Cyclic Pressure and Shear Stress Regulate Matrix Metalloproteinases and Cathepsin Activity in Porcine Aortic Valves

Manu O. Platt[1], Yun Xing[2], Hanjoong Jo[1,3], Ajit. P. Yoganathan[1]

[1]The Wallace H. Coulter School of Biomedical Engineering, Georgia Institute of Technology, Atlanta, [2]School of Chemical & Biomolecular Engineering, Bioengineering Program, [3]Department of Cardiology, Emory University, Atlanta, Georgia, USA

Background and aim of the study: **It has been shown previously that the exposure of porcine aortic valve leaflets (PAVL) to elevated pressure or steady shear stress increases extracellular matrix (ECM) synthesis. It was hypothesized that exposure of the aortic valves to pressure or shear stress would regulate valvular expression of proteases such as matrix metalloproteinases (MMPs) and cathepsins.**
Methods: **Fresh PAVLs were exposed to cyclic pressure (170 mmHg at 1.167 Hz) or to a steady shear stress of 25 dyne/cm^2 for 48 h, with static culture serving as controls.**
Results: **Shear stress significantly inhibited cathepsin activity and cathepsin L protein expression, but increased MMP-2/9 activity. A cyclic hypertensive pressure condition also slightly decreased MMP-2/9 and cathepsin L activities.**
Conclusion: **For the first time, valvular cathepsin and MMP activity were seen to be directly regulated by mechanical forces such as pressure and shear stress. The down-regulation of cathepsin L activity correlates with an up-regulation of ECM synthesis; MMP-2 and -9 activities increased with ECM synthesis under shear stress, showing that these proteinases may serve different roles in valvular remodeling. This observation may have implications for understanding valve biology, as well as for valve tissue engineering.**

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Heart valve disease is a serious condition that affects a significant percentage of the population, both in the United States and worldwide. According to the American Heart Association statistics, valvular diseases cause almost 20,000 deaths each year, and are related to another 42,000 deaths. Current surgical interventions include valve repair or replacement, depending on the diagnosis. Although during the past century significant progress has been made in the development and improvement of prosthetic valves, there is to date no ideal replacement valve available. Recent advances in biotechnology have spurred an interest in heart valve tissue engineering, though the success of a tissue-engineered heart valve (TEHV) will rely heavily on an understanding of native valve biology which has, unfortunately, been studied only to a very limited degree.

Native heart valves - the aortic and mitral valves in particular - function in a harsh and complex surrounding mechanical environment to which the valvular structure constantly responds. Close correlations between mechanical stresses and heart valve biology have long been documented by clinical observations and animal studies (1-3). According to these findings, the structural components of the aortic valve undergo constant renewal in response to mechanical loading (1), and the sites of protein and glycosaminoglycan synthesis in the leaflets correlate with the areas of functional stress (2). Similarly, changes in mechanical loading alter the biosynthetic behavior of valve cells. For example, collagen synthesis in mitral valve leaflets was enhanced as a result of altered stress distribution due to left ventricular infarctions (4). Although, it is clear that mechanical factors play a very important role in heart valve biology, there is to date a paucity of information relating to this subject. These studies have shown that cultured porcine aortic valvular cells respond to applied shear stress in a unique way (5). Moreover, the mechanotransduction pathway seems to be different from the extensively studied vascular cell counterparts (5,6). Previous studies conducted in the present authors' laboratory showed that elevated pressure, both steady and cyclic, caused enhanced extracellular matrix (ECM) synthesis in porcine aortic heart valve leaflets (7,8). It is clear from

Address for correspondence:
Prof. Ajit P. Yoganathan, The Wallace H. Coulter School of Biomedical Engineering, Georgia Institute of Technology, 313 Ferst Drive, Suite 1121, Atlanta, GA 30332-0535, USA
e-mail: ajit.yoganathan@bme.gatech.edu

these studies that mechanical factors such as pressure and shear stress are important in valve ECM remodeling, though the molecular mechanisms involved remain largely unexplored. An increase in ECM synthesis, accompanied by a decrease in its degradation, may be induced by reducing the enzyme activity of matrix metalloproteinases (MMPs) and cathepsins, or by regulating their activity through protein inhibition.

Cathepsin L, a member of the papain family of lysosomal cysteine proteases, was first identified in lysosomes but has since been found also to have functions in other cellular compartments, including the nucleus (9), and even extracellularly in different disease conditions such as arthritis (10,11) and cancer (12). Intracellularly, cathepsin L is a powerful protease involved in protein turnover. Extracellularly, cathepsin L has powerful collagenase and elastase activities, which necessitates its tight regulation by pH, reducing environment, and protein inhibitors. In the cardiovascular system, cathepsin L has been found to be active within abdominal aortic aneurysms and atherosclerotic lesions (13), while an absence of cathepsin L in knockout mice caused dilated cardiomyopathy (14), again highlighting that although initially found in lysosomes, this protease is also involved in other aspects of physiology. It has been shown recently that steady laminar shear inhibits cathepsin L activity in mouse aortic endothelial cells (15). Other cathepsins have also been linked to cardiovascular disease, particularly cathepsins S and K, which have been shown to be associated with elastic lamina degradation during atherosclerosis plaque development (16,17), as well as in myxomatous heart valves (18).

The MMP family of proteases is another large member family that has been studied in the context of both vascular (19-21) and valvular remodeling (18,22,23).

In the present study, the hypothesis was examined that MMPs and cysteine-dependent cathepsins are regulated by the mechanical environment, particularly pressure and steady laminar shear stress. Thus, fresh porcine aortic valve leaflets were exposed to the highest pressure tested (mean 170 mmHg, 1.167 Hz) and to a steady laminar shear stress of 25 dyne/cm^2. These were the same pressure and shear stress conditions used in previous studies on valve ECM synthesis (7,8).

Materials and methods

Tissue collection and culture

Fresh porcine aortic valves were obtained aseptically from a local slaughterhouse (Holifield Farm, Covington, GA, USA) and transported to the laboratory in ice-cold sterile Dulbecco's phosphate-buffered saline (DPBS) (BioWhittaker, Walkersville, MD, USA). The three leaflets were cut from the annulus and bisected at the mid-line. One half was subjected to cyclic pressure or steady shear stress; the other half was exposed to atmospheric pressure to serve as a control. Each experiment was continued for 48 h, with a sample size ranging from six to 10 leaflets.

Cyclic pressure and shear stress experiments

Valve tissue was placed in 12-well tissue culture plates and located in an in-house-designed polycarbonate pressure chamber (7,8). The tissues were exposed to pressure conditions (150-190 mmHg, at a frequency of 1.167 Hz) for up to 48 h. For shear stress studies, the valve leaflets were mounted onto a modified parallel plate (24), with the ventricular surface facing the flow. A steady laminar shear stress of 25 dyne/cm^2 was applied to the leaflets for 48 h. Control leaflet tissues were placed in 12-well plates with 3 ml Dulbecco's modified Eagle's medium in each well, and incubated under static conditions at 37ºC, 5% CO_2 and atmospheric pressure.

Gelatin zymography and immunoblotting

Following mechanical exposure, one-half of a porcine aortic valve leaflet tissue was solubilized in lysis buffer (20 mM Tris-HCl (pH 7.5), 5 mM EGTA, 150 mM NaCl, 20 mM glycerol-phosphate, 10 mM NaF, 1 mM sodium orthovanadate, 1% Triton X-100, 0.1% Tween 20) (20) and centrifuged at 18,000 *g*. The supernatant (lysate) was removed and assayed for protein concentration using a modified Lowry method, while equal amounts of protein (20 μg) were loaded onto 12.5% SDS-PAGE with 0.2% gelatin and electrophoresed at 4°C. Proteins were renatured in 50 mM Tris buffer (pH 7.4) with 20% glycerol for cathepsins, or with 2.5% Triton X-100 for MMPs, and incubated overnight in assay buffer containing 0.1 M sodium acetate buffer (pH 5.5), 1 mM EDTA and 2 mM dithiothreitol (DTT) (25), with or without the presence of 1 μM E-64, the cathepsin inhibitor, or in 50 mM Tris-HCl (pH 7.4), 10 mM $CaCl_2$, 50 mM NaCl, and 0.05% Triton X-100 for MMPs. Gels were then rinsed with deionized water and stained with Coomassie blue.

Aliquots of lysates were also loaded onto 12.5% SDS-PAGE without gelatin and transferred to a polyvinyldifluoride membrane. Membranes were first probed with a goat anti-cathepsin L primary antibody (R&D), and then with a rabbit anti-goat secondary antibody conjugated to alkaline phosphatase. CDP-Star reagent was added and chemiluminescence detected with photographic paper.

Cathepsin activity assay

The remaining half of the porcine aortic valve leaflet tissue was homogenized in 0.15 M NaCl, and centrifuged at 18,000 *g* for 15 min, and the protein content

of the supernatant was assessed using a modified Lowry protein assay. The activity assay procedure was modified from that previously published (26): Brij 35 (0.1%; Sigma) was added to 25 µg total protein in a final concentration of 100 mM acetate (pH 5.5), 2.5 mM EDTA, 2 mM DTT for 2 min at 37°C to activate cathepsin L. Z-FR-AMC (Biomol; a synthetic substrate susceptible to cathepsin L hydrolysis) was then added to a final concentration of 5 µM for 10 min prior to reading amino-4-methylcoumarin (AMC) fluorescence released in a 96-well plate, with excitation at 360 nm and emission at 460 nm.

Immunohistochemistry

Leaflet tissues were snap-frozen in liquid nitrogen for cathepsin immunohistochemistry (IHC). The orientation of the leaflet was controlled by holding the tissue upright so that the cross-section contained the three layers while placing it in the viscous medium (OCT), after which the block was quickly dipped into liquid nitrogen. The block was cut into 7-µm sections which were stored at -80ºC until staining. Separate leaflets were embedded in paraffin and sectioned for MMP IHC. Frozen sections were thawed at room temperature and then immersed in acetone for 5 min. The slides were then washed twice in PBS (5 min each), followed by immersion in 0.3% H_2O_2 for 15 min to block the endogenous peroxidase. The slides were then incubated in 2% bovine serum albumin (BSA)/PBS for 15 min to suppress non-specific binding. The blocking step was followed immediately by primary antibody incubation (anti-cathepsin L, dilution 1:20 (Santa Cruz, S6501); anti-MMP-2, dilution 1:50 (Calbiochem) or anti-MMP-9, dilution 1:50 (Calbiochem)) for 1 h followed by two washes in PBS (5 min each). There followed the secondary antibody incubation (anti-goat IgG, 1:400) for 30 min, followed by two washes in PBS, and then incubation in ABC-peroxidase (Vector Laboratories, Burlingame, CA, USA) for 1 h. Subsequently, the slides were incubated in DAB (SK-4100; Vector Laboratories) until color development. The exact time for color development varied according to the amount of antigen in the tissue, but total development time was less than 10 min. The slides were then washed in water for 5 min and counterstained with hematoxylin. Cytoseal 60 (Richard Allan Scientific) was used as a resinous mounting agent, and the slides were covered and allowed to dry overnight before viewing with a Nikon Eclipse E600w microscope.

Results

Inhibition of cathepsin L and MMP2/9 activities by cyclic pressure

Aortic heart valve leaflets were cultured for 48 h under 170 mmHg cyclic pressure before homogenization. Gelatin zymography showed a 37% decrease in cathepsin activity under cyclic pressure compared to that of the static control (Fig. 1B). The cathepsin inhibitor, E64, completely blocked gelatinase activity. The decrease in cathepsin activity was not due to the change in cathepsin L protein level, as shown by Western blotting with the specific antibody (Fig. 1A). MMP2/9 activity showed a minor, but significant decrease of 17% by cyclic hypertensive pressure (Fig. 1C). Fresh aortic valve leaflets contained minimal proMMP-2 activity and some cathepsin L activity (Fig. 1D).

Cathepsin L expression in the aortic valve leaflet

As shown by the IHC staining with the cathepsin L antibody (Fig. 2), cathepsin L protein was diffusely expressed in the spongiosa, ventricularis and fibrosa

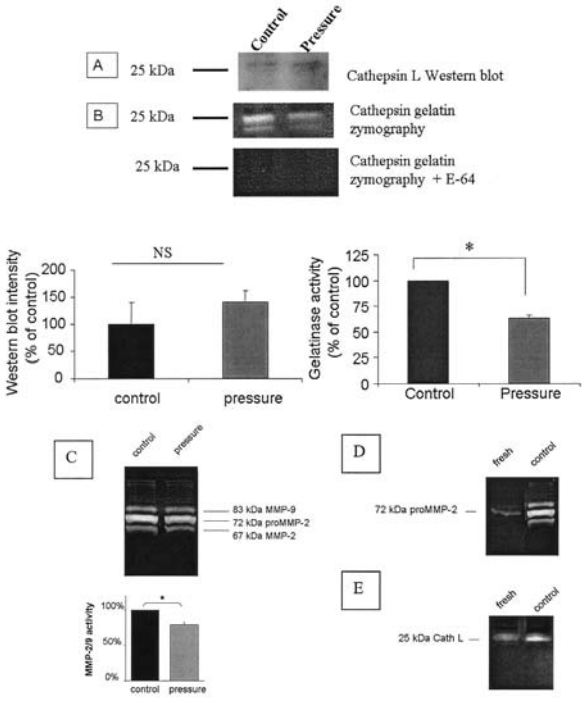

*Figure 1: Effect of cyclic pressure on cathepsin L activity in aortic valve leaflets. Fresh porcine aortic valve leaflets were exposed to hypertensive pressure (cyclic: 1.167 Hz, 170 mmHg) or control (atmospheric pressure) for 48 h. Equal protein content of leaflet lysates was analyzed by Western blotting with the cathepsin L antibody (A) and gelatin zymography for cathepsins (B) or for MMPs (C). A) Representative Western blot and (B) gelatin zymography in the absence (upper) or presence (lower) of E-64. The bar graph shows gelatinase activity as determined by densitometric quantitation of the Western blot and zymogram (mean ± SEM, n = 4, *p <0.05). Also shown are gelatin zymography from fresh aortic valve leaflets for MMP-2/9 (D) and cathepsin L (E).*

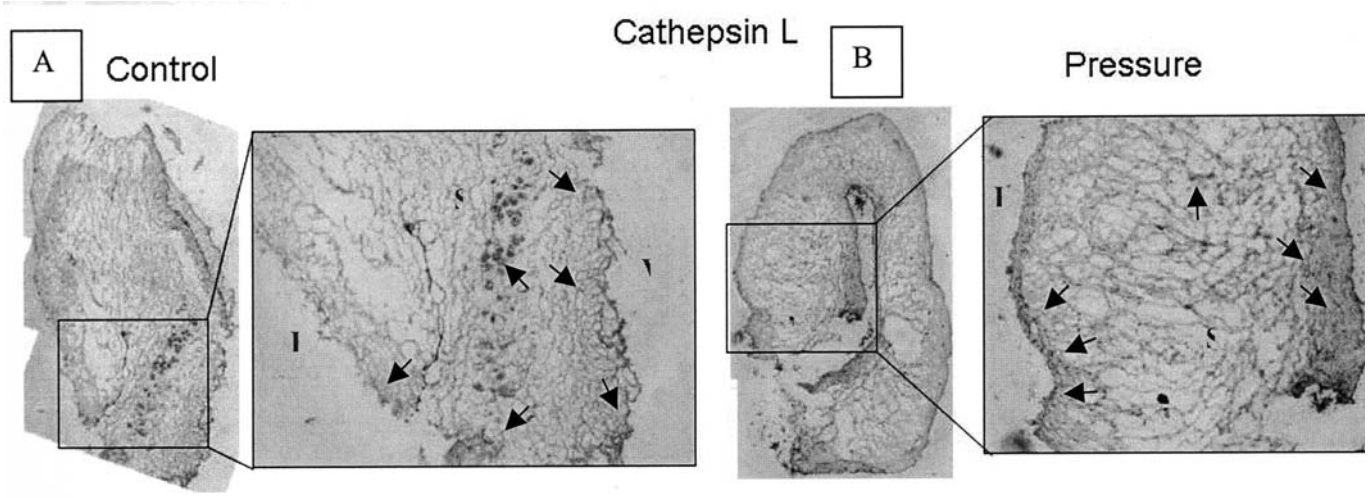

Figure 2: Cathepsin L content in aortic valve leaflet layers. Pig valve aortic leaflets exposed to cyclic pressure (170 mmHg at 1.17 Hz) or static controls for 48 h were frozen-sectioned, immunostained with the cathepsin L antibody, and counterstained with hematoxylin and eosin. Light microscopy images were taken using a 4× objective. Cathepsin L expression is indicated by the brown staining and arrows. The image shown is representative of four leaflets. V: Ventricular side of the leaflets.

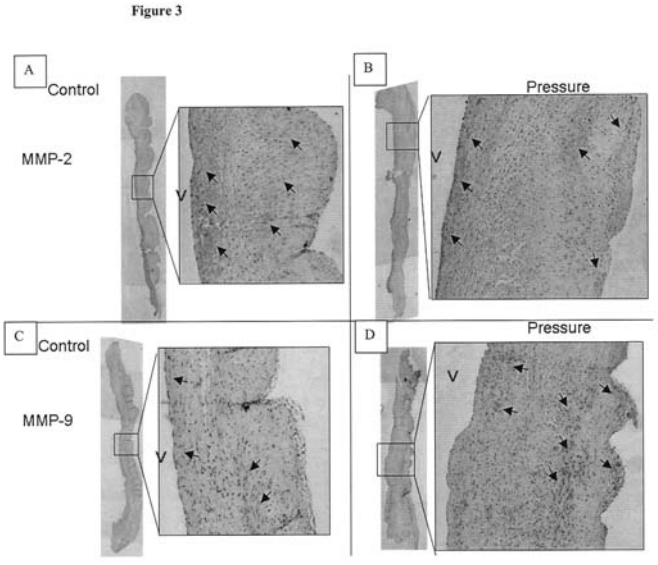

Figure 3: MMP-2 and MMP-9 immunohistochemistry on aortic valves under static conditions and cyclic pressure. Pig valve aortic leaflets exposed to cyclic pressure (170 mmHg at 1.17 Hz) or static controls for 48 h were paraffin-embedded and immunostained with the MMP-2 (A, B) or MMP-9 (C, D) antibodies. MMP-2 staining is stronger in the ventricularis than in other layers. The images shown are representative of four leaflets. V: Ventricular side; arrows indicate positive staining.

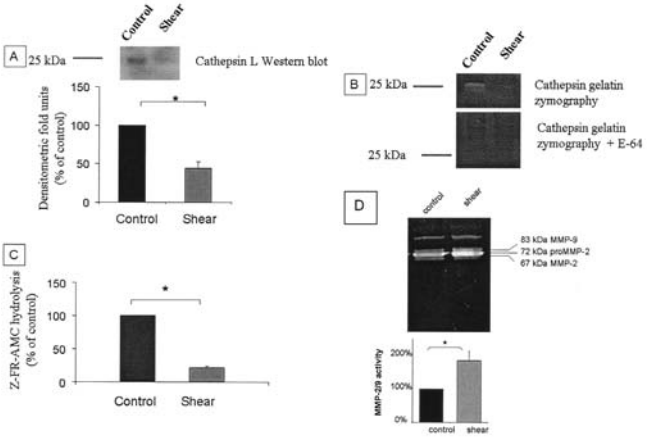

*Figure 4: Steady shear stress decreased cathepsin activity and cathepsin L protein expression, but increased MMP-2 activity in aortic valve leaflets. Porcine aortic valve leaflets exposed to steady laminar shear stress (25 dyne/cm^2) or no flow (as static control) for 48 h were frozen and lysed. Equal aliquots of lysates were analyzed by Western blotting with cathepsin L antibody (A) and gelatin zymography for cathepsins (B) or for MMPs (D). Representative blots (A) and zymography (B) in the absence (upper) or presence (lower) of E-64, the cathepsin inhibitor, are shown. The bar graph is the densitometric analysis of Western blots or zymography (mean ± SEM, n = 4, *p <0.05). In (C), lysates were used to determine cathepsin activity using the synthetic peptide (Z-FR-AMC) substrate (mean ± SEM, n = 4, *p <0.05).*

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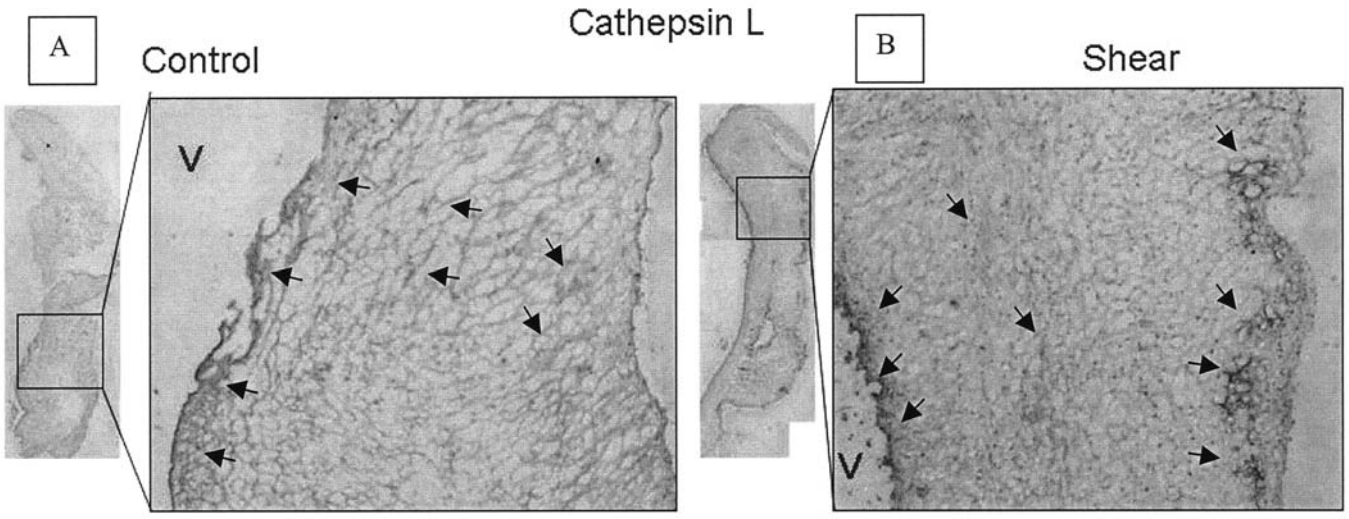

Figure 5: Cathepsin L immunohistochemistry in response to shear stress. Cathepsin L immunohistochemistry staining in leaflets exposed to shear stress was carried out as described in Figure 2. Note that the distribution of cathepsin L was also different between shear and control. Under shear, the majority of the staining was found in the ventricularis, while in the control cathepsin L was diffusely distributed in the ventricularis and spongiosa (n = 3). V: Ventricular side; arrows indicate positive staining.

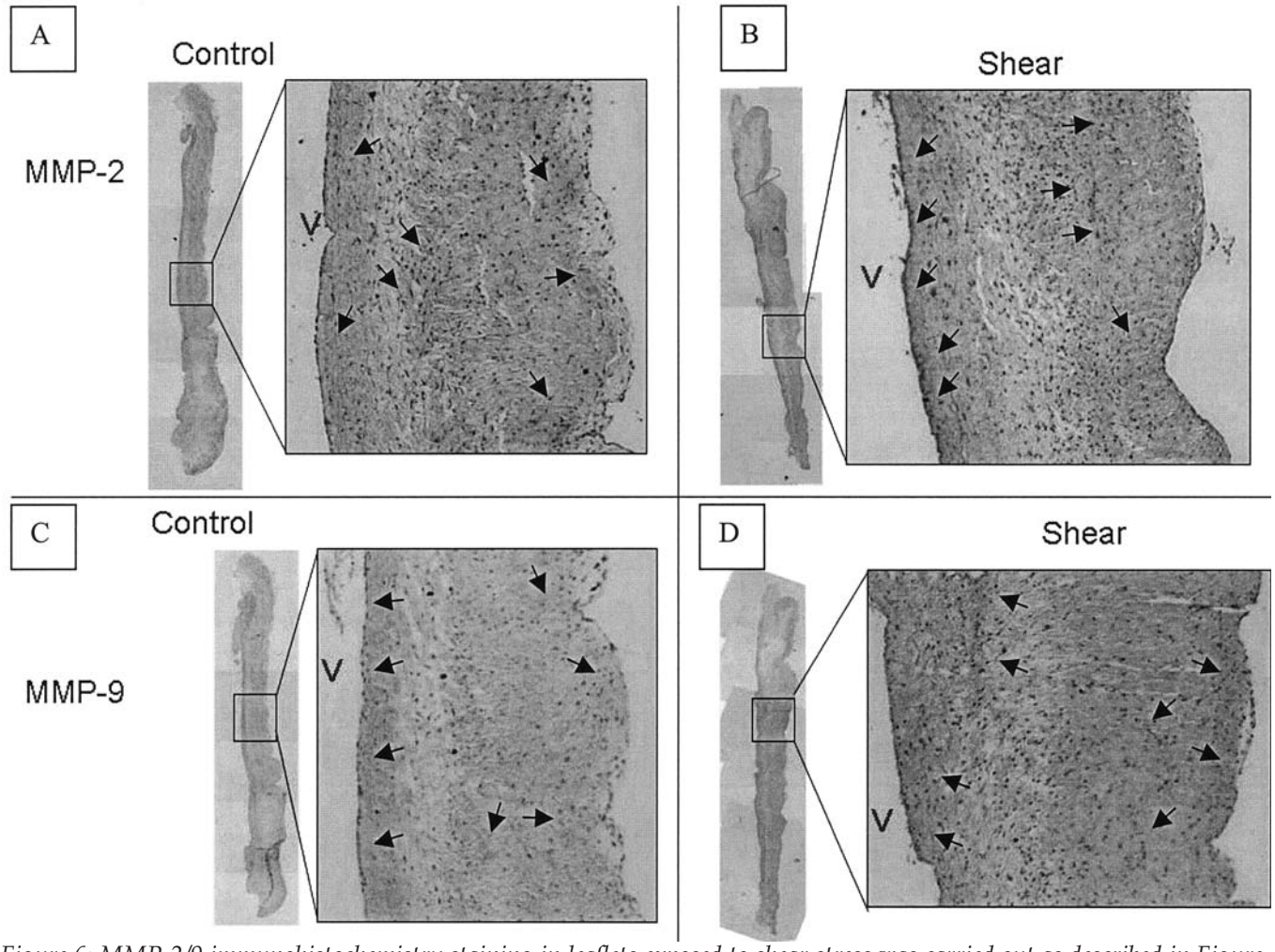

Figure 6: MMP-2/9 immunohistochemistry staining in leaflets exposed to shear stress was carried out as described in Figure 2. Under control and shear conditions, strong staining was found in the ventricularis (n = 5). V: Ventricular side; arrows indicate positive staining.

regions when cultured under either atmospheric (Fig. 2A) or cyclic pressure conditions (Fig. 2B).

MMP-2 expression in aortic valve leaflets

MMP-2 expression was stronger in the ventricularis region than the spongiosa region of the aortic valve leaflets under both atmospheric (Fig. 3A) and cyclic pressure (Fig. 3B) conditions. MMP-9 expression was distributed similarly between both regions (Fig. 3C and D).

Inhibition of cathepsin L by steady shear stress

Exposure to shear stress (25 dynes/cm^2 for 48 h) led to a decrease in cathepsin L protein levels, as shown by Western blotting of porcine aortic heart valves (Fig. 4A). In addition, shear stress significantly inhibited cathepsin activity as determined by two independent assays, namely gelatin zymography (Fig. 4B) and cathepsin peptide substrate assay (Fig. 4C). The cathepsin-specific activity was demonstrated by the complete inhibition of gelatin proteolysis by E-64 (Fig. 4B), and the inhibition of shear-induced cathepsin L-like activity is further supported by the synthetic substrate Z-FR-AMC, which has a preferential specificity toward cathepsin L (Fig. 4C). In contrast to this, shear stress increased the activity of MMP2/9 in the aortic valve leaflets, as shown by gelatin zymography (Fig. 4D).

The area of the valve leaflet in which cathepsin L expression was affected by laminar shear was identified by IHC staining with the cathepsin L antibody. Cathepsin L was diffuse in the leaflet under static culture, but stained more strongly in the ventricularis after exposure to steady laminar shear stress (Fig. 5). Overall, MMP-2 and -9 staining in the leaflets showed no remarkable differences between control and shear groups, though there was stronger signal in the ventricularis region compared to the spongiosa (Fig. 6A-D).

Discussion

The results of the present study showed that hypertensive pressure caused a significant reduction in cathepsin L activity and a minor reduction in MMP2/9 activity in porcine aortic valve leaflets, whereas a steady laminar shear stress decreased cathepsin activity and cathepsin L expression but increased MMP2/9 activity. Previous results reported by the present authors showed significant increases in collagen content under identical mechanical conditions (7,8). It appears that there is an inverse relationship between collagen synthesis and cathepsin activity under the stimulation of hypertensive pressure or steady shear stress. As the main function of cathepsins is to degrade collagen, the observed higher collagen content under hypertensive pressure/shear stress conditions is most likely due, in part, to a down-regulation of the collagenolytic activities. The net collagen content of valve tissue is determined by a dynamic balance between new synthesis and degradation by collagenases such as cathepsins and MMPs.

The novel finding of the present study was that the cathepsins, which are collagenolytic proteases, are regulated by mechanical factors. Both, an increase in synthesis and a decrease in degradation would lead to higher total collagen content in the leaflet tissue. Previously, it had been shown that cathepsin L is inhibited by steady shear stress in aortic endothelial cells (15), but in the present study it is reported for the first time that cathepsin L expression is regulated by cyclic pressure and shear stress, and appears to correlate with the ECM renewal/remodeling in normal heart valve tissue. Previous studies have indicated the involvement of cathepsins K and S in myxomatous mitral valves (18), but to date no report has been made on cathepsins in normal healthy heart valves. In addition to cathepsin L, the protein content and gelatinase activity of MMP-2 and MMP-9 were also studied because of their well-known role in ECM remodeling (27,28), as well as in valve pathology (23,29-31). Similar changes of MMP-2 and -9 were induced by hypertensive pressure, but the opposite occurred with steady shear stress in the valve tissue. These two protease families may play different roles in ECM remodeling under shear stress; for example, one family might be responsible for the degradation of old components, while the other is important in the organization of the newly synthesized matrix.

In contrast to the shear effect, cyclic pressure inhibited cathepsin L activity without altering its protein level. This raises the possibility that cyclic pressure may regulate cathepsin activity either by controlling the cathepsin inhibitors such as cystatin C, or by directly inhibiting cathepsin L activity through post-translational modifications.

The IHC staining results showed that cathepsin L was expressed throughout the layers of the valve leaflets (even the interstitial cells), but that the MMPs showed stronger staining in the ventricularis than in the spongiosa. Immortalized endothelial cells increase MMP-2/9 activity by steady shear stress compared to static culture (32), which might be the case in this ex-vivo valvular model, leading to increased MMP-2/9 activity by shear stress. As the IHC for cathepsin L and MMP-2/9 was not conclusive in terms of specific protein content, no difference was observed in cell type expression of these proteases. Moreover, Western blotting and zymography data were used as quantitative indicators of protease activity in the total porcine aortic valve exposed to various mechanical conditions.

The importance of flow characteristics in tissue remodeling and disease progression has been studied both by engineers and pathologists. The aortic valve experiences a complex hemodynamic environment whereby three distinct flow regions exist: the aortic wall of the sinus of Valsalva is characterized with a low, disturbed flow; the side of the leaflets facing the aorta with a low-shear, laminar flow; and the side facing the ventricle with a high-shear, laminar flow (33).

Clinical implications

The significance of these results is two-fold. First, they help to improve an understanding of the acute responses of the aortic valve to hypertensive pressure. Recent clinical retrospective studies have indicated that hypertension is a risk factor for valvular disease such as aortic valve stenosis. However, it is not yet clear how hypertensive pressure is involved in this pathophysiology, especially during the early stages of the disease. Results from the present study showed that hypertensive pressure could induce changes in valve matrix synthesis within 48 h, indicating that valvular matrix remodeling might contribute to the development of aortic valve stenosis in hypertensive patients. The involvement of cathepsins in these processes implicates this protease family as a potential drug target which might slow the remodeling process induced by abnormal mechanical stimuli. In addition, these results offer improved strategies for the development of tissue-engineered heart valves. A tissue-engineered heart valve created with biodegradable scaffolds or ECM needs to have controlled degradation. The present results suggest that culture under flow can reduce cathepsin-mediated proteolysis, and that this could extend the life of the scaffold in the valve construct.

In conclusion, the present study, for the first time, showed that cathepsin L and MMP-2 and -9 might be involved in valvular ECM remodeling induced by hypertensive pressure, while cathepsin L might also contribute to remodeling under regions of stagnant flow.

Acknowledgements

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References

1. Weber KT, Sun Y, Katwa LC, Cleutjens JP, Zhou G. Connective tissue and repair in the heart. Potential regulatory mechanisms. Ann N Y Acad Sci 1995;752:286-299
2. Schneider PJ, Deck JD. Tissue and cell renewal in the natural aortic valve of rats: An autoradiographic study. Cardiovasc Res 1981;15:181-189
3. Willems IE, Havenith MG, Smits JF, Daemen MJ. Structural alterations in heart valves during left ventricular pressure overload in the rat. Lab Invest 1994;71:127-133
4. Quick DW, Kunzelman KS, Kneebone JM, Cochran RP. Collagen synthesis is upregulated in mitral valves subjected to altered stress. Am Soc Artif Intern Organs J 1997;43:181-186
5. Butcher JT, Penrod AM, Garcia AJ, Nerem RM. Unique morphology and focal adhesion development of valvular endothelial cells in static and fluid flow environments. Arterioscler Thromb Vasc Biol 2004;24:1429-1434
6. Simmons CA, Grant GR, Manduchi E, Davies PF. Spatial heterogeneity of endothelial phenotypes correlates with side-specific vulnerability to calcification in normal porcine aortic valves. Circ Res 2005;96:792-799
7. Xing Y, He Z, Warnock JN, Hilbert SL, Yoganathan AP. Effects of constant static pressure on the biological properties of porcine aortic valve leaflets. Ann Biomed Eng 2004;32:555-562
8. Xing Y, Warnock JN, He Z, Hilbert SL, Yoganathan AP. Cyclic pressure affects the biological properties of porcine aortic valve leaflets in a magnitude and frequency dependent manner. Ann Biomed Eng 2004;32:1461-1470
9. Goulet B, Baruch A, Moon NS, et al. A cathepsin L isoform that is devoid of a signal peptide localizes to the nucleus in S phase and processes the CDP/Cux transcription factor. Mol Cell 2004;14:207-219
10. Keyszer GM, Heer AH, Kriegsmann J, et al. Comparative analysis of cathepsin L, cathepsin D, and collagenase messenger RNA expression in synovial tissues of patients with rheumatoid arthritis and osteoarthritis, by in situ hybridization. Arthritis Rheum 1995;38:976-984
11. Taubert H, Riemann D, Kehlen A, et al. Expression of cathepsin B, D and L protein in juvenile idiopathic arthritis. Autoimmunity 2002;35:221-224
12. Rousselet N, Mills L, Jean D, Tellez C, Bar-Eli M, Frade R. Inhibition of tumorigenicity and metastasis of human melanoma cells by anti-cathepsin L single chain variable fragment. Cancer Res 2004;64:146-151
13. Liu J, Sukhova GK, Yang JT, et al. Cathepsin L

expression and regulation in human abdominal aortic aneurysm, atherosclerosis, and vascular cells. Atherosclerosis 2006;184:302-311
14. Stypmann J, Glaser K, Roth W, et al. Dilated cardiomyopathy in mice deficient for the lysosomal cysteine peptidase cathepsin L. Proc Natl Acad Sci USA 2002;99:6234-6239
15. Platt MO, Ankeny RF, Jo H. Laminar shear stress inhibits cathepsin L activity in endothelial cells. Arterioscler Thromb Vasc Biol 2006; http://atvb.ahajournals.org/cgi/reprint/01.ATV.0000227470.72109.2bv1
16. Sukhova GK, Shi GP, Simon DI, Chapman HA, Libby P. Expression of the elastolytic cathepsins S and K in human atheroma and regulation of their production in smooth muscle cells. J Clin Invest 1998;102:576-583
17. Sukhova GK, Zhang Y, Pan JH, et al. Deficiency of cathepsin S reduces atherosclerosis in LDL receptor-deficient mice. J Clin Invest 2003;111:897-906
18. Rabkin E, Aikawa M, Stone JR, Fukumoto Y, Libby P, Schoen FJ. Activated interstitial myofibroblasts express catabolic enzymes and mediate matrix remodeling in myxomatous heart valves. Circulation 2001;104:2525-2532
19. Berceli SA, Jiang Z, Klingman NV, et al. Differential expression and activity of matrix metalloproteinases during flow-modulated vein graft remodeling. J Vasc Surg 2004;39:1084-1090
20. Galis ZS, Johnson C, Godin D, et al. Targeted disruption of the matrix metalloproteinase-9 gene impairs smooth muscle cell migration and geometrical arterial remodeling. Circ Res 2002;91:852-859
21. Lessner SM, Martinson DE, Galis ZS. Compensatory vascular remodeling during atherosclerotic lesion growth depends on matrix metalloproteinase-9 activity. Arterioscler Thromb Vasc Biol 2004;24:2123-2129
22. Dreger SA, Taylor PM, Allen SP, Yacoub MH. Profile and localization of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in human heart valves. J Heart Valve Dis 2002;11:875-880;discussion 880
23. Soini Y, Satta J, Maatta M, Autio-Harmainen H. Expression of MMP2, MMP9, MT1-MMP, TIMP1, and TIMP2 mRNA in valvular lesions of the heart. J Pathol 2001;194:225-231
24. Weston MW, Yoganathan AP. Biosynthetic activity in heart valve leaflets in response to in vitro flow environments. Ann Biomed Eng 2001;29:752-763
25. Desmazes C, Gauthier F, Lalmanach G. Cathepsin L, but not cathepsin B, is a potential kininogenase. Biol Chem 2001;382:811-815
26. Barrett AJ, Kirschke H. Cathepsin B, Cathepsin H, and cathepsin L. Methods Enzymol 1981;80(Pt.C):535-561
27. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: The good, the bad, and the ugly. Circ Res 2002;90:251-262
28. Lehoux S, Lemarie CA, Esposito B, Lijnen HR, Tedgui A. Pressure-induced matrix metalloproteinase-9 contributes to early hypertensive remodeling. Circulation 2004;109:1041-1047
29. Bracher M, Simionescu D, Simionescu A, Davies N, Human P, Zilla P. Matrix metalloproteinases and tissue valve degeneration. J Long Term Eff Med Implants 2001;11:221-230
30. Edep ME, Shirani J, Wolf P, Brown DL. Matrix metalloproteinase expression in nonrheumatic aortic stenosis. Cardiovasc Pathol 2000;9:281-286
31. Koullias GJ, Korkolis DP, Ravichandran P, Psyrri A, Hatzaras I, Elefteriades JA. Tissue microarray detection of matrix metalloproteinases, in diseased tricuspid and bicuspid aortic valves with or without pathology of the ascending aorta. Eur J Cardiothorac Surg 2004;26:1098-1103
32. Magid R, Murphy TJ, Galis ZS. Expression of matrix metalloproteinase-9 in endothelial cells is differentially regulated by shear stress. Role of c-Myc. J Biol Chem 2003;278:32994-32999
33. Mehrabian M, Demer LL, Lusis AJ. Differential accumulation of intimal monocyte-macrophages relative to lipoproteins and lipofuscin corresponds to hemodynamic forces on cardiac valves in mice. Arterioscler Thromb 1991;11:947-957