A role for plasma transforming growth factor-β and matrix metalloproteinases in aortic aneurysm surveillance in Marfan syndrome?

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**ABSTRACT**

**Background:** We have previously shown that the angiotensin-converting enzyme (ACE) inhibitor perindopril reduced aortic diameter by 3–7 mm in Marfan syndrome (MFS) patients. Excessive signalling by the transforming growth factor-β (TGF-β) has been implicated in the development of aortic dilatation. We hypothesised that reduction in plasma TGF-β and matrix metalloproteinase (MMP) levels would correlate with reduction in plasma TGF-β and matrix metalloproteinase (MMP) levels.

**Methods:** 17 MFS patients (aged 15–55 (mean ± SD)) on standard β-blocker therapy were randomised to also receive perindopril (n = 10) or placebo (n = 7) for 24 weeks in a double blind study. Aortic root diameters were measured at four sites via transthoracic echocardiography. Venous blood samples were analysed for latent and active TGF-β, MMP-2 and MMP-3 levels.

**Results:** Perindopril significantly reduced aortic root diameters relative to placebo in both end-systole and end-diastole (by 6.0 ± 5.5 mm/m², p < 0.001). In addition, compared to placebo perindopril significantly reduced plasma TGF-β levels by 14.0 ± 4.5 ng/ml (p = 0.01), active TGF-β levels by 4 ± 1 ng/ml (p = 0.02), MMP-2 levels by 22 ± 1 ng/ml (p < 0.001), and MMP-3 levels by 5 ± 1 ng/ml (p < 0.001). There were moderately strong correlations between the pre/post intervention change in aortic diameters and the change in plasma TGF-β (r = 0.61–0.76, p = 0.001–0.04) and active TGF-β (r = 0.59–0.73, p = 0.002–0.02), MMP-2 (r = 0.49–0.75, p = 0.001–0.007), and MMP-3 plasma levels (r = 0.81–0.83, p < 0.0001).

**Conclusions:** Plasma TGF-β, MMP-2 and MMP-3 should be further explored in longitudinal trials as potential prognostic indicators of progression of aortic dilatation and response to therapy in MFS.

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1. Introduction

Marfan syndrome arises from a defect in the gene encoding the key extracellular matrix protein, fibrillin-1 [1]. It has long been thought that aortic root aneurysm in MFS is caused by deficiency in fibrillin-1, resulting in fragmentation of elastin and a weakened aorta which is prone to rupture. More recent understanding of the regulatory functions of microfibrils in the extracellular matrix suggests an alternative pathogenesis of MFS. In fibrillin-1 deficient mice, Dietz et al. [2] demonstrated that many of the clinical features of Marfan syndrome are due to abnormal levels of activation of transforming growth factor-β (TGF-β). TGF-β is a potent stimulator of inflammation, fibrosis, and activation of certain matrix metalloproteinases (MMPs).

This combination of structural microfibril matrix abnormalities, dysregulation of matrix homeostasis mediated by excess TGF-β, and abnormal cell–matrix interaction results in the phenotypic features of MFS [3]. Ongoing destruction of the elastic and collagen lamellae and medial degeneration lead to increased aortic stiffness, decreased distensibility and progressive aortic dilatation and rupture.

Aortic stiffness is elevated in patients with MFS contributing to aortic dilatation and rupture, the major cause of premature death in this population [4]. We have recently reported that the angiotensin-converting enzyme (ACE) inhibitor perindopril reduced arterial stiffness by 60% and aortic diameters by 3–7 mm in patients with MFS already receiving β-blocker therapy [5]. Generally, antihypertensive drugs reduce arterial stiffness passively through mean arterial pressure reduction; however, ACE inhibitors may have additional effects on arterial wall structure contributing to a reduction in arterial stiffness and diameter [6].

We hypothesised that the perindopril induced reduction in aortic diameter in our previous study [5] would correlate with reduction in plasma TGF-β and MMP plasma levels, particularly
Each sample was assayed twice and averaged. All samples were assayed in random order and in a blinded fashion. The co-efficient of variation between all duplicate assays was 5% and results were averaged to obtain a single value for each sample. Unique standard curves were constructed for each bead and sample analyte concentration determined. This approach delivers the sensitivity of ELISA.

2. Materials and methods

As previously described 17 patients (aged 33±5 years (mean±SD)) on standard β-blocker therapy were randomised to receive either perindopril (8 mg once daily, n=10) or matching placebo (n=7) for 24 weeks in a double blind study [5].

2.1. Standard echocardiography

Two-dimensional, M-mode, and Doppler echocardiograms were obtained with a commercially available cardiac ultrasound system, using a 2.5-MHZ transducer. Aortic root measurements were made in two-dimensional parasternal long-axis view at end-diastole (peak of R wave on electrocardiogram) at the level of the left ventricular outflow tract, sinuses of Valsalva, supra-aortic ridge, and proximal ascending aorta 1–2 cm above the supra-aortic ridge according to the method of Roman et al. [9]. All measurements were made using the leading edge technique on up to five cardiac cycles and averaged. The severity of aortic and mitral regurgitation was graded semi-quantitatively using colour Doppler jet area criteria [10]. Echocardiographic evidence of mitral valve prolapse was evaluated using established echocardiographic criteria [11]. A single sonographer blinded to the clinical data performed the echocardiograms and all echocardiograms were also assessed by two experienced cardiologists blinded to patient identification and treatment. Results are expressed relative to body surface area (BSA) calculated according to Roman et al. [9].

2.2. Biochemical analysis

Venous blood samples were drawn from each patient at baseline and following 24 weeks of therapy. Samples were subsequently analysed for active and latent TGF-β using enzyme-linked immunosorbent assay (ELISA, Pharmacia Max® ImmunoAssay System, Piscataway, NJ). Minimum assay sensitivity 32 pg/ml. A fluorokine MAP-Human MMP Base Kit (R&D Systems, USA) was used to measure MMPs using Luminex xMAP® platform (BioRad Laboratories, Inc., CA, USA); this assay consists of multiplexed sandwich ELISA for the quantitative measurement of MMP-2 (minimum assay sensitivity 38.9 pg/ml) and MMP-3 (minimum assay sensitivity 2.6 pg/ml). Each sample was assayed twice and averaged. All samples were assayed in random order and in a blinded fashion. The co-efficient of variation between all duplicate assays was 5% and results were averaged to obtain a single value for each sample. Unique standard curves were constructed for each bead and sample analyte concentration determined. This approach delivers the sensitivity of ELISA.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo group (baseline)</th>
<th>Placebo group (24 weeks)</th>
<th>Perindopril group (baseline)</th>
<th>Perindopril group (24 weeks)</th>
<th>ΔPlacebo group</th>
<th>ΔPerindopril group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVOT (mm²/m²)</td>
<td>20.2±1.3</td>
<td>20.8±1.4</td>
<td>20.7±0.8</td>
<td>19.3±0.8</td>
<td>0.6±0.2</td>
<td>−1.5±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sinuses of Valsalva (mm²/m²)</td>
<td>20.9±0.8</td>
<td>21.6±1.0</td>
<td>21.1±0.6</td>
<td>19.2±0.5</td>
<td>0.7±0.1</td>
<td>−1.8±0.2</td>
<td>&lt;0.001</td>
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<tr>
<td>Supra-aortic ridge (mm²/m²)</td>
<td>17.3±1.1</td>
<td>17.7±1.0</td>
<td>18.1±0.6</td>
<td>15.1±0.6</td>
<td>0.6±0.1</td>
<td>−3.0±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ascending aorta (mm²/m²)</td>
<td>19.0±0.8</td>
<td>19.4±0.6</td>
<td>18.8±0.5</td>
<td>17.5±0.5</td>
<td>0.4±0.2</td>
<td>−1.2±0.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviation: LVOT, left ventricular outflow tract. Aortic diameters are indexed to body surface area. All parameters are mean±SEM.

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Latent TGF-β (ng/ml)</th>
<th>Active TGF-β (ng/ml)</th>
<th>MMP-2 (ng/ml)</th>
<th>MMP-3 (ng/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>p value</td>
<td>r value</td>
<td>p value</td>
</tr>
<tr>
<td>ΔLVOT (mm²/m²)</td>
<td>0.49</td>
<td>0.04</td>
<td>0.74</td>
<td>0.002</td>
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<tr>
<td>ΔSinuses of Valsalva (mm²/m²)</td>
<td>0.76</td>
<td>&lt;0.0001</td>
<td>0.59</td>
<td>0.02</td>
</tr>
<tr>
<td>ΔSupra-aortic ridge (mm²/m²)</td>
<td>0.51</td>
<td>0.03</td>
<td>0.73</td>
<td>0.002</td>
</tr>
<tr>
<td>ΔAscending Aorta (mm²/m²)</td>
<td>0.51</td>
<td>0.03</td>
<td>0.66</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Abbreviations: LVOT, left ventricular outflow tract; TGF-β, transforming growth factor-β; MMP, matrix metalloproteinase. Aortic diameters are indexed to body surface area.
measured at all sites and the changes in both latent and active TGF-β, MMP-2 and MMP-3 plasma levels (Table 2), with v values ranging from 0.49 to 0.83.

4. Discussion

We demonstrate a highly significant association between reduction in aortic diameter in patients with MFS and reduction in plasma TGF-β and MMP plasma levels, particularly MMP-2 and MMP-3. These correlations further highlight the pivotal role of TGF-β in the pathogenesis of MFS.

Several recent molecular studies have challenged the paradigm that MFS results exclusively from the production of abnormal fibrillin-1 [12]. These studies have recognized that the extracellular matrix proteins play a crucial role in the regulation of cytokine bioavailability in the vascular system, specifically the release of vascular endothelial growth factor and TGF-β [13]. TGF-β is secreted as a large latent complex (LLC; composed of TGF-β, latency-associated peptide and latent TGF-β binding protein), which is sequestered by the extracellular matrix [14]. Fibrillin-1 shares homology with TGF-β binding proteins, and the TGF-β latent complex specifically binds to fibrillin-1 domains [15]. TGF-β signalling requires release of the mature TGF-β embedded in the extracellular matrix and basement membranes by proteases or MMPs in a tightly controlled manner to allow interaction with its receptor complexes, and initiation of downstream signalling cascades [16].

These findings led to the hypothesis that fibrillin-1 may participate in the regulation of TGF-β signalling. Indeed, Neptune et al. [17] demonstrated that a reduced amount of fibrillin-1 may result in deficiency of the LLC sequestration and enhanced TGF-β signalling, resulting in many manifestations of MFS.

TGF-β is also an up-stream mediator of matrix metabolism and MMPs are known to play a crucial role in aneurysm progression [18]. In the current study we concentrated specifically on MMP-2 and MMP-3 which are up-regulated by TGF-β in MFS [19]. The role of MMP-9 in aortic aneurysm progression remains controversial. Although previous studies of aneurysm tissue from patients with MFS [8,19] and animal models [20] suggest that the expression of MMP-9 also plays a role in MFS, current studies indicate that levels of circulating MMP-9 have no clinical use in aortic aneurysm surveillance [21]. MMP-9 plasma levels were not measured in the current study.

Furthermore, TGF-β also mediates the effects of angiotensin II on extracellular matrix modelling and vascular fibrosis. In MFS, angiotensin II is elevated [22] and signalling through both the angiotensin type 1 receptor (AT1) and angiotensin type 2 (AT2) receptor pathways contributes to aortic degeneration. In addition, AT2 receptor downregulation is associated with cystic medial degeneration in MFS and contributes to aortic rupture [22]. Nagashima et al. also demonstrated that an ACE inhibitor but not an AT1 receptor blocker prevented cystic medial degeneration and aortic dissection in mice fed β-aminopropionitrile monofumarate, which induces dissection.

In 2007 our group demonstrated that perindopril therapy for 24 weeks reduced aortic diameters (relative to placebo) in both systole and diastole in patients with MFS on standard β-blocker therapy possibly through attenuation of TGF-β signalling, further highlighting the pivotal role this cytokine plays in the pathogenesis of MFS [5]. The present data further support this notion, through demonstration of moderately strong correlations between reduction in aortic diameters and reduction in both latent and active plasma TGF-β levels as well as MMP-2 and MMP-3 after perindopril therapy for 24 weeks.

Although, the current study was limited due to its small size and relatively short duration, it provides a valid basis for further larger scale clinical trials. Specifically, the relationships demonstrated raise the need for further longitudinal, non-interventional trials investigating levels of TGF-β and MMPs in MFS patients and how these relate to aortic diameter at follow-up.

In conclusion, future therapy directed at the TGF-β axis may ultimately prove effective at preventing the aortic complications of Marfan syndrome. Given the emerging role of TGF-β in abdominal aortic aneurysms [25], Duchenne muscular dystrophy, organ fibrosis, and cancer progression [13], this marker may also have broader application.

5. Conflicts of interest

None.

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References