction of AR (baseline) and during the surgical procedure to confirm the presence and severity of the lesion. It was performed again 60 days after the experimental induction of AR in these rats. Rats with AR in the OM treatment (n=8) and placebo, a physiologic saline solution, (0.9% NaCl) (n=10) groups received equal volumes (12 mL/kg body weight) of OM (1.2 mg/kg/h) or placebo, respectively, via a 30 min infusion through the femoral vein. Rats that underwent the sham intervention (n=6) received the same dose of OM.

Plasma levels of sST2 and NT-proBNP

Blood samples were obtained from each rat by venipuncture under general anesthesia (1.5% inhaled isoflurane) on the day of the surgery, immediately before (baseline), and 2 months after the induction of AR (or sham procedure, i.e., before infusion of OM or placebo), and 1, 2, and 7 days thereafter. The samples were collected into vacuum blood collection tubes (BD Vacutainer, Plymouth, UK) with ethylenediaminetetraacetic acid as the anticoagulant. The tubes were centrifuged immediately after collection at 3,000 rpm and 4 °C for 15 min to obtain plasma, which was separated in multiple aliquots and stored at −80 °C until analysis. The plasma levels of sST2 were measured using a rat ST2 ELISA Kit (MYBIOSOURCE, San Diego, CA, USA). The detection range for sST2 assay kit was 62.5 pg/mL–2000 pg/mL. The plasma levels of NT-proBNP were measured using a rat NT-proBNP ELISA Kit (MYBIOSOURCE, San Diego, CA, USA). The plasma levels of sST2 and NT-proBNP were measured using

electrochemiluminescence immunoassay GLOMAX multidetection system (Promega Corporation, Madison, WI, USA). The lower limit of detection for the NT-proBNP assay kit was 5 pg/mL, with a functional sensitivity of <50 pg/mL, and a working range (imprecision profile ≤10% coefficient of variation) that extended to ~35,000 pg/mL. The assays were carried out as per the manufacturer's instructions. The results were presented as the mean value of duplicated experiments. Laboratory technicians were blinded as to the specific characteristics of each sample.

Statistical analysis

Results are presented as means ± standard deviation (SD). Data were analyzed using a two-factor analysis of variance (ANOVA) for repeated measures. Intergroup differences were tested using a two-way ANOVA. If the F ratio of the ANOVA reached the threshold p-value of <0.05, further comparisons were made using the parametric Student's t-test. A p-value of <0.05 was considered to be significant (SPSS 23.0, IBM Corp., Armonk, NY). Correlations were analyzed parametrically by Pearson's rank correlation.

Results

Impact of surgically induced AR on echocardiographic findings

The impact of surgically induced AR was evaluated 60 days after the completion of the procedure. Values obtained from these rats are shown in Table 1. Our findings were consistent with the diagnosis of severe AR (e.g., PHT <200 ms) in each of the 18 rats that underwent this procedure. By contrast, no AR was detected in the six rats in the sham control group.

Plasma NT-proBNP levels detected in response to surgically induced AR

The baseline levels of plasma NT-proBNP were 244 ± 48 pg/mL in the 18 rats that were designated to undergo surgical induction of AR. About 60 days after the induction of AR, the plasma levels of NT-proBNP were not significantly different, i.e., 231 ± 35 pg/mL in this experimental cohort (Figure 1). We also detected no significant differences in plasma NT-proBNP levels among the six rats in the sham control group (235 ± 37 pg/mL at baseline vs. 280 ± 66 pg/mL measured 60 days later). We identified no correlations between plasma NT-proBNP levels and echocardiographic parameters, blood pressure, or body weight.

Table 1: Echocardiographic measurements in rats (n=18) at baseline and 60 days after induction of AR. Correlations of plasma NT-proBNP and sST2 levels with echocardiographic variables and invasive arterial pressure measurements at baseline and 2 months (60 days) after the induction of AR.

Parameter[d]	Baseline	2 Months	r (NT-proBNP)	r (sST2)
FS, %	40 ± 6	32 ± 7[c]	0.014	0.12
EF, %	75 ± 10	64 ± 12[c]	0.08	0.13
SWTs, mm	2.72 ± 0.57	2.64 ± 0.59	0.017	0.04
SWTd, mm	1.94 ± 0.53	2.11 ± 0.64	0.08	−0.065
LVESD, mm	5.0 ± 1.0	7.5 ± 1.3[c]	−0.02	−0.06
LVEDD, mm	8.3 ± 1.1	11.1 ± 1.3[c]	−0.033	−0.028
HR, BPM	309 ± 50	291 ± 43	−0.096	−0.14
LVOT, mm	2.48 ± 0.18	2.76 ± 0.18[c]	−0.14	0.12
PWTs, mm	2.86 ± 0.46	2.87 ± 0.69	−0.083	0.21
PWTd, mm	1.85 ± 0.45	1.87 ± 0.41	0.17	0.34[a]
PEP, ms	24 ± 9	23 ± 13	0.014	0.08
LVET, ms	78 ± 5	86 ± 8	0.08	0.2
ST, ms	103 ± 13	108 ± 17	0.05	0.017
DT, ms	99 ± 28	101 ± 30	0.06	0.1
RR, ms	201 ± 30	208 ± 28	0.08	0.12
PEP/LVET	0.30 ± 0.10	0.26 ± 0.15	−0.25	0.11
ST/RR	0.53 ± 0.11	0.52 ± 0.10	0.03	−0.08
ARPht, ms		91 ± 25		
SV, mL	0.30 ± 0.08	0.52 ± 0.12[c]	−0.1	0.03
CO, mL/min	96 ± 29	151 ± 41[c]	−0.08	0.02
LVOT VTI, mm	65 ± 13	87 ± 15[c]	−0.081	−0.03
BP sys, mmHg	116 ± 6	122 ± 15	−0.09	−0.05
BP dia, mmHg	78 ± 7	60 ± 10[c]	0.05	−0.12
Weight, g	486 ± 76	562 ± 66[c]	−0.004	0.45[b]
LV mass	1,042 ± 394	1747 ± 558[c]	0.047	0.02
σd, dyn/cm^2	88 ± 25	87 ± 30	−0.06	0.18
σmax, dyn/cm^2	264 ± 72	348 ± 125[c]	−0.15	−0.014
σEs, dyn/cm^2	69 ± 24	124 ± 52[c]	−0.02	−0.02
RWT	0.46 ± 0.15	0.34 ± 0.10[c]	0.08	0.24

Values presented are means ± SD; [a]p<0.05; [b]p<0.01; [c]p<0.001; [d]FS, fractional shortening; EF, ejection fraction; SWTs, septal wall thickness in systole; SWTd, septal wall thickness in diastole; LVESD, left ventricle end-systolic diameter; LVEDD, left ventricle end-diastolic diameter; HR, heart rate; LVOT, left ventricle outflow tract diameter; PWTs, posterior wall thickness in systole; PWTd, posterior wall thickness in diastole; PEP, pre-ejection period; LVET, left ventricular ejection time; ST, systolic time; DT, diastolic time; RR, interval between successive R waves; ARPht, aortic regurgitation pressure half-time; SV, stroke volume; CO, cardiac output; VTI, velocity-time integral; BP, blood pressure; σ, wall stress; σmax, maximum wall stress; σd, end-diastolic wall stress; σEs, end-systolic wall stress; RWT, relative wall thickness.

Plasma NT-proBNP levels detected in response to administration of OM or placebo

The plasma NT-proBNP levels were measured on day 0 as well as on days 1, 2, and 7 after OM or placebo treatment (i.e., days 60, 61, 62, and 67 post-procedure) of rats with
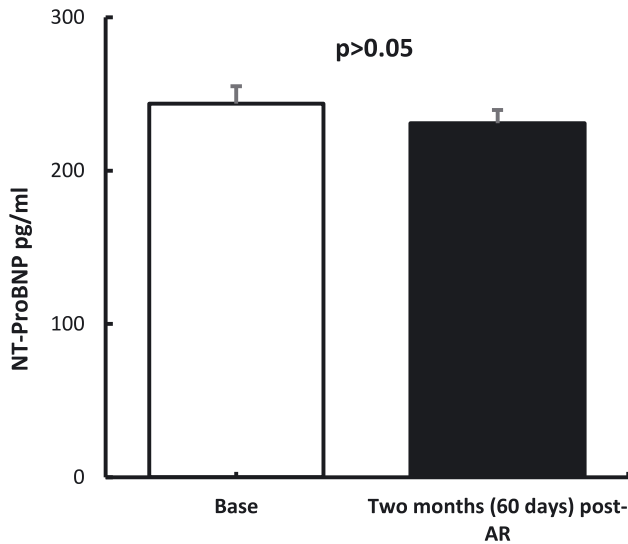
Figure 1: Plasma NT-proBNP levels in adult male rats (n=18) at baseline and 60 days after induction of AR (day 60). Values shown (pg/mL) are means ± SDs.
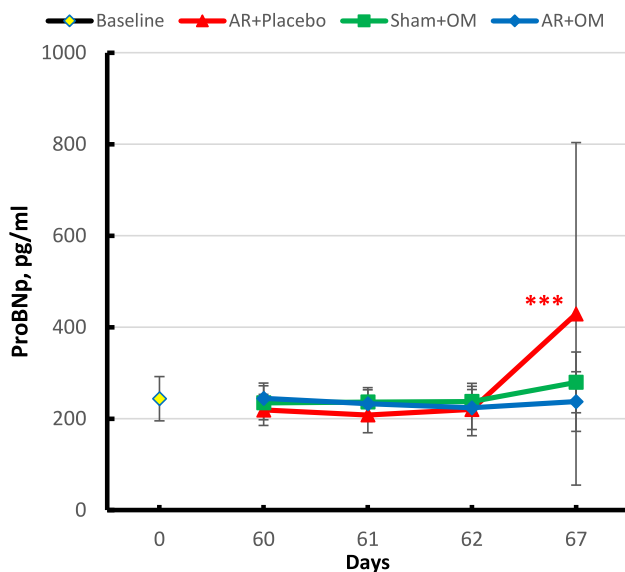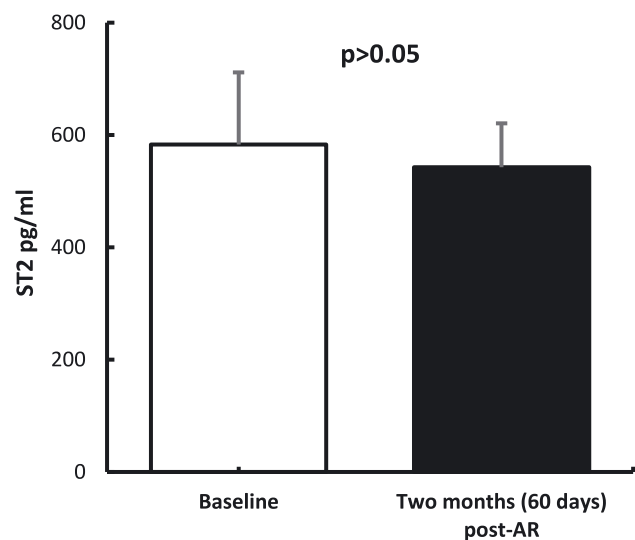

Figure 2: Plasma NT-proBNP levels. Shown are values at baseline (day 0) and at days 60, 61, 62, and 67 after induction of AR or sham procedure in response to administration of OM or placebo in rats with AR (n=8 or 10, respectively) and OM infusion only in rats in the sham control group (n=6).Values shown are means ± SDs; ***$p<0.001$.

experimentally induced AR and sham control rats treated with OM (Figure 2). Interestingly, the plasma NT-proBNP levels increased significantly in the rats with surgically induced AR on day 7 after infusion of placebo (219 ± 34 pg/mL

on day 0 vs. 429 ± 374 pg/mL on day 7; $p<0.001$). We also observed significant increases in plasma NT-proBNP when comparing levels detected on day 1 and day 2 vs. day 7 (208 ± 39 pg/mL and 220 ± 57 pg/mL, respectively vs. 429 ± 374 pg/mL on day 7; $p<0.001$). We observed no significant changes when comparing levels detected on days 0, 1, and 2 (days 60, 61, and 62 post-procedure) to one another. Likewise, we observed no significant changes in plasma levels of NT-proBNP in either the sham (n=6) or surgically induced AR group (n=8) when comparing levels detected at day 0 to those on 1, 2, and 7 after infusion with OM.

## Plasma sST2 levels detected in rats with surgically induced AR

As shown in Figure 3, we detected no significant differences in plasma sST2 in rats when comparing levels detected before and 2 months after surgical induction of AR (583 ± 514 pg/mL vs. 543 ± 340 pg/mL). We also detected no significant changes in the levels of plasma sST2 in rats in the sham group at these time points. However, we did identify significant correlations between plasma levels of sST2 and PWTd as determined by echocardiography ($r=0.34$, $p<0.05$). We also identified a significant correlation between plasma sST2 and body weight ($r=0.45$, $p<0.01$), as shown in Figure 4.

Figure 3: Plasma sST2 levels in adult male rats (n=18) at baseline and 60 days after the induction of AR. Values shown (pg/mL) are means ± SDs.

(A)

n=18

(B)

n=18
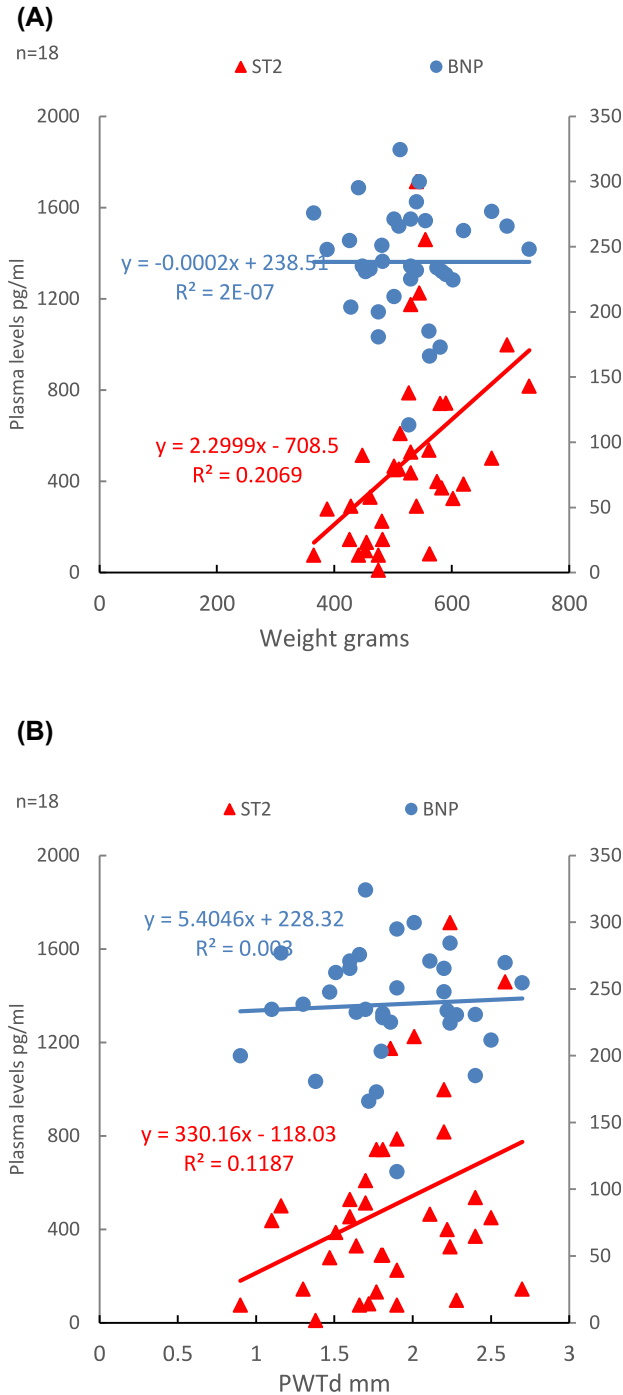
Figure 4: (A) Correlations between plasma NT-proBNP and sST2 levels and body weight (n=18 rats) at baseline and 60 days after the induction of AR (p<0.01); (B) correlations between plasma NT-proBNP and sST2 levels and PWTd (n=18 rats) at baseline and 60 days after the induction of AR; *p<0.05.
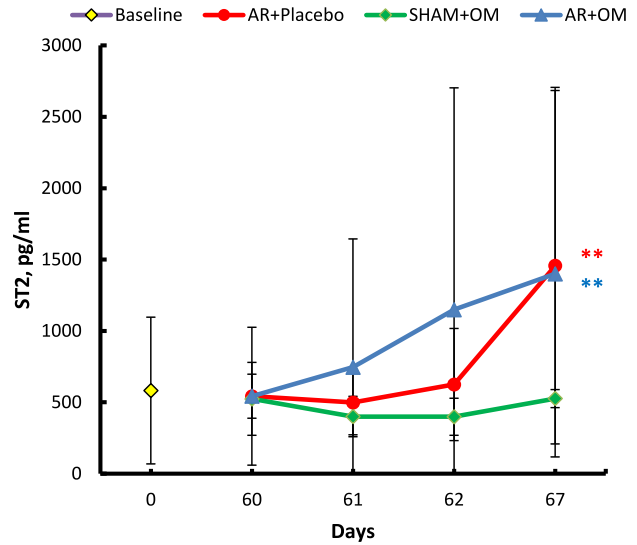
Figure 5: Plasma sST2 levels. Shown are values at baseline (day 0) and at days 60, 61, 62, and 67 after induction of AR or sham procedure in response to administration of OM or placebo in rats with AR (n=8 or 10, respectively) and OM infusion only in rats in the sham control group (n=6). Values shown are means ± SDs; **p<0.01.

Plasma sST2 levels detected in response to administration of OM or placebo

The plasma sST2 levels measured on day 0 and days 1, 2, and 7 after OM or placebo treatment (i.e., days 60, 61, 62, and 67 post-procedure) of rats with experimentally induced AR and sham control rats treated with OM were assessed (Figure 5). The plasma sST2 levels increased significantly on day 7 when compared to that on day 0 in rats with AR after infusion of placebo (543 ± 154 pg/mL vs. 1,457 ± 1,248 pg/mL, p<0.01). Significant increases in plasma sST2 were also observed when comparing results obtained on day 1 (day 61) and day 2 (day 62) vs. day 7 (day 67; 498 ± 225 pg/mL and 625 ± 393 pg/mL, respectively, vs. 1,457 ± 1,248 pg/mL, p<0.01). Among the rats with AR who were treated with OM, we detected significant increases in sST2 on day 7 after the infusion (543 ± 483 pg/mL at day 0 vs. 1,401 ± 1,284 pg/mL, p<0.01). However, we observed no significant differences in plasma sST2 when comparing levels detected on days 1 and 2 vs. day 7. No significant differences in plasma sST2 levels were detected in any of these comparisons in the sham group.

Discussion

Our findings included several notable results. First, contrary to our initial hypothesis and despite the severity of cardiac dysfunction revealed by the echocardiographic findings (particularly, those documenting changes in LV parameters), we detected no significant changes in plasma levels of NT-proBNP at 2 months after surgical induction of AR when compared to that in baseline. As might be anticipated from these results, we detected no significant correlations between echocardiographic parameters and plasma NT-proBNP levels. As one explanation for this finding, we consider the possibility that compensatory mechanisms might develop in the LV in response to chronic severe AR. For example, the LV can adapt to the volume overload by developing eccentric hypertrophy and increased mass. In this situation, the LV volume/mass ratio remains within normal limits. Likewise, the LVEF is maintained by increased preload, and, despite an increase in the end-systolic diameter and pressure early in the course of the disease, the end-systolic wall stress is maintained within the normal range by a compensatory increase in wall thickness [15]. In a previous study, Song et al. [16] reported that NT-proBNP levels may reflect time-dependent structural and/or functional changes in the LV. Similarly, Weber and colleagues [17] found that NT-proBNP levels were associated with clinical symptoms in patients with chronic AR, notably with dyspnea. Interestingly, none of the rats in our study developed dyspnea. Another possible explanation considers the long time span between the induction of AR and the first biochemical assessment. Given the biological half-life of NT-proBNP, the plasma levels detected at 2 months after induction of AR may be significantly influenced by the number of cardiomyocytes available for and engaged in its production.

It is unlikely that the significant increase in NT-proBNP in the rats with AR treated with a placebo infusion can be explained by an increase in wall stress as a result of volume overload *per se* because the two groups of rats (AR and sham) that were treated with OM received the same volume of liquid, yet no modification of the plasma NT-proBNP levels were observed in the latter group. However, the volume overload not counteracted by concomitant OM administration in the presence of AR could explain this observation. This result is consistent with an OM-mediated reduction in myocardial wall stress in AR, as reported by a previous study [18]. Of note, NT-proBNP expression is promoted not only by mechanical stretch, but also by proinflammatory, oxidative, and trophic stimuli [19].

Our results also revealed that plasma sST2 levels underwent no significant change from baseline levels at

60 days after induction of AR, despite the impact of this procedure on LV function to the opposite of our working hypothesis. Najjar et al. [20] reported that there were no significant differences in sST2 levels when comparing healthy controls to subjects with HF with a preserved EF. This is in contrast to Weinberg et al. [5] who reported that sST2 levels increased significantly as early as 1 day after experimentally induced myocardial infarction (MI). Interestingly, 3 days after MI, serum sST2 levels were similar to those of the unmanipulated control mice. This group also reported a significant increase in ST2 levels detected in human subjects 1 day after experiencing an MI when compared to values obtained at 2 weeks and 3 months thereafter. Collectively, these results suggest that elevations in circulating sST2 levels may represent a transient response to acute myocardial stress that diminishes over time and ultimately returns to baseline levels after several weeks to months. Such mechanisms could, thus, account for observations.

In this study, we identified significant positive correlations between sST2 levels with body weight and PWTd. Interestingly, we observed no significant increases in PWTd at 2 months after the induction of AR. The explanation for this observation is unclear given the overall absence of changes in sST2 after AR induction. We can only speculate that the stress imposed on cardiomyocytes during diastole secondary to the overload linked to AR as described in human subjects may have played a role [21]. The gene encoding sST2 is induced under conditions of myocardial overload associated with MI, because the myocardial tissue that remains viable is required to bear more stress.

In our model, no associations were identified between plasma sST2 levels with LV function and geometry, including LV volume and mass. This finding is consistent with the results reported in other recent studies that also demonstrated no specific associations between sST2 levels and LV function or geometry, as assessed by echocardiography in human subjects [9, 20]. Likewise, we observed a correlation between sST2 levels and weight only in the rats with surgically induced AR. This correlation may reflect the impact of myocardial stress, ventricular remodeling, and/or fibrosis [5]. It is also possible that the surgical induction of AR results in a chronic inflammatory response. Of note, sST2 has been implicated in numerous inflammatory diseases [22] and in cardiovascular pathophysiology. No correlations between these parameters were detected in the sham control group despite the significant weight gain.

The significant increase in plasma sST2 levels observed in rats with AR in response to administration of the placebo may be directly related to acute cardiomyocyte stretch due to volume overload and mechanical stress [21]. Volume overload in a setting of a fragilized hemodynamic status as

a result of AR could conceivably induce similar changes in sST2 than those already discussed with NT-proBNP. By contrast, no changes in plasma sST2 levels were detected in the sham control rats at days 1, 2, and 7 after OM administration, possibly since cardiomyocytes in the control rats can support this acute volume overload.

Changes in sST2 levels in rats with AR who received volume overload and OM differ completely from those already discussed with NT-proBNP. We believe that this is an important finding of our study as it highlights a differential regulation of NT-proBNP and sST2 in AR. Rønning et al. [23] reported that the administration of OM prolonged the systolic ejection time in LVs that were dilated due to AR. This effect may be associated with limitations on LV distensibility observed in diastole. Interestingly, OM had an opposite effect in animal models of cardiac ischemia. This is consistent with the previous findings that OM reduces the rate of relaxation and increases passive tension in isolated cardiomyocytes while at rest [24]. This will limit the extent to which the cardiomyocytes can undergo additional stretch or distension, including that required to compensate for AR. In such circumstances, NT-proBNP remained unchanged, whereas sST2 increased markedly in our study suggesting that passive muscle tension with OM has distinct and subtle deleterious effects in the setting of AR, not reliably assessed by modifications in NT-proBNP. Furthermore the effects of OM are concentration, time, and species-dependent [25].

Limitations

This study has several limitations. First, our analysis included only a small number of rats. Second, we did not measure plasma NT-proBNP and sST2 at any time between the induction of AR and the initiation of experimental infusions 2 months later. Thus, we do not have a clear sense of events that may have taken place during this interval. Third, we did not perform echocardiographic or any invasive hemodynamic measurements on days 1, 2, and 7 after the OM or placebo infusions. Thus, we were unable to evaluate the relationships between these parameters and the changes in plasma sST2 and NT-proBNP concentrations observed. Finally, we used an ELISA kit marked "research use only" to assay plasma sST2 levels. There may be considerable differences between the results from this commercial assay and others that are currently in wide use [26].

Conclusions

Among our findings, we noted that plasma levels of sST2 correlated positively with both PWTd and body weight in

rats with chronic severe AR. Interestingly, NT-proBNP and sST2 levels were significantly higher in these rats after infusion of placebo, a finding that may be linked to pressure overload. Importantly, plasma sST2 to the opposite of NT-proBNP increased after therapeutic infusion with OM. These results may reflect LV dysfunction associated with the administration of OM in the presence of AR, a drug whose mechanism of action has not been fully determined.

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