Marfan syndrome: Getting to the root of the problem
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No beneficial effect of general and specific anti-inflammatory therapies on aortic dilatation in Marfan mice.


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Abstract

Aims: Patients with Marfan syndrome have an increased risk of life-threatening aortic complications, mostly preceded by aortic dilation. In the FBN1C1039G/+ Marfan mouse model, losartan decreases aortic root dilation. We recently confirmed this beneficial effect of losartan in adult patients with Marfan syndrome. The straightforward translation of this mouse model to man is reassuring to test novel treatment strategies. A number of studies have shown signs of inflammation in aortic tissue of Marfan patients. This study examined the efficacy of anti-inflammatory therapies in attenuating aortic root dilation in Marfan syndrome and compared effects to the main preventative agent, losartan.

Methods and Results: To inhibit inflammation in FBN1C1039G/+ Marfan mice, we treated the mice with losartan (angiotensin II receptor type 1 inhibitor), methylprednisolone (corticosteroid) or abatacept (T-cell-specific inhibitor). Treatment was initiated in adult Marfan mice with already existing aortic root dilation, and applied for eight weeks. Methylprednisolone- or abatacept-treated mice did not reveal a reduction in aortic root dilation. In this short time frame, losartan was the only treatment that significantly reduced aorta inflammation, transforming growth factor-beta (TGF-β) signaling and aortic root dilation rate in these adult Marfan mice. Moreover, the methylprednisolone-treated mice had significantly more aortic alcian blue staining as a marker for aortic damage.

Conclusion: Anti-inflammatory agents do not reduce the aortic dilation rate in Marfan mice, but possibly increase aortic damage. Currently, the most promising therapeutic drug in Marfan syndrome is losartan, by blocking the angiotensin II receptor type 1 and thereby inhibiting pSmad2 signaling.
Chapter 11: Anti-inflammatory therapies in Marfan mice

Introduction

Marfan syndrome is a monogenic connective tissue disorder, caused by mutations in the gene encoding fibrillin-1 (*FBN1*).\(^1\) The major feature of Marfan syndrome is development of aortic aneurysms, especially of the aortic root, which subsequently may lead to aortic dissection and sudden death.\(^2\)\(^-\)\(^6\)

In a well-known Marfan mouse model with a cysteine substitution in *FBN1* (C1039G), losartan effectively inhibits aortic root dilation by blocking the angiotensin II type 1 receptor (AT1R), and thereby the downstream production of transforming growth factor (TGF)-β.\(^7\) The destructive role for TGF-β was confirmed since neutralizing TGF-β antibodies inhibited aortic root dilation in Marfan mice and inhibited the activation of TGF-β-downstream transcription factor Smad2.\(^7\) Increased Smad2 activation is usually observed in human Marfan aortic tissue and considered crucial in the pathology of aortic degeneration.\(^8\) Even though the response to losartan was highly variable, we recently confirmed the overall beneficial effect of losartan on aortic dilation in a cohort of 233 human adult Marfan patients.\(^9\) The direct translation of this therapeutic approach from the Marfan mouse model to the clinic, exemplifies the extraordinary power of this mouse model to test novel treatment strategies, which are still necessary to achieve optimal personalized care.

In aortic tissue of Marfan patients, inflammation is observed, which may contribute to aortic aneurysm formation and is the focus of the current study. In the *FBN1* hypomorphic mgR Marfan mouse model, macrophages infiltrate the medial smooth muscle cell layer followed by fragmentation of the elastic lamina and adventitial inflammation.\(^10\) Furthermore, fibrillin-1 and elastin fragments seem to induce macrophage chemotaxis through the elastin binding protein signaling pathway in mice and human Marfan aortic tissue.\(^11\)\(^-\)\(^12\) Increased numbers of CD3\(^+\) T-cells and CD68\(^+\) macrophages were observed in aortic aneurysm specimens of Marfan patients, and even higher numbers of these cell types were shown in aortic dissection samples of Marfan patients.\(^13\) In line with these data, we demonstrated increased cell counts of CD4\(^+\) T-helper cells and macrophages in the aortic media of Marfan patients and increased numbers of cytotoxic CD8\(^+\) T-cells in the adventitia, when compared to aortic root tissues of non-Marfan patients.\(^14\) In addition, we showed that increased expression of class II major histocompatibility complex (MHC-II) genes, *HLA-DRB1* and *HLA-DRB5*, correlated to aortic root dilation in Marfan patients.\(^14\) Moreover, we found that patients with progressive aortic disease had increased serum concentrations of Macrophage Colony Stimulating Factor.\(^14\)

All these findings suggest a role for inflammation in the pathophysiology of aortic aneurysm formation in Marfan syndrome. However, it is still unclear whether these inflammatory reactions are the cause or the consequence of aortic disease. To interfere with inflammation, we studied three anti-inflammatory drugs in adult *FBN1*\(^{C1039G+/+}\)
Marfan mice. Losartan is known to have AT1R-dependent anti-inflammatory effects on the vessel wall, and has proven effectiveness on aortic root dilation upon long term treatment in this Marfan mouse model. Besides losartan, we will investigate the effectiveness of two anti-inflammatory agents that have never been applied in Marfan mice, namely the immunosuppressive corticosteroid methylprednisolone and T-cell activation blocker abatacept. Methylprednisolone preferentially binds to the ubiquitously expressed glucocorticoid receptor, a nuclear receptor, modifying inflammatory gene transcription. Abatacept is a CTLA4-Ig fusion protein that selectively binds T-cells to block CD28-CD80/86 co-stimulatory activation by MHC-II positive dendritic cells and macrophages.

In this study, we investigate the effect of these three anti-inflammatory agents on the aortic root dilation rate, the inflammatory response in the aortic vessel wall, and Smad2 activation in adult Marfan mice.

Methods

Animal and study design

*FBN1*<sup>C1039G/+</sup> Marfan mice have a C57Bl6J background and are maintained as a heterozygous breeding colony in our animal facility. For breeding we used wild-type females and Marfan males to prevent death of Marfan females during pregnancy and labour. The mice included in the treatment groups were an equal mix between males and females. Polymerase chain reaction (PCR) was used to identify Marfan mice and wild-type littermates. Mice were housed in a temperature-controlled environment with 12 hour light/dark cycles and had access to food and water ad libitum. All animal protocols were approved by the Institutional Animal Welfare Committee of the Academic Medical Centre Amsterdam in the Netherlands.

Treatment was started at 8 weeks of age and was continued for 8 weeks. There was no difference in weight between Marfan and wild-type mice (males and females together and equal distribution per group; 32 ± 19 gram versus 28 ± 19 gram, respectively, p=0.243). Treatment dosage in the losartan group was 0.6 g/L orally given in drinking water, which was used in previous studies. The two novel anti-inflammatory treatment groups received methylprednisolone 12 mg/kg or abatacept 10 mg/kg based on equal dosage in humans and previously documented dosages in mice. The mice were injected three times a week by intraperitoneal (i.p.) injections of 300 μL each time. Placebo-treated Marfan mice were 1) injected i.p., three times a week with saline or 2) were not treated at all. There was no difference between the two Marfan placebo groups on aortic dilation, medial area and elastic lamina breaks and therefore the groups were pooled. All groups contained n=11 mice per group, except the Marfan placebo group,
which consisted of n=12 mice. At the end of the treatment period, the mice were sacrificed by an overdose of ketamine/xyazine anaesthesia. Subsequently, the mice were slowly perfused with phosphate-buffered saline (PBS; 1 min) and fixative (1:5 diluted Shandon Formal-Fixx (Thermo Scientific); 1 min), through the heart. As a reference for baseline aortic dimensions and to be able to calculate the aortic root dilation rate, wild-type and Marfan mice were sacrificed at 8 weeks of age.

**Histology and Immunohistochemistry**

Specimens of mouse hearts, containing the aortic root and part of the ascending aorta, were stored in fixative overnight at 4°C. Tissues were embedded in paraffin and then sectioned from the middle of the heart (around the mitral valves) towards the aortic arch into 7μm sections and used for histological analyses. A standardized reference point for aortic root diameter quantification was set at the first section of the aortic root where the valve leaflets (or remnants) were not present any longer. To perform immunohistochemistry, consecutive sections were taken at this specific location.

Sections were stained with haematoxylin and eosin and were photographed (Leica Microsystems, QWin software). Image analysis software (Adobe Photoshop CS5) was used to measure the aortic wall thickness (medial area) and the aortic root perimeter (luminal circumference). The luminal aorta diameter was calculated from the perimeter. The cell nuclei were counted in two views with 200x amplification. To visualize the elastic fibres of the aortic wall, sections were stained with Lawson stain. The degree of fragmentation of the elastic fibres was examined by a pathologist (TR) blinded to the genotype and treatment group. The number of elastic lamina breaks was counted within the aortic media of each mouse. Alcian blue staining was performed to visualize acidic polysaccharide accumulation, such as glycosaminoglycans, at areas of aortic damage and quantified (corrected for medial area) with QWin software (Leica Microsystems). Nuclear Fast red was used as counterstain for nuclei.

Immunohistochemical examinations were carried out after deparaffinization and rehydration. Endogenous peroxidase activity was quenched by 20-minute incubation in 1% H₂O₂ and epitope retrieval was heat-induced for 10 minutes in citrate buffer pH6. Tissue sections were incubated overnight at 4°C with antibodies recognizing CD45 (clone 30 F-11, eBioscience), Mac3 (M3/84, Pharmingen) or pSmad2 (kindly provided by Peter ten Dijke). The sections were subsequently washed in tris buffered saline (TBS) and incubated with a rabbit anti-rat IgG secondary antibody (DAKO E0468) for 1h (for CD45). The sections were incubated for 30 minutes with a horseradish peroxidase (HRP) conjugated anti-rabbit-IgG polymer (BrightVision, ImmunoLogic) for CD45 and pSmad2 and with HRP-conjugated donkey anti-rat-antibody (Jackson Lab) for Mac3. After washing, antigen detection was performed by development with diaminobenzidine tetrachloride (DAB). The sections were then mounted in Pertex and analysed. The presence of
CD45, Mac3 and pSmad2 was quantified by QWin software and expressed as positive area corrected for the total aortic wall (expressed in arbitrary units (AU)), including the intima, media and adventitia. As negative control we used normal rabbit serum, diluted similarly as the pSmad2 antiserum, which revealed no nuclear staining (data not shown). Due to the limited number of sections at the specific aortic root location, we measured one section per mouse for each staining, n≥11 per group, by an investigator blinded to the treatment.

**Statistical analysis**

Statistical analysis was performed using the Kruskal–Wallis one-way analysis of variance. When the Kruskal-Wallis test leads to significant results, the two-sided Mann-Whitney U test was performed. The Spearman's rank correlation was used for the correlation between CD45 or Mac3 and aortic dilation rate. Data are presented as median ± range. The CD45, Mac3, alcian blue and pSmad2 measurements are plotted on log scale to improve the comparison, the horizontal lines reflect the median, and the vertical lines reflect the minimum and maximum measured values. Since we compared two novel treatment groups, p<0.025 was considered statistically significant. Data analysis was performed using the SPSS statistical package (19.0 for Windows; SPSS Inc., Chicago, Illinois, USA).

**Results**

**Increased inflammation in FBN1<sup>C1039G/+</sup> Marfan mouse aortic root**

To evaluate the presence of inflammation in the FBN1<sup>C1039G/+</sup> Marfan mouse model, we quantified the presence of leukocyte and macrophage migration into the medial and adventitial layer of the aortic wall (Figures 1 and S1). Leukocyte migration (CD45) into the aortic wall was significantly increased in the Marfan placebo group as compared to wild-type mice (2.4 ± 10 versus 0.8 ± 1, p<0.001, Figure 1A). Macrophages influx (Mac3) is considered detrimental to vascular integrity and these inflammatory cells were thus specifically analysed. Significantly more macrophages were present in the vessel wall of the Marfan placebo mice as compared to the wild-type mice (1.9 ± 11 versus 0.9 ± 3, p=0.003, Figure 1B).

**Aortic histology upon anti-inflammatory treatment**

Leukocyte migration (CD45) into the aortic wall was significantly reduced by losartan (1.1 ± 2, p=0.004). Methylprednisolone (1.4 ± 3, p=0.050) and abatacept (1.6 ± 2, p=0.149), did not reduce leukocyte infiltration significantly, when compared to Marfan placebo mice (Figure 1A), although methylprednisolone showed a trend. However, macrophage influx was significantly reduced by losartan (0.6 ± 5, p=0.022), methylprednisolone (1.0
as well as by abatacept (1.0 ± 2, p=0.010) (Figure 1B). Thus, the two novel anti-inflammatory treatment strategies predominantly reduce macrophage influx into the vessel wall.

As a measure of changed morphology of the vessel wall, we determined the thickness of the smooth muscle cell containing medial layer of the aortic root (Figure 2A). The area of the aortic media was significantly increased in Marfan mice, compared to wild-type mice (0.32 ± 0.1 versus 0.24 ± 0.1 mm², p=0.004), which was not changed by losartan (0.30 ± 0.1 mm², p=0.767). Abatacept did not show a difference (0.36 ± 0.2 mm², p=0.148), while methylprednisolone showed a trend towards an increased thickness of the medial layer (0.35 ± 0.4 mm², p=0.066). The number of smooth muscle cells (cell nuclei) were similar between untreated Marfan mice (227 ± 19 cell nuclei) and methylprednisolone- (220 ± 28 cell nuclei, p=0.674) or abatacept-treated Marfan mice (231 ± 28 cell nuclei, p=0.786).

The degree of elastic lamina breaks in the medial layer is a measure of vascular damage and was compared between treatment groups. Placebo-treated Marfan mice showed a significant increase in elastic lamina breaks (Marfan: 12 ± 20 versus wild-type: 3 ± 9, p<0.001, Figure 2B). None of the treatment groups preserved the vascular integrity by decreasing the number of elastic lamina breaks within the medial layer. However, methylprednisolone showed a trend towards increased number of elastic lamina breaks (25 ± 31, p=0.076).

In Marfan patients, it is known that alcian blue staining detects areas of cystic medial necrosis. At sites of smooth muscle cell death and elastic lamina breaks, acidic polysaccharides such as glycosaminoglycans (GAG) accumulate. Therefore, alcian blue staining is performed to visualize the medial necrosis in the various Marfan treatment groups.
Interestingly, the methylprednisolone group showed a significant increase in alcian blue staining as compared to the Marfan placebo-treated mice \( (p=0.010) \), and abatacept revealed a trend in increased GAG accumulation \( (p=0.066) \), suggesting that these anti-inflammatory treatment strategies are potentially harmful. In conclusion, all anti-inflammatory treatment groups, including losartan, revealed decreased macrophages within the aortic wall, but none of these drugs improved aorta morphology in this short time frame. Methylprednisolone-treated mice seemed to have even more aortic damage.

Losartan inhibits the aortic dilation rate, which is not affected by the other drugs
To study whether all three anti-inflammatory drugs used in this study have an effect on aortic root dilation in Marfan syndrome, we measured the aortic root diameters in tissue

![Figure 2](image)

**Figure 2.** Aortic wall thickness, elastin breaks and GAG accumulation. (A) The area of the aortic media of placebo-treated Marfan mice was significantly thickened compared to wall thickness in wildtype mice. Methylprednisolone showed a trend towards enhanced thickening of the aortic media in Marfan mice \( (* \ p=0.066) \). (B) There were significantly more elastic lamina breaks in the aortic wall of Marfan mice compared to wildtype mice. Methylprednisolone revealed a trend towards enhanced elastic lamina breaks in the aortic media in Marfan mice \( (* \ p=0.076) \). (C) There was enhanced alcian blue positive area in the aortic media of methylprednisolone-treated mice, as compared to Marfan placebo mice, as a marker for medial necrosis. Abatacept showed a trend towards increased GAG accumulation as visualized by alcian blue \( (* \ p=0.066) \). (D) Alcian blue staining (blue) is present in the media (black line) in placebo-treated Marfan mice, yet it is more pronounced in the Methylprednisolone-treated aortic root. Pink stain=cytoplasm, red dots=nuclei, A=adventitia, L=lumen
sections. Losartan showed a protective effect on aortic root dilation when treatment started at 6 weeks of age and persisted during 6.5 months. We started treatment in adult mice at 8 weeks of age. The Marfan mice then already showed a significant increase in aortic root diameter when compared to wild-type littersmates (0.62 mm ± 0.09 versus 0.55 mm ± 0.10, p=0.007). After a treatment period of only 8 weeks, the aortic root diameter was dilated more pronounced in placebo-treated Marfan mice compared to the diameter of wild-type mice (1.15 mm ± 0.21 versus 0.98 mm ± 0.27, respectively, p<0.001). Losartan could significantly attenuate aortic root diameter enlargement in this short time frame in Marfan mice (1.09 mm ± 0.23, p=0.023). However, methylprednisolone (1.15 mm ± 0.37, p=0.898) and abatacept (1.21 mm ± 0.46, p=0.847) did not inhibit aortic root dilation.

We calculated the aortic root dilation rate by using the aortic root diameters of wild-type and Marfan mice that were sacrificed at the age of 8 weeks old (initiation of treatment) and 16 weeks old (termination of treatment). Placebo-treated Marfan mice demonstrated a significantly increased aortic root dilation rate, when compared to wild-type mice (+0.52 ± 0.24 mm/2 months versus +0.43 ± 0.25 mm/2 months, p=0.004, Figure 3). Losartan was again the only drug that inhibited the aortic root dilation rate significantly (+0.47 ± 0.25, p=0.025). Methylprednisolone and abatacept did not show any significant change in the aortic root dilation rate when compared to placebo-treated Marfan mice (+0.55 ± 0.34, p=0.848 and +0.58 ± 0.43, p=0.876, respectively).

For the correlation between inflammation and aortic root diameter / aortic root dilation rate we included each individual mouse of this experiment. As expected from earlier observations in human Marfan patients and the mgR Marfan mice, the number of leukocytes in the vessel wall (CD45) correlates with aortic root diameter (r=0.563, p<0.001), and with aortic root dilation rate (r=0.405, p=0.003). The number of infiltrated

![Figure 3](image)

**Figure 3.** Aortic dilatation in Marfan mice reduced by losartan. The aortic root dilatation rate was determined. Placebo-treated Marfan mice had a significantly higher dilatation rate compared to wildtype mice. Losartan attenuated the aortic root dilatation rate in Marfan mice significantly, whereas the other treatment strategies did not change the aortic root dilatation rate compared to placebo-treated Marfan mice.
macrophages (Mac3) correlates with aortic root diameter ($r=0.304$, $p=0.012$), but surprisingly not with aortic root dilation rate ($r=0.185$, $p=0.177$).

**Aortic Smad2 signaling**

AT1R and TGF-β signaling are considered detrimental in Marfan syndrome; therefore we also investigated activation of its downstream transcription factor Smad2 in the aortic root. We measured phosphorylated Smad2 (pSmad2) in the nucleus of aortic endothelial cells (intima), smooth muscle cells (media) and fibroblasts (adventitia) and inflammatory cells locally present. In placebo-treated Marfan mice, nuclear pSmad2 was increased compared to wild-type littermates ($4.0 \pm 11$ versus $2.8 \pm 10$, $p=0.022$, Figure 4A). Methylprednisolone or abatacept did not show a change in pSmad2 compared to placebo-treated Marfan mice ($6.2 \pm 9$, $p=0.511$ and $4.7 \pm 9$, $p=0.793$, respectively). Significantly, losartan decreased nuclear pSmad2 staining ($1.6 \pm 5$, $p=0.003$), which is almost absent in the smooth muscle cells (Figure 4B). In conclusion, where all three anti-inflammatory treatments responded equally in decreasing the macrophage influx into the aortic wall, a decrease in total leukocytes or pSmad2 was only observed in the losartan-treated mice. We hypothesize that a reduced macrophage influx alone interferes with extracellular matrix homeostasis, while additional suppression of leukocyte influx and pSmad2 signaling reduces aortic dilation (Figure 5).

**Figure 4.** Aortic SMAD2 signaling. (A) Phosphorylation of Smad2 (pSmad2) and localization in the nucleus of vascular cells in the aortic wall (positive area/total aortic wall area) is expressed in arbitrary units (AU). pSmad2 was significantly reduced by losartan treatment, as compared to placebo-treated Marfan mice. The other anti-inflammatory drugs did not affect the number of pSmad2-positive nuclei. (B) An example of pSmad2 staining in placebo-treated Marfan mice and reduced pSmad2 in losartan-treated Marfan mice. A=adventitia, L=lumen, line indicates media.

**Discussion**

In the present study we showed increased vascular inflammation in the aortic root of adult Marfan mice, which was significantly reduced by short term losartan treatment,
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We demonstrate that the increased inflammatory profile of the human Marfan aorta is also observed in the aortic vessel wall of adult $FBN1^{C1039G/+}$ Marfan mice. Therefore, we chose to intervene with the established general anti-inflammatory drug methylprednisolone which activates the glucocorticoid receptor that is protective in vascular disease, as summarized in a recent review.\(^{21}\) When treating Marfan mice with methylprednisolone, a significant decrease in macrophage influx was demonstrated. However, an increase in GAG accumulation was observed, while the aortic dilation rate remained the same. This indicates that glucocorticoids should not become the drug of choice to prevent aortic dilation in Marfan syndrome, especially when taking into consideration that these drugs have severe side effects in chronic use.

We previously revealed that MHC-II genes $HLA-DRB1$ and $HLA-DRB5$ correlate in Marfan patients with an increased aortic root dilation rate.\(^{14}\) Therefore, we chose to treat Marfan mice with abatacept, which blocks T-cell activation by MHC-II positive antigen presenting cells. Abatacept has been shown to effectively inhibit atherosclerosis in mice\(^{22}\) and to reduce renin-angiotensin-aldosterone (RAAS)-induced hypertension.\(^{23}\) In the aortic vessel wall, thus disturbing ECM homeostasis, which may be potentially harmful.

**Figure 5.** Proposed mechanism. Losartan is currently the only drug that effectively inhibits aortic root dilatation in mice and men, and specifically targets the angiotensin-II receptor type 1. Losartan clearly decreases TGF-β/pSmad2 signaling, decreases total leukocyte and macrophage influx into the vessel wall, and diminishes aortic root dilatation. TGF-β is known to polarize macrophages into a repair phenotype and at the same time induces collagen synthesis and matrix metalloproteinase activity to degrade extracellular matrix proteins (ECM). Methylprednisolone and abatacept decreased macrophage influx significantly, which resulted in increased GAG accumulation in the aortic vessel wall, thus disturbing ECM homeostasis, which may be potentially harmful.

accompanied by decreased nuclear pSmad2 in the vessel wall and prevention of aortic root dilation.
Marfan mice, abatacept treatment resulted in a decreased macrophage influx into the aorta, yet abatacept did not protect from aortic dilation.

An underestimated aspect of vascular inflammation is the variety in inflammatory responses. Vascular inflammation either promotes or repairs damage. Here, we observed an increased influx of inflammatory cells in Marfan placebo mice, and a clear correlation between leukocyte presence in the vessel wall and aortic dilation rate. Yet, a correlation between macrophages and aortic dilation rate was not significant, while methylprednisolone and abatacept predominantly reduced macrophage influx. Even though we did not further characterize the leukocyte populations, it seems that leukocytes, other than macrophages, may be detrimental in aortic dilation, while the macrophages may promote vascular repair in Marfan syndrome. In immunology, TGF-β (abundantly present in Marfan) is mostly known as an anti-inflammatory factor, promoting resolution of inflammation by skewing macrophages towards a protective “repair” phenotype.

The increased accumulation of GAG in the aortic media of methylprednisolone-treated mice, suggests that there is increased vascular damage upon use of this immunosuppressive drug, which may be harmful upon long term treatment. In line with these data, Lindeman et al. presented a case study in which a patient with an abdominal aortic aneurysm (AAA) had a sudden increase in aortic dilation rate (from 3.4 cm to 7.0 cm in 27 months) upon immunosuppressive therapy (combination therapy containing glucocorticoids) after kidney transplantation. In addition, in 18 patients with abdominal or thoracic aneurysms, the aneurysm dilation rate was increased from 0.46 cm/year before transplantation to 1.0 cm/year after transplant operation and the start of immunosuppressive drugs. Similarly, in the Blotchy mouse aneurysm model, aortic rupture occurred upon glucocorticoid treatment. So, based on these and our data, a similar phenomenon may occur in Marfan patients with existing aorta dilation, when using glucocorticoids. In general, the anti-inflammatory drugs did not contribute to the improvement of aorta pathology in Marfan mice, suggesting that the macrophage influx is rather a consequence of aortic damage than the cause of aortic dilation in Marfan syndrome. However, a beneficial effect of the anti-inflammatory drugs after longer treatment or in older Marfan mice with more severe aortic inflammation cannot be excluded.

In this 8-week treatment period in adult Marfan mice, losartan consistently reduced vascular inflammation, nuclear pSmad2 and most importantly aortic root dilation, despite lack of improvement in medial thickness or elastin breaks. Our treatment strategy could therefore be considered as a rapid screening approach for novel drugs in Marfan syndrome. Losartan is the first treatment targeting the underlying aortic pathophysiology in Marfan syndrome and is effective in reducing aortic dilation rate in patients and mice with Marfan syndrome. Losartan is an AT1R-blocker, which counteracts the effect of angiotensin II-mediated detrimental signaling cascades; including TGF-β production,
pSmad2 signaling, increasing blood pressure, reactive oxygen species generation, and induction of a pro-inflammatory response.\textsuperscript{31-33} Thus increased leukocytes (other than macrophages) and TGF-\(\beta/p\)Smad2 by angiotensin II-induced signaling seems to be the underlying devastating pathway of Marfan syndrome.\textsuperscript{34} Recently, a study has demonstrated epigenetic changes in the Smad2 promoter in vascular smooth muscle cells derived from human thoracic aneurysm tissue.\textsuperscript{35} This study highlights the important role of Smad2 and TGF-\(\beta\) in thoracic aortic aneurysms. In addition, mutations in the TGF-\(\beta\) receptor genes (TGFBR1 and TGFBR2) result in Marfan-like syndromes with aortic aneurysms and dissections as well, named ‘Loeys-Dietz Syndrome’.\textsuperscript{36}

Besides losartan therapy, doxycycline, an antibiotic with anti-inflammatory and matrix metalloproteinases (MMP) inhibition capacities,\textsuperscript{37} reduced aortic root dilation rate in two different mouse models of Marfan syndrome (\(FBN1^{C1039G/+}\) and \(FBN1^{m8r/m8r}\)).\textsuperscript{38-40} It has been suggested that doxycycline reduces aortic root dilation rate through the TGF-\(\beta\) and pSmad2 pathway.\textsuperscript{38-41} TGF-\(\beta\) stimulates the expression of MMP in vascular cells. Furthermore, MMP can activate TGF-\(\beta\) through proteolytic degradation of the latent TGF-\(\beta\) complex.\textsuperscript{42} In conclusion, doxycycline may reduce aortic dilation rate in Marfan mice by inhibiting TGF-\(\beta\)-induced MMP production and by inhibiting MMP-induced release of TGF-\(\beta\), rather than by reducing inflammation. However, in the only trial in patients with aneurysms, doxycycline presented an unexpected increase in aortic diameter of 0.8 mm after 18 months, when compared to the placebo AAA patients.\textsuperscript{43}

In conclusion, the use of anti-inflammatory drugs methylprednisolone and abatacept did not protect against progressive aortic root dilation in Marfan mice. So far, losartan is the only drug that can prevent aortic dilation in adult Marfan mice and patients. Inhibition of macrophage influx did not reduce the aortic diameter and aortic root dilation rate. Thus, macrophages do not seem to play a major role in promoting aortic pathology. Hence, inhibition of inflammation may be potentially harmful in Marfan patients. When long-term immunosuppressive therapy is needed in Marfan patients, the aorta should be carefully monitored for excessive dilation.

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