ORIGINAL ARTICLE

Basic Study

Bicuspid aortic valve hemodynamics does not promote remodeling in porcine aortic wall concavity

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Abstract

AIM: To investigate the role of type-I left-right bicuspid aortic valve (LR-BAV) hemodynamic stresses in the remodeling of the thoracic ascending aorta (AA) concavity, in the absence of underlying genetic or structural defects.

METHODS: Transient wall shear stress (WSS) profiles in the concavity of tricuspid aortic valve (TAV) and LR-BAV AAs were obtained computationally. Tissue specimens excised from the concavity of normal (non-dilated) porcine AAs were subjected for 48 h to those stress environments using a shear stress bioreactor. Tissue remodeling was characterized in terms of matrix metalloproteinase (MMP) expression and activity *via* immunostaining and gelatin zymography.

RESULTS: Immunostaining semi-quantification results indicated no significant difference in MMP-2 and MMP-9 expression between the tissue groups exposed to TAV and LR-BAV AA WSS ($P = 0.80$ and $P = 0.19$, respectively). Zymography densitometry revealed no difference in MMP-2 activity (total activity, active form and latent form) between the groups subjected to TAV AA and LR-BAV AA WSS ($P = 0.08$, $P = 0.15$ and $P = 0.59$, respectively).

CONCLUSION: The hemodynamic stress environment present in the concavity of type-I LR-BAV AA does not

cause any significant change in proteolytic enzyme expression and activity as compared to that present in the TAV AA.

Core tip: The bicuspid aortic valve with left-right cusp fusion (LR-BAV) generates a stress overload on the ascending aorta (AA) convexity, which promotes aortic medial degeneration and aortic dilation. While the wall concavity is generally spared from the disease, the protective role of the local hemodynamics has not been demonstrated. This study aimed at comparing matrix metalloproteinase biology in AA concavity tissue subjected to the local hemodynamic stresses generated by a tricuspid aortic valve (TAV) and a LR-BAV. The results suggest that the fluid stresses in the TAV AA and LR-BAV AA concavity result in similar MMP expressions and activities.

INTRODUCTION

The bicuspid aortic valve (BAV) is present in 1%-2% of the general population[1-3] and is the most common cardiac anomaly. Despite its seemingly low incidence, the BAV is responsible for causing more valvular and vascular disease compared to all other congenital heart defects combined[4]. Unlike the normal tricuspid aortic valve (TAV) which consists of three leaflets, the BAV forms with only two[5-7]. While there are different BAV morphogenic phenotypes[8-10], the most common is referred to as the type-I BAV and is characterized by the presence of two cusps of unequal size and one fibrous raphe marking the site of fusion on the larger leaflet[5]. Type-I BAV patients not only have a higher susceptibility to develop valvulopathies that usually require surgical intervention and valvular replacement, they are also associated with increased risk of aortopathies such as aortic dilation, dissection and aneurysm[11-13]. In particular, type-I BAVs with fusion between the left- and right-coronary cusps (LR subtype) has emerged as the most aggressive in terms of risk for secondary aortopathy[14-16]. This subtype tends to result in asymmetric dilation patterns that localize to the convex region of the thoracic ascending aorta (AA) but spare the wall concavity[14,15,17].

Previous clinical studies have demonstrated that the degenerative remodeling of the aortic wall in type-I

BAV patients is accompanied by increased expression of matrix metalloproteinase-2 (MMP-2) and MMP-9[16,18-20] in the disease-prone wall convexity relative to the wall concavity[14,15,21]. Those proteolytic enzymes degrade key extracellular matrix components such as collagen and elastin[22]. The respective expression and activity of those enzymes and their tissue inhibitors regulate the balance between extracellular matrix synthesis and resorption[23]. A perturbation of this delicate equilibrium can result in the progressive degeneration of the vascular wall and the loss of vessel wall integrity[24].

Interestingly, type-I LR-BAVs have been shown to generate perturbed hemodynamics characterized by a valvular jet skewed toward the non-coronary leaflet and increased shearing friction force [*i.e.,* wall shear stress (WSS)] on the convexity of the thoracic AA[25-31]. While those observations suggest a role for hemodynamics in the pathogenesis of BAV aortopathy[32-34] and despite the clear evidence for the existence of flow abnormalities in BAV aortic wall regions vulnerable to dilation, the causative effects of those abnormalities on the local weakening of the aortic wall have not been fully established. Underlying challenges hampering the *in vivo* assessment of this hemodynamic theory include the possible existence of genetic anomalies in the aortic wall, as well as the paucity of hemodynamic data in non-dilated BAV aortas. To circumvent those issues, *ex vivo* methodologies enabling the replication of the native BAV AA WSS environment on genetically normal and non-dilated AA tissue have been developed.

In our previous *ex vivo* study[35], we isolated the impact of TAV and LR-BAV hemodynamic stresses on the remodeling of the AA convexity. The WSS environments in the convex region of a TAV AA and a normal (non-dilated) LR-BAV AA were quantified computationally[28,35] and replicated in the laboratory using a shear stress bioreactor[36,37]. The remodeling response of porcine aortic tissue extracted from the AA convexity and exposed to those environments for 48 h was investigated *via* immunostaining, immunoblotting and zymography. Exposure of normal aortic tissue to BAV AA WSS resulted in increased MMP-2 and MMP-9 expressions and MMP-2 activity but similar fibrillin-1 content relative to the TAV AA WSS treatment. While this study demonstrated the susceptibility of the hemodynamic stresses experienced by the BAV AA convexity to focally mediate aortic medial degradation, the apparent protective effects of the LR-BAV hemodynamics on the AA concavity and the asymmetric development of dilation in the LR-BAV AA require further investigation. Therefore, the objective of the present study was to isolate *ex vivo* the impact of LR-BAV hemodynamic stresses on the remodeling of the disease-protected AA concavity, with a focus on MMP expression and activity.

MATERIALS AND METHODS

WSS characterization and in vitro generation
The temporal WSS variations experienced by the

Table 1 Wall shear stress signal characteristics in the concavity of the tricuspid aortic valve and left-right bicuspid aortic valve ascending aorta

	Maximum (Pa)	Minimum (Pa)	TSM (Pa)	OSI
TAV AA WSS	3.2	-2.75	0.77	0.49
LR-BAV AA WSS	3.3	-3.63	1.06	0.18

WSS: Wall shear stress; LR-BAV: Left-right bicuspid aortic valve; TAV: Tricuspid aortic valve; AA: Ascending aorta; OSI: Oscillatory shear index; TSM: Temporal shear magnitude.

concave region of a TAV AA and LR-BAV AA were obtained computationally using a previously published and validated fluid-structure interaction (FSI) model of a human aorta subjected to idealized TAV and LR-BAV flows[28]. Briefly, a realistic model of a human aortic arch was reconstructed based on histological slices (Visible Human Project, National Library of Medicine). 3D transient velocity profiles matching physiologic TAV and LR-BAV average flow rates were prescribed at the model inlet. The dynamic WSS profiles experienced by the TAV AA and LR-BAV AA concavity were captured in a rectangular region (dimensions: 8 mm × 15 mm) centered on the wall concavity and located 1 cm above the left-coronary leaflet (Figure 1A). The two WSS waveforms share important similarities both qualitatively and quantitatively, as indicated by their peak values and average magnitude temporal shear magnitude (TSM) over one cardiac cycle (Table 1). Importantly, as compared to the TAV, which generates a nearly perfectly sinusoidal WSS signal, the LR-BAV generates a double negative WSS peak, which tends to lower the signal oscillatory shear index (OSI).

The two WSS environments were replicated in the laboratory using our previously described and validated cone-and-plate bioreactor[35,37]. Briefly, the device consists of a cylindrical chamber filled with culture medium and containing a cone rotating above a stationary mounting plate (Figure 1B). The rotation of the cone generates a flow and thus, a WSS on the surface of the plate, whose intensity τ at a radial location r is directly proportional to the cone angular velocity ω:

$$\omega = [(h + r\alpha)/\mu r]\tau \qquad (1)$$

where μ is the dynamic viscosity of the culture medium (0.95×10^{-3} kg/m per second), α is the cone half angle ($0.5°$), and h is the distance between the cone apex and the mounting plate (200μm)[36]. The two angular velocity waveforms producing the TAV AA and LR-BAV AA WSS profiles obtained computationally were programmed into the servo drive (Gemini GV6k, Parker Hannafin) controlling the cone motion. The details of this protocol have been previously published[35].

Tissue harvest and preparation
The experiments were conducted on porcine aortas (6-12 mo) acquired from a local abattoir (Martin's Custom

Butchering, Wakarusa, IN, United States) due to their structural similarities with human aortas and their well-characterized antibody specificities. Aortic tissue was harvested after on-site dissection of the hearts within 10 min of slaughter and was transported to the laboratory in sterile, ice-cold Dulbecco's Phosphate Buffered Saline (PBS, Sigma-Aldrich). This protocol has been previously implemented in our laboratory and has been shown to preserve endothelium integrity and cellular viability[35,36,38]. All subsequent procedures were performed in a sterile flow hood. Upon arrival to the laboratory, the aortas were cut longitudinally in order to expose the inner endothelial surface. Consistent with our previous study on the effects of BAV flow on AA convexity[35], two circular specimen (7-mm diameter) were excised from the AA concavity, 8 and 15 mm above the sinus of the left-coronary leaflet (*i.e.,* region least prone to dilation[14,15,39]). Samples were then randomized into fresh controls and experimental samples. Six samples were mounted to the circular plate using a button suturing technique, which has been shown not to affect the WSS level generated on the tissue[35]. The native orientation of the tissue relative to blood flow was maintained by aligning the longitudinal axis of the samples with the direction of cone motion (*i.e.,* tangential direction). Tissue conditioning to WSS was performed in an incubator maintaining a temperature of 37 ℃ and a CO_2 level of 5% for 48 h (duration sufficient for acute mechanosensitive remodeling processes to become evident in aortic tissue[34,35,40]). The system was continuously perfused with standard culture medium (Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 3.7 g/L sodium bicarbonate, 0.05 g/L ascorbic acid, 10% non-essential amino acid solution and 1% penicillin-streptomycin; all from Sigma-Aldrich) at a rate of two bioreactor volumes per hour. The perfusion system was flushed and replenished with fresh medium every 12 h.

Groups
Two experimental groups and one control group were considered to isolate the impact of TAV AA and LR-BAV AA hemodynamics on the acute remodeling response of the AA concavity: (1) fresh porcine tissue excised from the concavity of the ascending aortic wall (control); (2) fresh porcine tissue excised from the concavity of the ascending aortic wall and subjected to the local TAV AA WSS; and (3) fresh porcine tissue excised from the concavity of the ascending aortic wall and subjected to the local BAV AA WSS.

Biological analyses
Following WSS conditioning, the samples were harvested and washed three times with sterile PBS. The samples were then either frozen in optimal cutting medium for future immunostaining analysis flash frozen in liquid nitrogen for future gelatin zymography analysis.

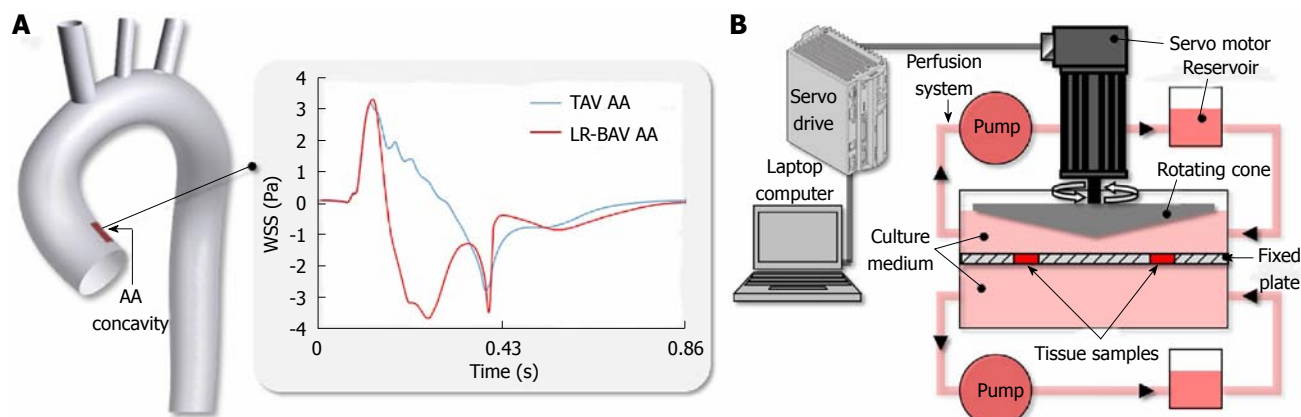Immunostaining: The OCT blocks were cut into

Figure 1 *Ex vivo* methodology. A: Temporal wall shear stress (WSS) signals captured computationally in the concavity of the tricuspid aortic valve (TAV) ascending aorta (AA) and left-right bicuspid aortic valve (LR-BAV) AA (adapted from Cao *et al*[28]); B: Shear stress bioreactor used to condition porcine AA tissue to TAV AA and BAV AA WSS.

5-μm sections using a Microm 505E cryostat (Microm International GmbH) and mounted on glass slides. The region occupied by the tissue section was circled with a fluid block pen (Immunotech) after 20 min on a heater at 37 ℃. Sections were then rinsed for 20 min in PBS. Blocking [10% Goat Serum (Sigma), 0.2% TritonX-100 (Sigma), 1% dimethyl sulfoxide (Thermo Fisher Scientific)] was performed at room temperature for 1 h. Next, MMP-2 (1:200, EMD Millipore) or MMP-9 (1:200, EMD Millipore) primary antibody was diluted in blocker and slides were incubated overnight at 4 ℃ with shaking. The following day, PBS was used to rinse the sections 3 times and secondary antibody (1:100, Santa Cruz) was incubated for 2 h at room temperature in PBS. Sections were rinsed 3 more times in PBS for 5 min each before counterstaining with 1 4',6-Diamindino-2-phenylindole (DAPI, Sigma) and mounted with fluorescence mounting medium (Dako). Coverslipped sections were stored at 4 ℃. Fluorescence immunohistochemistry (IHC) performed on a Nikon E600 microscope was used to identify regions positively stained for MMP-2 and MMP-9 on each slide. MMP-2 and MMP-9 expression was assessed semi-quantitatively using ImageJ (National Institutes of Health, Bethesda, MD) over three image fields per sample, following our previously published methodology[38,41-43]. The overall intensity of MMP immunopositive expression was measured and normalized by the total number of cells present over each image field.

Gelatin zymography: Proteolytic activity of enzymes MMP-2 and MMP-9 was quantified using gelatin zymography. The collected supernatant protein content was quantified using a bicinchoninic acid protein assay (BCA, Pierce). Tissue lysates were loaded in equal amounts (20 μg) on a 10% zymogram gel (Bio-Rad). Gels were resolved by sample buffer (Bio-Rad) followed by 1 h incubation in developing buffer (Bio-Rad) at 37 ℃ and 5% CO_2. Stain solution (G-Biosciences) was added at room temperature, then gels were destained in deionized water at room temperature. Gels were scanned and the digital images were converted to 8-bit grayscale images before being processed in ImageJ for densitometric analysis following our previously published protocol[42,43].

Statistical analysis
Consistent with our previous study, each analysis was performed on a sample size of $N = 3$ and was quantified as mean ± SE. This sample size was shown previously to generate significant biological differences between convexity tissue specimens subjected to TAV and BAV flows[35]. Normalization to the fresh control was performed in all experimental groups. Significance was determined using ANOVA followed by a Bonferroni post-hoc test using the software SAS (SAS Institute Inc). The threshold for statistical significance was set at a P value of 0.05. Those analyses were reviewed by a biomedical statistician (Dr. Ick H Jin, Department of Applied and Computational Mathematics and Statistics, University of Notre Dame, Notre Dame, IN, United States).

RESULTS

TAV and LR-BAV hemodynamic stresses generate similar MMP expression levels in AA concavity tissue
MMP-2 and MMP-9 immunostaining results are shown in Figure 2. In fresh tissue, MMP-2 and MMP-9 expressions were moderate and consistently localized in the medial layer (Figure 2A). While MMP-2 and MMP-9 were also detected in the same region in specimens subjected to WSS, MMP-9 expression was reduced in the samples subjected to BAV AA WSS. Semi-quantification of the IHC images (Figure 2B) revealed statistically similar MMP-2 expression across the different groups (TAV AA WSS: 1.2-fold increase *vs* control, $P = 0.73$; BAV AA WSS: 1.1-fold increase *vs* controls, $P = 0.95$) and no statistical difference between the two experimental groups subjected to WSS ($P = 0.80$). While MMP-9 semi-quantification indicated a significant reduction in MMP-9 expression in tissue exposed to BAV AA WSS (0.3-fold increase *vs* control, $P = 0.01$), it revealed no statistical difference between the fresh controls and the
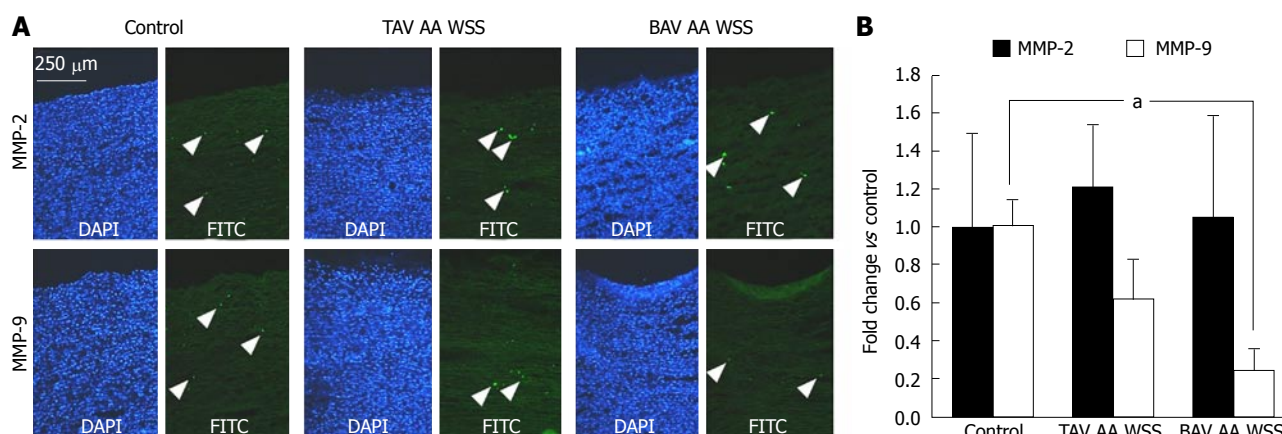
Figure 2 Matrix metalloproteinase immunohistochemistry. A: Matrix metalloproteinase (MMP)-2 and MMP-9 immunostaining with DAPI and FITC filters (blue cell nuclei, green positively stained cells); B: Semi-quantitative results ([a]$P < 0.05$ *vs* control). DAPI: 4',6-diamidino-2-phenylindole; FITC: Fluorescein isothiocyanate.

specimens subjected to TAV AA WSS (0.6-fold increase *vs* control, $P = 0.20$) or between the two conditioned groups ($P = 0.19$).

TAV and LR-BAV hemodynamic stresses generate similar MMP activities in AA concavity tissue

MMP-2 and MMP-9 zymography results are shown in Figure 3. MMP-9 was undetectable in the zymograms. While the active form of MMP-2 was hardly detectable in the fresh controls, both latent and active MMP-2 were found in both experimental groups subjected to WSS (Figure 3A). Neither latent nor active form of MMP-9 was detected in the zymogram. Therefore, the densitometric quantification of MMP activity was only performed on MMP-2. Total MMP-2 activity was quantified as the sum of active and latent forms of MMP-2 (Figure 3B). Tissue specimens subjected to TAV AA WSS exhibited a significant increase in total MMP-2 activity relative to the fresh controls (1.5-fold increase, $P = 0.02$). While BAV AA WSS also resulted in increased total MMP-2 activity relative to the fresh controls (1.5-fold increase), the difference was not statistically significant ($P = 0.37$). No significant difference was also detected between the two experimental groups subjected to WSS ($P = 0.08$). Although the densitometric analysis performed on the latent form of MMP-2 (Figure 3C) indicated lower contents in groups subjected to TAV AA WSS (0.76-fold increase) and tissue subjected to BAV AA WSS (0.69-fold increase) relative to fresh tissue, the differences were not statistically significant ($P = 0.17$ and $P = 0.12$, respectively). No statistical difference was found between the two experimental groups subjected to WSS ($P = 0.59$). Lastly, tissue conditioning to WSS resulted in a dramatic increase in expression of active MMP-2 relative to the fresh controls, in which active MMP-2 expression was hardly detectable (TAV AA WSS: 5713-fold increase *vs* controls, $P = 0.004$; BAV AA WSS: 3877-fold increase *vs* controls, $P = 0.0008$; Figure 3D). The difference in active MMP-2 expression between the two conditioned groups remained non-significant ($P = 0.15$), but both groups exhibited a

conversion from pro-(latent) to active MMP-2 after 48 h of conditioning.

DISCUSSION

We conducted an *ex vivo* study to investigate the isolated effects of BAV hemodynamic stresses on the biological remodeling of porcine AA tissue excised from the concavity of the aortic wall. The primary contribution of this study is the indication that, in the absence of any underlying congenital defect, the local hemodynamics experienced by the LR-BAV AA concavity does not trigger any acute upregulation of enzymatic protease expression or activity in the aortic medial layer.

The immunostaining and zymography analyses evidenced the absence of significant change in MMP expression and activity between the aortic wall speci-mens subjected to TAV AA and LR-BAV AA WSS. Those results complement our previous study on the effects of BAV flow on the biology of the AA convexity, which revealed the susceptibility of the WSS overload experienced by the convexity of the LR-BAV AA to promote aortic medial degradation *via* MMP-dependent pathways[34,35,40]. Those observations are consistent with the asymmetric presentation of aortic dilation observed in type-I LR-BAV patients and the higher vulnerability of the AA wall convexity to aortopathy[14,15,44]. The similarities in the hemodynamic stress environments present in the concavity of the TAV AA and LR-BAV AA evidenced in the present study, combined with the absence of difference in the remodeling activity of tissue exposed to those environments support a hemodynamic etiology of BAV aortopathy.

The demonstration of causality between the asymmetric BAV flow patterns and the asymmetric expression of secondary BAV complications has already been established in the context of BAV calcification, which typically affects primarily the fused leaflets exposed to WSS overload but spares the non-coronary leaflet subjected to relatively normal WSS levels[42,45]. Therefore, while the involvement of underlying genetic
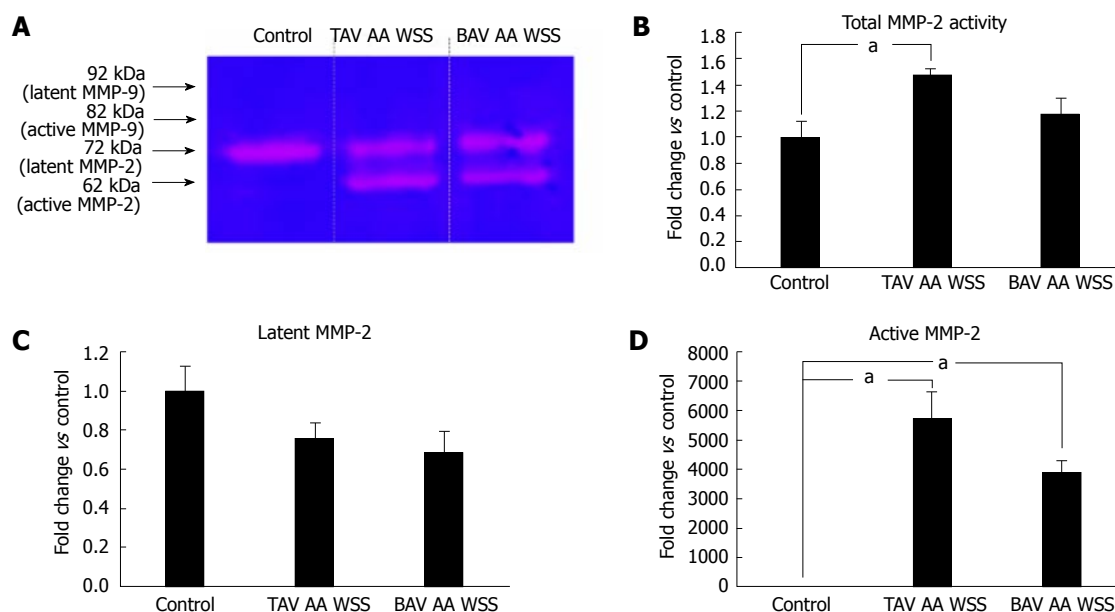
A

92 kDa
(latent MMP-9) →
82 kDa
(active MMP-9) →
72 kDa
(latent MMP-2) →
62 kDa
(active MMP-2) →

Control   TAV AA WSS   BAV AA WSS

B

Total MMP-2 activity

Fold change *vs* control

Control   TAV AA WSS   BAV AA WSS

C

Latent MMP-2

Fold change *vs* control

Control   TAV AA WSS   BAV AA WSS

D

Active MMP-2

Fold change *vs* control

Control   TAV AA WSS   BAV AA WSS

Figure 3 Matrix metalloproteinase gelatin zymography. A: Zymogram showing latent and active forms of matrix metalloproteinase (MMP)-2 and MMP-9 in fresh controls and tissue conditioned to tricuspid aortic valve (TAV) ascending aorta (AA) and bicuspid aortic valve (BAV) AA wall shear stress (WSS); B: Densitometry results for total MMP-2 enzymatic activity; C: Latent MMP-2; D: Active MMP-2 ($^aP < 0.05$ *vs* control; MMP-9 data not shown as MMP-9 was absent from the protein lysates).

abnormalities in BAV leaflets and BAV AA tissue cannot be ruled out, the present study provides one more evidence in support of the key role played by blood flow and hemodynamic stresses in BAV disease.

While no difference in protease expression and activity was detected between the tissue groups subjected to TAV AA and BAV AA WSS, some changes were measured between the fresh controls and the conditioned groups. First, tissue subjected to WSS exhibited a significant upregulation of active MMP-2 relative to fresh tissue. Although this result requires further investigation, it is important to note that, while the bioreactor was able to subject the samples to WSS, it eliminated all other forces normally present in the native environment, such as stretch and pressure. Those forces have been shown to play a critical role in the maintenance of vascular homeostasis and MMP-2 regulation[46,47]. Second, tissue exposure to LR-BAV WSS resulted in a significant MMP-9 downregulation relative to the fresh controls as suggested by immunostaining, while MMP-2 levels remained unchanged. This difference in biological response may be related to the specific mechanosensitivity of the aortic endothelium to WSS. In fact, studies conducted in our laboratory in the context of valvular tissue have demonstrated the differential sensitivity of valvular endothelial cells to WSS magnitude, directionality and frequency[41,43,48]. Following the same concept, the cells lining the aortic wall may be able to detect changes in different WSS characteristics and transduce them into different biological responses. As a result, despite the qualitative similarity between the WSS waveforms captured in the concavity of the TAV AA and LR-BAV AA, the minor differences in magnitude and directionality, as quantified by the TSM and OSI (Table 1), may be sufficient to

drive a differential biological response.

MMP-9 expression was below detection level in tissue lysates and zymograms. The near absence of MMP-9 expression following exposure of aortic concavity tissue to WSS proves to be an interesting phenomenon. Animal models have indicated that MMP-2 and MMP-9 work synergistically to promote aneurysm formation[49] and that MMP-9 knockout mice are unable to develop aneurysms even under the presence of increased MMP-2 activity[50]. Therefore, the absence of MMP-9 in fresh tissue and tissue subjected to TAV AA and BAV AA WSS, which prevents the possible downstream pathological effects of MMP-2/MMP-9 synergies, may protect the aortic wall against dilation and aneurysm formation. Further *ex vivo* investigations on the combined effects of WSS, MMP-2 and MMP-9 and the modulation of the remodeling response through MMP-2/MMP-9 synergies will be necessary to test this hypothesis.

Although relationships between WSS abnormalities and biological perturbations in the aortic endothelium have been previously evidenced[51-54], more work needs to be done to isolate the impact of hemodynamic stress abnormalities on tissue remodeling. The results collected from the present study combined with those from our previous study[35] on the local effects of TAV and LR-BAV AA hemodynamic stresses on AA remodeling suggest the susceptibility of BAV hemodynamic stresses to mediate aortic medial degradation on the disease-prone wall convexity, while sparing the wall concavity. These observations suggest a critical role played by hemodynamic stresses in the development of BAV asymmetric aortopathies.

Finally, several limitations should be discussed. The demonstration of a role for WSS in the differen-

tial remodeling state of the BAV AA convexity and concavity relied on the retrospective comparison of the present data obtained with AA concavity tissue with our previous data obtained with AA convexity tissue[35]. While ideally both sets of experiments should have been carried out using tissue specimens from the same animal, the availability of a single shear stress bioreactor prevented such mode of operation. However, the same methodology (sample size, culture techniques, biological endpoints, assays) as in the previous study was implemented to allow for the direct comparison of the results.

Second, the study only focused on the effects of WSS and neglected other important mechanical signals (stretch, pressure) normally found in the native environment. This choice is justified by two arguments. First, the objective of the study was to isolate the potential role played by WSS in the remodeling of the BAV AA concavity, in the absence of any other biochemical and mechanical signals. Second, the mechanical characterization of the TAV and BAV aortic wall provided by the FSI model revealed the absence of substantial differences in circumferential stretch and pressure between the TAV AA and BAV AA (average pressure difference: 0.4% in the convexity and 0.5% in the concavity; average stretch difference: 0% in the convexity and 0.3% in the concavity). The analysis of the WSS in both anatomies revealed more contrasted environments (TSM difference: 94% in the convexity and 38% in the concavity), which motivated and justified the investigation of their mechanobiological impact.

Third, while the study would benefit from a larger sample size, the absence of significant differences in the remodeling response of TAV AA and BAV AA concavity tissue is in agreement with the data reported in larger clinical studies that examined the asymmetric nature of aortic dilation and the spatiotemporal patterns of MMP expression in BAV AAs[14-16,55,56]. In addition, the same sample size in our previous study was able to demonstrate statistically significant biological differences between convexity specimens subjected to TAV AA and BAV AA WSS. In this context, the absence of statistical differences in the remodeling response of concavity tissue samples subjected to TAV AA and BAV AA flow is likely to be a reflection of the low impact of the concavity WSS environment on the local tissue biology rather than the consequence of a small sample size.

COMMENTS

Background
The asymmetric dilation of the ascending aortic (AA) wall downstream of the bicuspid aortic valve (BAV) is marked by aortic medial degeneration through upregulation of matrix metalloproteinase (MMP) expression and enzymatic activity. BAV AA dilation typically localizes to the AA wall convexity, which is the region impinged by the skewed BAV orifice jet and subjected to abnormally high hemodynamic wall shear stress (WSS). While previous studies have established a link between the WSS overload on the AA wall convexity and the upregulation of remodeling activity, the ability of the hemodynamic environment present in

the BAV AA concavity to maintain vascular homeostasis has not been formally demonstrated.

Research frontiers
The common classification of BAV aortopathy as an inherited disorder has guided the implementation of aggressive surgical modalities including reduction aortoplasty, aortic root replacement and AA replacement. However, those procedures have been associated with a significant mortality rate when treating aortic dilation in BAV patients. Such outcome questions the suitability of those surgical approaches and justifies the need to elucidate the etiology of BAV aortopathy. The characterization of the remodelling pathways involved in the disease and the interacting mechanisms of micro-scale mechanotransduction and macro-scale hemodynamics are current hotspots in BAV and vascular research.

Innovations and breakthroughs
The authors' previous *ex vivo* characterization of the isolated effects of tricuspid aortic valve (TAV) AA and BAV AA WSS on the biology of the AA convexity revealed the ability of BAV AA hemodynamic abnormalities in the wall convexity to promote aortic medial degeneration *via* MMP-2 and MMP-9-dependent pathways. The present study is a logical extension of the authors' previous work as it investigates the contribution of the WSS environment in the seemingly disease-protected concavity of the LR-BAV AA on MMP biology. The absence of difference in the remodeling response of tissue subjected to the local WSS normally present in the TAV AA and LR-BAV AA concavity justify at least partially the asymmetric dilation pattern typically observed in LR-BAV patients and isolate, for the first time, the key role played by hemodynamic stresses in BAV aortopathogenesis.

Applications
The demonstration of a hemodynamic pathway of BAV aortic dilation may switch the clinical focus from developing aggressive surgical procedures aimed at eliminating the presumed genetically weakened AA wall to investigating new modalities aimed at normalizing BAV aorta hemodynamics or inhibiting pharmacologically the pathological remodeling cascade at an early age.

Terminology
The BAV is the most common cardiac anomaly and consists of two leaflets instead of the three present in the normal TAV. The most common LR-BAV morphotype results from the fusion between the left- and right-coronary cusps. WSS is the frictional fluid force resulting from the relative motion between the aortic wall and the surrounding blood flow. MMP-2 and MMP-9 are two proteolytic enzymes that degrade collagen, elastin and fibronectin, which are fundamental protein components of the aortic media.

Peer-review
The manuscript by Atkins *et al* proposes an *ex-vivo* model of wall shear stress applied to the lesser curvature (concavity) of porcine thoracic aorta. The *ex-vivo* model reproduces in a bioreactor the shear stress generated by a TAV and by a LR-BAV, with the aim of dissecting the role played by this single local hemodynamic factor on vascular wall remodeling of aortic concavity.

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