



Correlation between MMP and TIMP levels and elastic moduli of ascending thoracic aortic aneurysms

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ARTICLE INFO

Article history:

Received 14 March 2018

Received in revised form 5 July 2018

Accepted 5 July 2018

Keywords:

Elastic Modulus

MMPs/TIMPs levels

Ascending thoracic aortic aneurysm

ABSTRACT

Objective: The objective of this preliminary investigation is to determine if there is a relation between the biological levels of matrix metalloproteinases and tissue inhibitor of matrix metalloproteinase (TIMP) and the elastic moduli of the ascending aortic wall in patients with ascending thoracic aortic aneurysms (ATAA). **Methods:** Circumferential specimens from twelve patients with ATAA were obtained from the greater curvature and their tensile properties (maximum elastic modulus) were tested uniaxially. The levels of MMP1, 2, 3, 8, and 9 as well as TIMP1 and 2 were determined in these aortic wall specimens using MMP/TIMP antibodies array.

Results: Direct relations were found between MMP2 and the elastic modulus of the ascending aorta wall ($R^2 = 0.52$) and between MMP9 and TIMP1 ($R^2 = 0.63$). However, weak positive relation was found between MMP2 and TIMP2 ($R^2 = 0.23$). We found inverse relations between MMP3 and MMP8 levels and the elastic module. There were no relations between MMP1 and MMP9 levels and the elastic modulus of aortic wall.

Conclusions: This preliminary study looks at the relationship between the elastic moduli and the MMPs/TIMPs levels found in aortic wall specimens. Given that the value of the elastic moduli can be obtained non-invasively, a close relation might permit to infer the value of MMPs and TIMPs levels from the non-invasive determination of the elasticity of the aortic wall. By allowing the non-invasive determination of the mechanical and biological properties of the aorta in in-vivo, the method proposed here might improve the prediction of outcomes of ascending aortic aneurysms. This is a very preliminary study (small sample size) and the outcomes of this study cannot be used as final conclusions and should be verified in further studies with larger sample of patients.

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1. Introduction

Ascending thoracic aortic aneurysm (ATAA) is a life-threatening condition that would benefit from a tool that predicts its rupture early [1–7]. Studies of the biomechanical properties of ATAA have shown some contradictory results in reporting the values of elastic modulus of ATAA [8–16]. For example, Vorp et al. [12] determined the tensile strength and maximum tissue stiffness of ATAA tissue in a uniaxial tensile testing machine by stretching to failure tissue strips in longitudinal or circumferential orientation. Surprisingly they did not find any difference in the strength between circumferential and longitudinal specimens in either aneurysmal or control tissues. However, Khanafer et al. [8] illustrated that the maximum elastic moduli of ATAA were significantly higher in circumferential than in longitudinal orientation. Duprey et al. [9] measured in vitro the biomechanical behavior of ATAA using uniaxial tensile testing. They also reported that the maximum elastic

modulus was significantly higher in the circumferential than in the longitudinal specimens. Iliopoulos et al. [13] did not find a significant relation between tissue strength and ATAA diameter or patient age. They showed, however, - like Duprey et al. [9] - a higher stiffness in the circumferential than in the longitudinal direction. Conversely, Okamoto et al. [11, 17] studied the elastic properties of ascending aortas in Marfan, bi-valve and tri-valve patients and found that the mean strength was significantly lower in older patients, so that age could affect the rupture risk of ATAA.

It is recognized that Matrix Metalloproteases (MMPs) play a major role in the degradation of collagen and other extracellular matrix macromolecules [18–24]. Notably, MMP-2 and MMP-9 were found to be critical in the destructive processes that accompany aneurysmal growth, as evidenced by inhibition of experimental AAA development in mice with a targeted disruption of the MMP9 or MMP2 gene [18]. However the precise relevance of MMPs in human ATAA formation is less well known. Ikonomidis et al. [24] demonstrated that the deletion of the TIMP1 gene results in increased and continued progression of thoracic aortic aneurysm in mice. The vast majority of studies of the

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mechanical properties of ATAA have focused on the isolated measurements of these markers. None of them have sought to correlate quantitatively or temporally the elastic properties and the MMP levels of aneurysmal ascending aortas. Vallabhaneni et al. [25] measured the tensile strength of the aneurysm wall and the MMP concentration (MMP-2 and MMP-9) in the same sample of aortic tissue. The aortic specimens were tested in a tensile testing apparatus at a deformation rate of 1 mm/min (which is at least 100 times slower than the normal aortic tissue strain force). The authors claimed that the localized “hot spots” of MMP hyperactivity could lead to focal weakening of the aneurysm wall and rupture at relatively low levels of intraluminal pressure. The authors did not study the relation between elasticity and levels of both MMPs and TIMPs.

One can note from the above review that there were no studies conducted in the literature investigating if there is possible relationship between mechanical and biological properties. Therefore, the aim of this exploratory study is to determine whether there is a relation between the biological levels of MMPs and TIMPs and the elastic moduli of samples of ATAA.

2. Material and methods

2.1. Human aortic tissue specimens

Fresh tissue samples were obtained from the operating room from patients undergoing surgical repair of ATAA at the Frankel Cardiovascular Center of the University of Michigan. All procedures were carried out in accordance with the guidelines of the Institutional Review Board of the University of Michigan. The aorta wall specimen was cut into circumferential strips with custom-designed tissue cutters of 30 mm strips were obtained from the 3×30 mm dimensions. An average of 41 \pm 3 \times 30 mm ascending aorta. For each patient, one strip was processed for MMPs/TIMPs assay while the rest were tested mechanically. Multiple measurements were utilized and their average value was used to calculate the elastic modulus. All the strips were circumferential specimens obtained from the anterior wall of the ascending aorta. These tissues were placed in gauze wetted with saline and refrigerated at 4 °C and tissue testing was performed within 24 h of harvest. The recovered tissue was equilibrated to room temperature by immersion in fresh phosphate-buffered saline. Adipose tissue was removed from the surface of the adventitia and the 3 \times 30mm strips were tested uniaxially. The original dimensions of the sample (thickness and width) were measured at zero stress state.

2.2. Mechanical tissue testing

Mechanical tissue testing were carried out in a tensile testing machine (Instron® model 5542). Adhesive sandpaper of fine grit to avoid damaging the tissue was applied to the surface of the jaws of the pneumatic grips to prevent slippage during the test. It was found during preliminary testing that an aspect ratio of at least 2 was required to ensure a free shear deformation and local narrowing of the tissue between the grips. During testing, the tissue was kept wet by a spray of phosphate buffering solution. Each specimen was preconditioned by 10 cycles of 1 N load at 500 mm/min to eliminate the effect of hysteresis. Then tensile testing was performed until failure. Load and stretch was continuously recorded by the data acquisition software Merlin® provided by Instron®. The load-stretch curve was derived to obtain the stress-strain relationship. The maximum elastic modulus was measured from the maximum slope of the stress/strain curve using true stress- true strain definition as recommended by Khanafer et al. [16]. Data processing and statistical analysis were performed on Excel 2012® (Microsoft Corporation).

2.3. MMP and TIMP level measurements in aortic tissues

The aortic strip was minced into small pieces and wash in ice cold Tris-buffered L of RayBio® Cell Lysis Buffer supplemented with saline (TBS) to remove the blood. 500 with protease inhibitors were added to the washed tissue and homogenized and sonicated several times to extract the proteins out of the tissues. Insoluble cellular debris were separated by centrifuging the tissue extract at 7000 rpm for 5 min and the clear supernatant was saved as the tissue extract. Protein concentration of the tissue extract was measured by BCA kit (Thermo Scientific, Pittsburgh, PA). The MMP array membrane was incubated with 200 μ g of tissue lysate overnight at 4 °C as outlined by the manufacturer (RayBio® Tech). Briefly, the membranes were incubated in the blocking buffer for 30 min and then the tissue extract diluted in blocking buffer was added to it for overnight at 4 °C. The membranes were washed in Wash Buffer I and Wash Buffer II and then incubated with biotin conjugated anti-cytokine antibodies diluted in blocking buffer for 2 h at room temperature. The membranes were washed as before and incubated with HRP-conjugated streptavidin diluted in blocking buffer for 2 h and washed again as usual. The membrane was incubated with detection buffer for 2 min and then exposed to X-ray films to capture the image of the proteins that were detected by the anti-cytokine antibodies. The images on the films were scanned and the individual protein spots (MMP1, MMP2, MMP3, MMP8, MMP9, TIMP1, TIMP2) were quantified densitometrically by ImageJ software (NIH). The values of each individual protein spot were divided by the values from the positive control present on each membrane to get a relative number which reflect the relative amount of the protein present in each tissue with respect to the positive control.

2.4. Statistical analysis

Data processing and statistical analysis were performed using Minitab software (Minitab v14). The coefficient of determination denoted R² is used in this investigation to determine if there is a relation between elastic moduli and the levels of MMPs and TIMPs.

3. Results

Forty one-3 \times 30 circumferential strips from 12 patients with ATAA (8 Males and 4 Females) with a mean age of 66.8 ± 7.6 SD years were obtained from the greater curvature for uniaxial testing of their tensile properties. MMP1, 2, 3, 8, and 9 as well as TIMP1 and 2 levels were determined in these aortic wall specimens (RayBio® Tech). The MMP/TIMP assays were negatives in two patients. This explains why only 10 data points were available for study. Figs. 1 through 6 show the relation between the elastic properties of the aortic wall and the biological levels

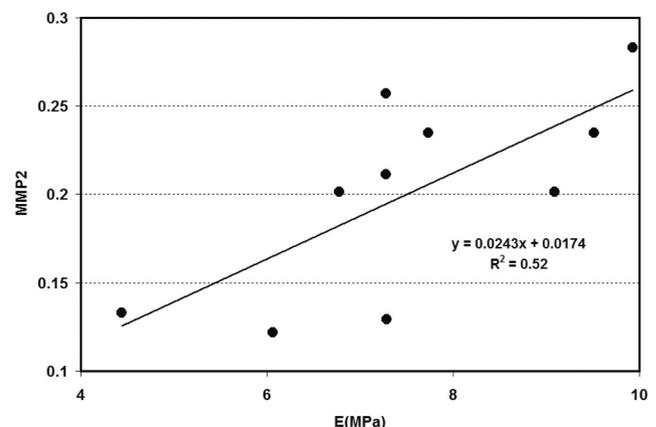


Fig. 1. Relation between MMP2 and the elastic modulus of the ATAA.

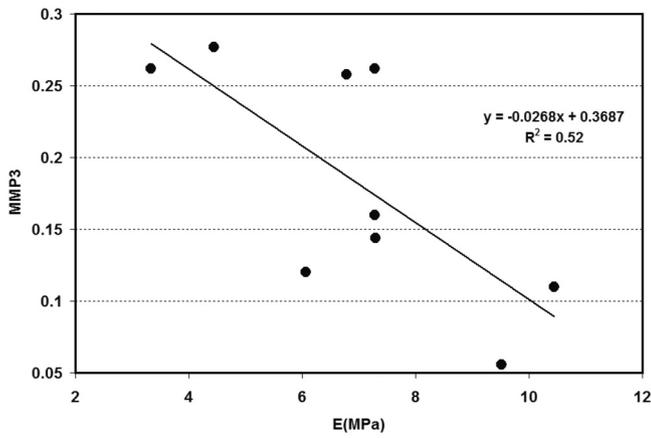


Fig. 2. Relation between MMP3 and the elastic modulus of the ATAA.

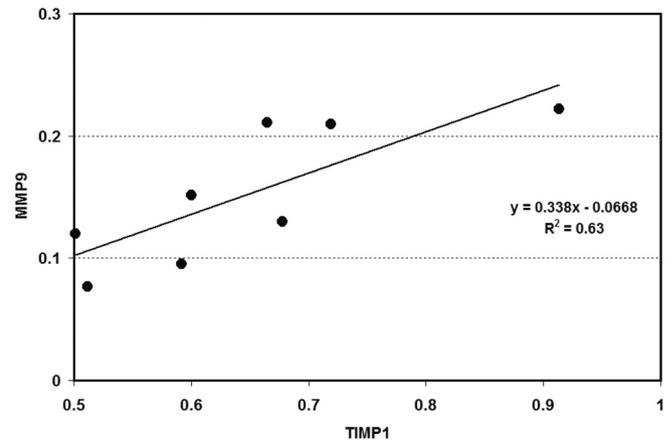


Fig. 5. Relation between MMP9 and the TIMP1.

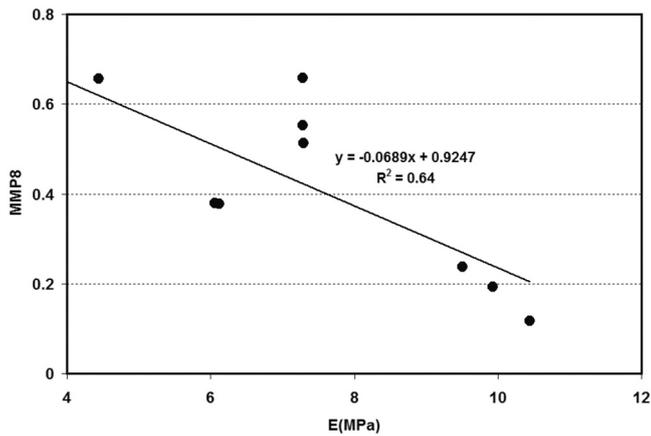


Fig. 3. Relation between MMP8 and the elastic modulus of the ATAA.

4. Discussion

A relation between the levels of MMP's and the values of the elastic moduli of the aortic wall could be a potential tool for the prediction of growth of these aneurysms given that elastic measurements of a functioning aorta can be derived from CTA or MRI data. The modulus of elasticity is a reference that describes how a material will deform when subjected to a stress. The initiation, growth and eventual rupture of an aneurysm are prompted by changes in elasticity of the aortic wall. MMPs are important enzymes that play a critical role in extracellular matrix remodeling. There are over 28 known human MMPs that are commonly classified as collagenases, membrane type matrix metalloproteinases, stromelysins and matrilysins. The catalytic activities of the MMPs are tightly regulated by tissue inhibitors of TIMPs, which consists of 4 members, i.e., TIMP1, 2, 3 and TIMP4. The ratios of MMPs and TIMPs are crucial for the maintenance of the normal architecture of the extracellular matrix (ECM) and this ratio is often disrupted under pathological conditions. For example, elastin, the main component of the arterial wall, gets degraded by MMPs in abdominal aortic aneurysm (AAA) where elevated levels of MMP2 and 9 have been found. To date, the existence of any relation between the elastic moduli and the biological levels (MMPs and TIMPs) in the walls of AATA has not been studied.

The results of this preliminary study did not find a direct relation between the stiffness of the aortic wall aneurysms and MMP1 levels. However, Fig. 1 demonstrates direct relation between MMP2 and the elasticity of the aortic wall ($R^2 = 0.52$). Figs. 2 and 3 show the inverse

of MMPs and TIMPs. There was no relation between MMP1 and the elastic modulus of the aortic wall ($R^2 = 0.0057$) and between MMP9 and elasticity ($R^2 = 0.018$) (Direct relation existed between MMP2 and elasticity as shown in Fig. 1 ($R^2 = 0.52$)). Figs. 2 through 4 illustrate inverse relations between MMP3, 8, and 9 and elasticity. Direct relation between MMP9 and TIMP1 ($R^2 = 0.63$) and weak positive association between MMP2 and TIMP2 ($R^2 = 0.23$) is shown in Figs. 5 and 6, respectively.

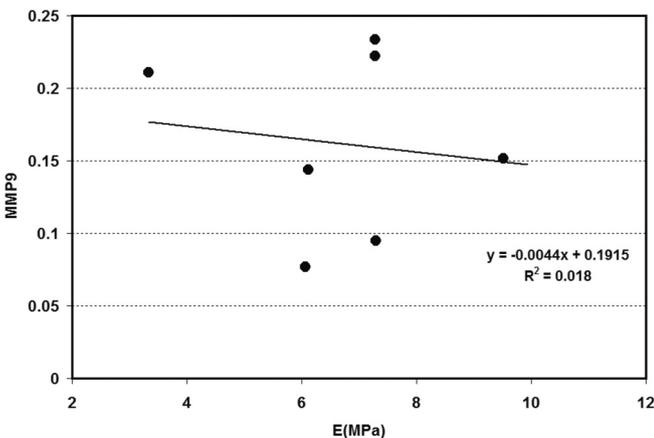


Fig. 4. Relation between MMP9 and the elastic modulus of the ATAA.

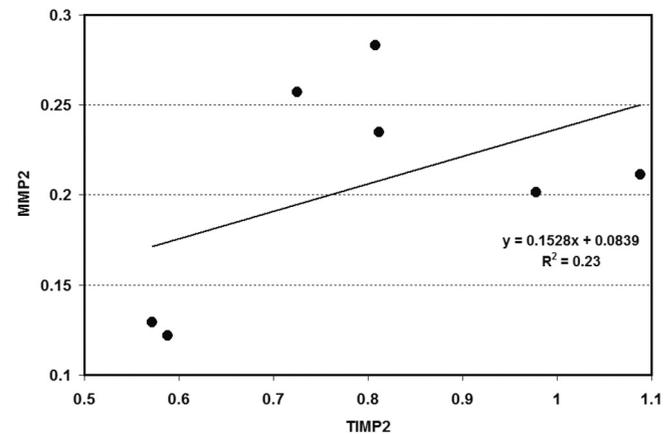


Fig. 6. Relation between MMP2 and TIMP2.

relation between MMP3, MMP8 and the elasticity of the ATAA wall. Fig. 4 shows a very weak inverse relation between MMP9 level and the elastic modulus of the aortic wall ($R^2 = 0.018$). MMP9 activity is associated with destruction of the elastic laminae of arteries and aneurysm formation in animals and humans [18, 26]. We also analyzed the relation between MMP and TIMP because an imbalance between MMPs and TIMPs may be a mechanism of thoracic aortic aneurysm formation [27]. MMPs are inhibited by specific endogenous TIMPs that can inhibit all active MMPs, albeit not with the same efficiency. For example, TIMP1 inhibits MMP1, MMP3, MMP7, and MMP9 while TIMP2 is an effective inhibitor of MMP2 [28]. Figs. 5 and 6 show positive relations between MMP9 and TIMP1 ($R^2 = 0.63$) and weak one between MMP2 and TIMP2 ($R^2 = 0.23$).

Since we can derive the elastic modulus of a live aorta from CTA scan data [29], we propose here an innovative approach that may permit to infer specific level of MMP by the non-invasive determination of the elastic modulus. Schlicht et al. [29] showed the feasibility of measuring the elastic properties of the arterial wall from CTA scan data and a good relation between the elastic values derived from the CTA data and those obtained in a tensile strength testing machine. If we can establish a relation between the elastic moduli and the MMPs/TIMPs levels in aortic wall specimens, we might be able to infer the value of the latter (biological properties) from the non-invasive determination of the former (mechanical properties) and consequently improving the prediction of outcomes of ascending aortic aneurysms. We are not implying causality or directionality of this relation. However, we anticipate that if the level of MMP correlates with the dilation of the aorta, a non-aneurysmal aorta with increased levels of MMP could be presumed to be at risk for thoracic aortic aneurysm. This requires a fairly large number of histochemistry analyses from non-dilated aortas.

5. Conclusions

MMPs play an important role in the pathophysiology of ATAA. High levels of MMPs were measured in patients with ATAA. The elastic moduli of aortic specimens from patients undergoing repair of ATAA were determined using uniaxial tensile testing machine and the levels of MMPs and TIMPs in the aortic wall were assessed. Our preliminary findings showed a positive relation between MMP9 and TIMP1. Direct relation was found between MMP2 and elasticity. However, MMP3 and 8 levels correlated inversely with elastic modulus. This investigation is considered a very preliminary study (small sample size) and the outcomes of this study cannot be used as final conclusions and should be verified in further studies. It may provide a foundation for the non-invasive determination of the mechanical and biological properties of the aorta in in-vivo.

Conflict of interest statement

None.

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