Extracellular matrix remodelling in human aortic valve disease: the role of matrix metalloproteinases and their tissue inhibitors

Olivier Fondard¹, Delphine Detaint¹, Bernard Iung², Christine Choqueux¹, Homa Adle-Biassette³, Mohamed Jarraja⁴, Ulrich Hvass⁵, Jean-Paul Couetil⁵, Dominique Henin³, Jean-Baptiste Michel¹, Alec Vahanian², and Marie-Paule Jacob¹*

¹INSERM U 460, Bâtiment 13, Hôpital Bichat-Claude Bernard, 46 rue Henri Huchard, 75877 Paris Cedex 18, France; ²Department of Cardiology, Hôpital Bichat-Claude Bernard, 46 rue Henri Huchard, 75877 Paris Cedex 18, France; ³Department of Pathology, Hôpital Bichat-Claude Bernard, 46 rue Henri Huchard, 75877 Paris Cedex 18, France; ⁴Banque de Tissus Humains, Hôpital Saint-Louis, 1 avenue Claude Vellefaux 75475, Paris Cedex 10, France; and ⁵Department of Cardiac Surgery, Hôpital Bichat-Claude Bernard, 46 rue Henri Huchard, 75877 Paris Cedex 18, France

Received 1 October 2004; revised 10 February 2005; accepted 3 March 2005; online publish-ahead-of-print 12 April 2005

Aims Aortic valve diseases are characterized by pathological remodelling of valvular tissue but the cellular and molecular effectors involved in these processes are not well known. The role of matrix metalloproteinase (MMP)-2, MMP-9, MMP-3, MMP-7, and tissue inhibitor of matrix metalloproteinase (TIMP)-1 and TIMP-2 are investigated here.

Methods and results Histological analysis of pathological valves [aortic stenosis (AS) (n = 49), aortic regurgitation (AR) (n = 23)] and control valves (n = 8) was performed. The main tissue abnormalities (calcification, inflammatory cells, and capillaries) observed in AS were less severe or absent in AR. However, both groups of pathological valves displayed similar histological signs of extracellular matrix (ECM) remodelling. Biochemical analysis of MMPs and TIMPs (gelatin and casein zymography and ELISA) was performed on valve extracts. MMP-2 activity was not significantly different in control and pathological valves. Increases in MMP-9 and MMP-3 in AS demonstrated an inflammatory state. Finally, there was a four- to seven-fold increase of TIMP-1 in pathological valves. TIMP-1, TIMP-2, and MMP-2 were synthesized by the valvular interstitial cells in primary culture.

Conclusion This study demonstrates the involvement of the MMP/TIMP system in ECM remodelling of both AS and AR. These findings provide evidence of inflammatory injury more severe in AS than in AR and involvement of mesenchymal cell response.

KEYWORDS Aortic stenosis; Aortic regurgitation; Matrix metalloproteinases; Tissue inhibitor of matrix metalloproteinases; Valves

Introduction Stenosis and regurgitation are the two clinical manifestations of aortic valve disease. Aortic stenosis (AS) is the most frequent valvular disease in the Western world. Its prevalence increases with age such that it affects ~3% of the elderly population and is the most common reason for valve replacement. When compared with AS, aortic regurgitation (AR) is a less frequent pathological condition.

Abnormal aortic valve function observed in AS and AR likely results from tissue remodelling, involving especially the extracellular matrix (ECM). The processes involved in the abnormal ECM accumulation or destruction are uncertain. In physiological conditions, the ECM of any tissue is maintained by a rigorously controlled balance between the synthesis and the breakdown of its component proteins. A disequilibrium between the synthesis of ECM components and their degradation leads to a pathological remodelling of the ECM. Matrix metalloproteinases (MMPs) and their endogenous inhibitors, tissue inhibitors of MMPs (TIMPs), play central role in the degradation process. Recent findings suggest that inflammation contributes to valve diseases. The link between inflammation and remodelling has been established in atherosclerosis, but has not been clearly established in valve disease.

Our hypothesis is that the ECM remodelling and the associated systems of degradation, especially the MMPs, play a major role in pathological valvular remodelling observed in AS and AR. MMP-2 is the main MMP secreted by mesenchymal cells, whereas MMP-3, MMP-9, and, to a lesser extent, MMP-7 are synthesized by inflammatory cells.

Our study, based on a histological and biochemical approach, evaluated the MMP/TIMP balance using AR and AS valves from patients who required surgery for severe aortic dysfunction. These pathological valves were...
compared with control valves obtained at the bank of human tissues. In addition, interstitial cells from pathological valves were cultured to analyse the contribution of these cells to the secretion of MMPs and TIMPs.

Methods

Patient population and valve collection
Seventy-two aortic valves were collected during valve replacement from 72 patients suffering from AS (n = 49) or AR (n = 23) at Bichat Hospital. Eight fresh aortic homografts discarded from therapeutic use were collected from eight explanted hearts during transplantation and were used as controls. Valves were dissected at the human tissue bank of Saint-Louis Hospital within 24 h after heart explantation without any preservation procedure. Characteristics of patients and controls are described in Table 1. No patient had acute rheumatic fever, and no patient with endocarditis was operated on during the acute phase.

Valve fragments were frozen in liquid nitrogen and kept at −80°C until used for protein extraction. The remaining fragments, when available, were either used for histological study (Control: n = 6; AS: n = 32; and AR: n = 9) or used for cell culture (Control: n = 2; AS: n = 9; and AR: n = 2), according to the quantity of remaining tissue after biochemical analysis.

Histological analysis
After fixation in a 4% buffered formaldehyde solution and embedding in paraffin, 5 μm sections were stained for histological examination using haematoxylin–phloxin–saffron, Masson’s trichrome, acid–afiavin–orcein, and Alcian Blue. Pathological findings (calcification, neovascularization, inflammatory infiltrates, etc.) and ECM alterations (disorganization of collagen bundles, increased loose connective tissue [myxoid tissue], fragmentation or stratification of elastic fibres) were evaluated using a semi-quantitative analysis performed by two independent investigators without knowledge of the origin of the tissues. For this purpose, three sections of each valve were observed at low magnification and divided into four to five fields. Each parameter was graded 0–3 per field and expressed as the mean value per valve.

Biochemical analysis

Protein extraction
Valve leaflet fragments were homogenized in a 2 mol/L guanidinium chloride solution containing 0.01 mol/L CaCl₂, 0.2% Triton X-100, and 0.05 mol/L Tris–HCl, pH 7.5 (3 mL/g wet weight) using a Polytron® as previously described. After centrifugation (10 000 g, 25 min, and 4°C), supernatants were dialyzed at 4°C during 48 h (Spectrapor membrane 6–8000 MWCO). The dialyzed extracts were centrifuged (10 000 g, 25 min, and 4°C) and protein concentration was determined by the Bradford assay (Bio-Rad). Antibodies to MMP-2, -3, -7, and -9 (Chemicon) were used at the concentration of 1 g/mL and horseradish peroxidase IgG (Dako) was used after dilution (1/1000). Peroxidase activity was detected using a chemiluminescence reagent (NEN, Perkin Elmer).

ELISA
TIMP-1 and TIMP-2 levels in valve extracts were measured using an ELISA assay (Amersham). The analysis of correlations between the TIMP-1 and the TIMP-2 concentrations and their inhibitory capacities visualized on reverse gelatin zymography,7 was performed using 20 valve extracts.

Cell culture
Intersitial cells, obtained by the explant method, were cultured in smooth muscle basal medium 2 (Promocell®) supplemented with 10% fetal calf serum. Immunohistochemical analysis using antibodies against vimentin, smooth muscle α-actin, desmin, smooth muscle-myosin, and prolyl-4-hydroxylase was performed on cells seeded in Labtek wells and fixed in 3.7% parafomaldehyde. After two or three passages, cells were seeded in 1.9 cm² wells and cultured to confluence. After 48 h in serum-free medium (deprivation), cells were further incubated in serum-free medium in the absence or in the presence of tumour necrosis factor (TNF)-α and interleukin (IL)-1β (2 and 20 ng/mL). At the end of an additional 48 h incubation, culture media were collected, cells were rinsed with PBS and lysed in Tris–HCl 0.05 mol/L, Triton 0.1%, and EDTA 0.01 mol/L, pH 7.5. After the measurement of protein concentration, MMP activities were analysed using gelatin or casein zymography and TIMP concentrations by ELISA in cell culture media and cell extracts.

Table 1 Description of controls and patients

<table>
<thead>
<tr>
<th>Description of controls and patients</th>
<th>Control (n = 8)</th>
<th>AS (n = 49)</th>
<th>AR (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (males/females)</td>
<td>4/4</td>
<td>30/19</td>
<td>20/3</td>
</tr>
<tr>
<td>Age (years)a</td>
<td>42</td>
<td>72</td>
<td>54</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degenerative</td>
<td>31</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Rheumatic</td>
<td>9</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Bicuspid</td>
<td>9</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Healed endocarditis</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Aortic valve area² (cm²)</td>
<td>0.64</td>
<td>(0.50–0.85)</td>
<td></td>
</tr>
<tr>
<td>Regurgitation gradeb</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as number or median value (25th–75th percentile).

*Age differed among the three groups (P < 0.0001): patients with AS were older than controls (P < 0.05) and patients with AR (P < 0.05).

**These parameters were evaluated during Doppler echocardiography.

Statistical analysis
Data were expressed as median values (25th–75th percentile) unless stated. Comparison between the three groups of valves was performed using the non-parametric Kruskall–Wallis analysis of
Results

Histological analysis

Microscopic observations of control valves are shown in Figure 1. The eight control aortic valves showed the three characteristic layers: the fibrosa composed mainly of collagen bundles, the spongiosa which consists of a proteoglycan matrix, and the ventricularis which contains several well-organized elastic lamellae (Figure 1A and B).

Microscopic observations and semi-quantitative analysis showed that tissue and ECM abnormalities were more frequent in the two pathological groups when compared with controls (Table 2). Calcification, inflammatory cells, and capillaries were significantly increased in AS compared with controls. Calcification was significantly more abundant in AS than in AR. Numbers of inflammatory cells and capillaries were also higher in AS than in AR but the differences did not reach statistical significance. Bone metaplasia, spumous cells, and cholesterol crystals were only observed in certain AS valves (3/32, 3/32 and 12/32, respectively).

The incidence of alterations of the ECM, that is, the disorganization of collagen bundles and the fragmentation or stratification of elastic fibres was significantly higher in AS and in AR when compared with controls. The disorganization of collagen bundles in the fibrosa of AS was due to the presence of calcified nodules (Figure 1F and G), whereas it was the result of the infiltration of loose connective tissue between the collagen bundles in AR (Figure 1I). The main difference in the ECM structure between AS and AR valves was the quantity of loose connective tissue, the main component of the spongiosa, which was higher in AR than in AS.

Biochemical analysis

MMP activity

The activity of the gelatinase MMP-2 was not different between control and pathological valves (P = 0.42), whereas MMP-9 activity was significantly different between the three groups (P = 0.005) (Figure 2C and D). MMP-9 activity was higher in AS than in AR. MMP-9/MMP-2 ratio differed between the three groups (P = 0.007). It was significantly increased in AS compared with AR (Figure 2E). This MMP-9/MMP-2 ratio was higher in AS than in controls, the difference being of borderline significance.

The activities of MMP-3 and MMP-7 were low in control valves (Figure 2F and G) and differed among the three groups (P = 0.02 and 0.04, respectively). MMP-3 activity was significantly higher in AS than in controls and the increase in MMP-7 activity in AS compared with controls was almost significant. These two MMP activities were not significantly different between AS and AR.

TIMP concentration

TIMP-2 and TIMP-1 were detectable in both normal and pathological aortic valves. TIMP-2 concentration did not differ among the three groups (P = 0.70) (Figure 3A), whereas TIMP-1 did (P < 0.0001): it was significantly higher in both pathologies than that in controls and higher in AS compared with AR. The median value of TIMP-1 level was seven times higher in AS than in controls and four times higher in AR than in controls (Figure 3B). The inhibitory capacity of TIMP-1 and TIMP-2 has been measured using reverse gelatin zymography (Figure 3C). The correlation between the measurement of TIMP-1 inhibitory capacity and its protein concentration was significant (r = 0.49, P = 0.04); the non-significant correlation for TIMP-2 (r = 0.35, P = 0.21) is probably due to the lack of sensitivity of its detection on reverse gelatin zymography.

The MMP-2/TIMP-2 ratio did not differ between control and pathological aortic valves (P = 0.44) (Figure 3D). The MMP-9/TIMP-1 ratio significantly differed among the three groups (P = 0.025) and tended to be lower in AR and AS than in controls (Figure 3E). This was mainly due to the marked increase in TIMP-1 content of pathological aortic valves. This ratio was significantly lower in AR than in AS.

Cell culture

Immuno-histological studies showed that interstitial cells in aortic valves and in culture were similarly labelled for smooth muscle-α-actin and prolyl-4-hydroxylase (Figure 4). Cells stained also positively for vimentin and negatively for desmin and smooth muscle-myosin. Varval interstitial cell phenotype was identical in valvular tissue and cell culture and was characteristic of myofibroblasts (Figure 4).

Valvular interstitial cells constitutively expressed pro-MMP-2 at the basal state (Figures 2A and 5) and showed a biphasic response when they were exposed to TNF-α or IL-1β. Pro-MMP-2 activity was increased at the concentration of 2 ng/mL and decreased at 20 ng/mL; but only the variations of pro-MMP-2 activity in cells treated with IL-1β were significant (Figure 5). Neither MMP-9 nor MMPs-3 and -7 were detected in the culture medium and cell layer either in basal conditions or after the addition of IL-1β or TNF-α (Figures 2A, 2B, and 5).

The media of non-stimulated interstitial cells contained both TIMP-1 (140 ± 45 ng/10 μg cell proteins) and TIMP-2 (1.40 ± 0.45 ng/10 μg cell proteins). Only TIMP-2 was present in the cell layer (2.34 ± 0.52 ng/10 μg cell proteins). Treatment of cells with IL-1β and TNF-α did not modify TIMP-1 and TIMP-2 concentrations.

Discussion

Histological and biochemical analyses of AS and AR valves consistently showed that there is a pathological remodelling of valvular ECM which involves the MMP/TIMP system. The main tissue abnormalities were observed in AS and they were less severe (inflammatory cells and capillaries) or not detected (calcification) in AR. However, these two groups of pathological valves displayed common histological signs of ECM remodelling. Despite the marked histological differences, the MMP-2 activity was similar in control and pathological valves. The increases of MMP-9 and MMP-3 in the pathological valves provided further proof of their inflammatory state, which was much more severe in AS than in AR. Finally, there was an overexpression of TIMP-1 in pathological valves. TIMP-1 as well as TIMP-2 and MMP-2 were synthesized by the valvular interstitial cells in culture.
Figure 1  Histological observation of control and pathological valves. Normal (A and B), AS (C–G), and AR (H and I) valves were stained with haematoxylin–eosin–saffron (A, C–E, and H) to analyse the tissue architecture, or with Masson’s trichrome (B, F, G, and I) to analyse the ECM. The three characteristic layers of an aortic valve are the fibrosa (F), the spongiosa (S), and the ventricularis (V). Calcification (asterisk), bone metaplasia (closed circle), capillaries (c), and inflammatory cells (arrow) are mainly detectable in AS. Original magnification: C and F: (25×, A, B, D, E, and G–I: 100×).

Table 2  Histological characteristics of control and pathological valves

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>AS (n = 32)</th>
<th>AR (n = 9)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue abnormalities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibro-calcific areas</td>
<td>0 (0–0)</td>
<td>1.5 (1.0–2.0)(^a)</td>
<td>0 (0–0)(^b)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>0 (0–0)</td>
<td>0.29 (0–0.58)(^a)</td>
<td>0 (0–0.2)</td>
<td>0.02</td>
</tr>
<tr>
<td>Neocapillaries</td>
<td>0 (0–0)</td>
<td>0.5 (0–1.0)</td>
<td>0 (0–0.2)</td>
<td>0.006</td>
</tr>
<tr>
<td>ECM abnormalities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen bundle disorganization</td>
<td>0.29 (0–0.4)</td>
<td>2 (1.67–2.29)(^a)</td>
<td>1.4 (1.0–1.75)(^a)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Loose connective tissue</td>
<td>0.42 (0–0.8)</td>
<td>0 (0–0.33)</td>
<td>1.0 (0.4–1.75)(^a)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Collagen bundles in the ventricularis layer</td>
<td>0 (0–0.25)</td>
<td>0.13 (0–0.5)</td>
<td>0.6 (0.25–1.0)</td>
<td>0.05</td>
</tr>
<tr>
<td>Elastic fibre fragmentation</td>
<td>0 (0–0)</td>
<td>1.0 (0.42–1.58)(^a)</td>
<td>1.0 (0.5–2.0)(^a)</td>
<td>0.003</td>
</tr>
<tr>
<td>Elastic fibre stratification</td>
<td>0.25 (0–0.6)</td>
<td>1.13 (0.67–2.0)(^a)</td>
<td>1.4 (1.25–2.0)(^a)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Each tissue and ECM abnormality was quantified as described in Methods. Results are expressed as median value (25th–75th percentile). P-value refers to the global comparison of the three groups (Kruskall–Wallis test).
\(^a\)P < 0.05 when compared with control valves.
\(^b\)P < 0.05 when compared with AS valves.
Figure 2. MMP activities in control valves, pathological valves, and valvular interstitial cells. MMP-2 and MMP-9 activities were visualized by gelatin zymography (A) and quantified in valve extracts (C and D). Results are expressed as densitometric units per 10 µg of extracted proteins. The MMP-9/MMP-2 ratio (E) was further calculated. MMP-3 and MMP-7 activities were visualized by casein zymography (B) and quantified in valve extracts (F and G). Results are expressed as densitometric units per 10 µg of extracted proteins. On each graph, individual values as well as median values, 25th percentile and 75th percentile are presented for each group. MyoF, valvular myofibroblasts.
Histological analysis

At the tissue level, calcification is the most common pathological finding in AS. However, bone formation was observed in only three out of 32 AS valves. Inflammatory cell infiltration, mainly composed of plasmocytes, and the process of angiogenesis were also observed in AS. AR differed from AS mainly by the absence of calcification, the lower content of inflammatory cells and the decreased number of neo-capillaries. These results are consistent with previous findings.8–11

Normal heart valves have a complex layered architecture and highly specialized ECM. The ECM is synthesized by interstitial cells and its composition differs in the three layers. The collagen bundles in the fibrosa provide strength and stiffness to maintain coaptation during diastole. The loose, watery connective tissue of the spongiosa, which contains mainly proteoglycans, confers flexibility and plasticity to the cusp. Elastic fibres present in the ventricularis layer extend during diastole and spontaneously recoil during

---

**Figure 3** TIMP concentrations in control and pathological valves. TIMP-2 (A) and TIMP-1 (B) were measured by ELISA. Results are expressed as nanograms per 100 µg of extracted proteins. The inhibitory capacity of TIMP-1 and TIMP-2 was evaluated using reverse gelatin zymography (C). The MMP-2/TIMP-2 (D) and MMP-9/TIMP-1 (E) were further calculated. Results are expressed as arbitrary units. On each graph, individual values as well as median values, 25th percentile and 75th percentile are presented for each group.

---

**Figure 4** Phenotypic analysis of valvular interstitial cells in the aortic valve and in the culture. Immunodetection of smooth muscle-α-actin (A and B) and prolyl-4-hydroxylase (C and D) was carried out on tissue sections (A and C) and cells (B and D). Original magnification: ×200 and ×400 (insets).
systole. Only minor abnormalities of the ECM were observed in our control valves. The extent of disorganization of collagen bundles, the fragmentation and stratification of elastic fibres were significantly increased in AS and AR when compared with controls, but were similar in AS and AR, except the loose connective tissue content which was greater in AR.

Thus, although the pattern of histological abnormalities were different in AR when compared with AS, they shared some common basic ECM abnormalities, raising our interest for the evaluation of the role of MMPs and TIMPs in this process.

Biochemical analysis

Previous studies demonstrated the presence of MMPs and TIMPs in control and pathological valves at the mRNA and at the protein level. However, our study is the first to report a quantitative and comparative analysis of MMPs (MMP-2, MMP-3, MMP-7, and MMP-9) and their tissue inhibitors (TIMP-1 and TIMP-2) in AR and AS human valves.

MMP activities and TIMP concentrations were normalized by the weight of extracted proteins, that is, on a cell protein basis as the ECM components, especially collagens and elastic fibres and calcium minerals are insoluble in the buffers used for extraction. On this cellular basis, the MMP-2 activity was not significantly different between control and pathological valves, that is, the mean MMP-2 quantity secreted per cell was similar in control and pathological valves. Conversely, other studies reported increased quantities of MMP-2 in AS when compared with controls. These apparent discrepancies can be partly explained by differences in study designs, in particular the expression of MMP-2 activity normalized by the weight of extracted proteins and the use of fresh homografts as controls in the present study.

In the present study, cell culture showed that MMP-2 was constitutively secreted by valvular interstitial cells in culture as it is secreted by other mesenchymal cells such as arterial smooth muscle cells. We showed that MMP-2 activity, secreted by valvular cells in culture, was influenced by the concentration of added inflammatory cytokines, as has been shown for arterial smooth muscle cells. Thus, the MMP-2 activity secreted by a cell may be increased or decreased in a pathological valve according to the local concentration of inflammatory cytokine. This could explained the absence of significant difference in the mean MMP-2 activity per microgram of extracted proteins between pathological and control valves.

Leucocytes (neutrophils, monocytes, and lymphocytes) synthesize small amounts of MMP-2, whereas valvular interstitial cells do not secrete MMP-9. This MMP-9 is concentrated in azurophil granules of neutrophils and is synthesized by macrophages. The MMP-9 activity was significantly increased in AS compared with AR valves and was higher in AS compared with controls, suggesting a higher degree of inflammation in AS when compared with AR. This is consistent with higher MMP-3 activity and also the significantly increased number of inflammatory cells observed on histological sections of AS. Therefore, in our study, consistent findings of histological and biochemical quantitative analyses suggest a possible link between inflammation and ECM remodelling in aortic valve diseases.

The contribution of the MMP activities to ECM remodelling cannot be investigated without the analysis of their specific inhibitors, the TIMPs. Our results showed that TIMP-1 was significantly increased and TIMP-2 was not modified in AS and AR valves compared with controls. TIMP-1 and TIMP-2 have been detected in azurophil granules of neutrophils and is synthesized by macrophages. The MMP-9 activity was significantly increased in AS compared with AR valves and was higher in AS compared with controls, suggesting a higher degree of inflammation in AS when compared with AR. This is consistent with higher MMP-3 activity and also the significantly increased number of inflammatory cells observed on histological sections of AS. Therefore, in our study, consistent findings of histological and biochemical quantitative analyses suggest a possible link between inflammation and ECM remodelling in aortic valve diseases.

The contribution of the MMP activities to ECM remodelling cannot be investigated without the analysis of their specific inhibitors, the TIMPs. Our results showed that TIMP-1 was significantly increased and TIMP-2 was not modified in AS and AR valves compared with controls. TIMP-1 and TIMP-2 have been detected in azurophil granules of neutrophils and is synthesized by macrophages. The MMP-9 activity was significantly increased in AS compared with AR valves and was higher in AS compared with controls, suggesting a higher degree of inflammation in AS when compared with AR. This is consistent with higher MMP-3 activity and also the significantly increased number of inflammatory cells observed on histological sections of AS. Therefore, in our study, consistent findings of histological and biochemical quantitative analyses suggest a possible link between inflammation and ECM remodelling in aortic valve diseases.
arteries. Whatever the inducer, the increased level of tissue was observed on histological sections of our pathological valves and hypoxia induces TIMP-1 in pulmonary arteries. Whatever the inducer, the increased level of TIMP-1 could limit the ECM remodelling in these end-stage pathological AS and AR valves. A similar increase in TIMP-1 levels has already been demonstrated in hypertensive arteries and varicose veins in comparison to control vessels, corresponding to a mesenchymal response to various stimuli (hypertension, hypoxia, etc.).

**Study limitations**

The number of control valves was small. This was the consequence of the deliberate choice to use fresh homografts as controls. The alternative of using autopsy samples as controls would enable age to be matched between pathologic and controls valves, but post-mortem hydrolysis of protein could impair the relevance of the quantitative analysis of MMPs and TIMPs. Another consequence of this choice is that patients with AS were older than both controls and patients with AR.

The analysis of the MMP and TIMP concentrations was performed on protein extracts of valves, resulting in the determination of mean activities and concentrations. Thus, although there was a significant increase in the mean TIMP-1 levels and mean TIMP-1/MMP-9 ratios in AS and AR valves, we cannot exclude that locally the MMP content may exceed the TIMP content, for example, when inflammatory cells are present. Furthermore, as capillaries are detectable in pathological valves, it cannot be excluded that some soluble plasma proteins are present in protein extracts.

This analysis of the ECM remodelling and its molecular effectors was performed in end-stage pathological aortic valves. This limitation is inherent to the analysis of surgically excised human valves. These findings cannot be extrapolated to the pathogenesis of valve disease at an earlier stage.

**Conclusions**

Our semi-quantitative histological analyses provide evidence of active tissue and ECM remodelling in AS and AR, and our biochemical analysis quantifies the implication of MMPs and TIMPs in this remodelling, which is more important in AS than in AR. The quantitative analysis provides new insights into the respective roles played by inflammatory injury and mesenchymal response in the pathogenesis of aortic valve disease. Consistent histological and biochemical findings further suggest a number of common features between the pathogenesis of aortic valve diseases and atherosclerosis. These common features could lead to further treatment strategies based on intensive control of risk factors and intervention in the inflammatory process.

**Acknowledgements**

This project was supported by INSERM (Institut de la Longévité) and a grant from the European Commission (TELASTAR, QLK6-CT-2001-00332). O.F. received a fellowship from the Fédération Française de Cardiologie. The authors thank Grazia Ferré, Patricia Joao Wa Khiflala, Laurence Le Men, and Isabelle Prevost for technical assistance, and Mary Osborne-Pellegrin for editorial expertise.

**References**


