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The Effect of a Nonpeptide Angiotensin II Type 2 Receptor Agonist, Compound 21, on Aortic Aneurysm Growth in a Mouse Model of Marfan Syndrome

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Introduction: Available evidence suggests that the renin–angiotensin–aldosterone (RAA) system is a good target for medical intervention on aortic root dilatation in Marfan syndrome (MFS). The effect of Compound 21 (C21), a nonpeptide angiotensin II type 2 receptor agonist, on aneurysm progression was tested.

Methods: Mice with a mutation in fibrillin-1 ($Fbn1^{C1039G/+}$) and wild-type mice were treated with vehicle, losartan, C21, enalapril, or a combination. Blood pressure, aortic root diameter, and histological slides were evaluated.

Results: All groups had a comparable blood pressure. Echographic evaluation of the aortic root diameter revealed a protective effect of angiotensin II type 1 receptor antagonist (losartan) and no effect of C21 treatment. None of the treatments had a beneficial effect on the histological changes in MFS.

Discussion: This study confirms that angiotensin II type 1 receptor antagonism (losartan) decreases aortic aneurysm growth in a mouse model of MFS. A nonpeptide angiotensin II type 2 receptor agonist (C21), at the doses studied, was ineffective. Future studies are warranted to further elucidate the exact role of the RAA system in aneurysm formation in MFS and identify alternative targets for intervention.

Key Words: aneurysm, Marfan syndrome, angiotensin II, novel drug therapy

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INTRODUCTION

Marfan syndrome (MFS) is a multisystem connective tissue disorder characterized by cardiovascular, musculoskeletal, and ocular abnormalities. Most life-threatening for these patients are thoracic aortic aneurysm (TAA) and dissection. In the past decades, life expectancy of Marfan patients increased due to earlier diagnosis and proper surgical management of aortic disease.[1] The optimal medical therapy for these patients is still not clear. Nowadays, treatment focuses mainly on interfering with hemodynamics, using beta-blockers.[2]

Marfan patients have a defect in fibrillin-1.[3] This is associated with disturbances in transforming growth factor-beta (TGF-β) and angiotensin II (Ang II) signaling.[4] These processes are accompanied by fragmentation of elastin, excess of metalloproteinases, and infiltration of macrophages, causing an inappropriate remodeling within the extracellular matrix and aortic root dilatation.[5] Evidence suggests that Ang II signaling might be a good target for medical intervention. In murine models of MFS, the angiotensin II type 1 receptor (AT_1R) antagonist losartan has been shown to effectively slow down aortic growth in comparison with enalapril (ACE-I).[6] The net effect of an AT_1R antagonist is not clear because these agents increase not only Ang II[7] but also angiotensin II type 2 receptor (AT_2R) expression.[8] AT_2R is known to exert antiproliferative, anti-inflammatory, and cardioprotective effects that partly counterbalance the effects of AT_1R stimulation.[9–11] Moreover, the protective effect of AT_1R blocker losartan was lost in fibrillin-1–deficient mice with disruption of AT_2R gene.[12] Therefore, it is believed that losartan shunts Ang II signaling through the AT_2R, which seems to have a protective role in the development of TAA.

Because C21 is the first highly selective nonpeptide AT_2R agonist,[13] it might be a good therapeutic agent for the prevention of aortic aneurysm growth in MFS. The compound previously showed to have several beneficial cardiovascular effects. It reduces myocardial fibrosis and vascular injury,[14] promotes vasodilatation,[15] reduces infarct size,[16] and delays the development of aortic atherosclerosis.[17] The primary aim of this study was to investigate whether C21 decreases aneurysm growth in a mouse model of the MFS.

METHODS

Murine Model

Approval for the project was obtained from the Ethical Commission at the KULeuven (number: P060/2012).

Male mice heterozygous for a cysteine substitution in an epidermal growth factor–like domain of fibrillin-1 ($Fbn1^{C1039G/+}$) were used.[5,18,19] These mice develop

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pathological changes in the aortic wall and aneurysms of the ascending aorta. Wild-type (WT) littermates (C56BL/6J) were used as controls. At an age of 8 weeks, mice were randomly assigned to 1 of the 8 groups (n = 14 per group) (Figure 1). Thereafter, treatment was started and continued for 6 months. In case of early mortality, an autopsy excluded aortic causes of death. Intraperitoneal (IP) injection [C21 vs. NaCl (0.9%)] was performed daily. Other drugs (losartan and enalapril) were administered through drinking water, in doses which were used in previous studies.[12] Originally, these doses were chosen because they achieve a comparable hemodynamic effect.[20] Echographic evaluation and blood pressure measurements were performed at 0, 3, and 6 months of treatment. Thereafter, an overdose of pentobarbital killed the animals. Immediately following sacrifice, the right atrial appendix was opened. Through a puncture of the left ventricle, the animal was flushed with phosphate buffered saline (pH 7.4) at a pressure of 100 mm Hg for 5 minutes. The last minute the abdominal aorta was transected, and latex was injected at low pressure into the left ventricular apex until visible from the abdominal aorta. The mice were placed in 10% buffered formaldehyde. Twenty-four hours later, the heart and aorta were removed en bloc, the atria cut away, and images taken. The aortic root and ascending aorta were dissected from the heart and stored in 70% ethanol.

To evaluate the effect of C21 treatment at young age, 10-day-old mice were injected daily until the age of 8 weeks (Figure 1). The treatment group received C21 (0.5 $mg \cdot kg^{-1} \cdot d^{-1}$) and the control group NaCl. WT littermates (C56BL/6J) served as controls. At the age of 8 weeks, echography and euthanasia were performed using the methodology described above.

Blood Pressure

Blood pressure was measured using the CODA noninvasive blood pressure system (Kent Scientific Corporation, Torrington, CT).[21] In summary, the animals were restrained on the warming platform for 20 minutes for 3 consecutive days. On the fourth and fifth days, after 5 minutes of acclimatization, blood pressure measurements were performed consisting of 10 cycles 3 times. The measurements of the fifth day were used for data collection only if the percentage of accepted cycles was more than 80%.

Echographic Evaluation

The aortic root of the mice was imaged using a high-resolution transthoracic echography (Vevo 770; VisualSonics, Toronto, Canada), and a 40-MHz transducer. Nair hair removal cream was used at least 1 hour before echocardiography. The mice were sedated with isoflurane inhalation. The aorta was imaged in the parasternal long axis view. Three separate measurements of the maximal internal diameter at the level of the sinuses of Valsalva during systole were obtained by an observer blinded to genotype and treatment arm (JS).

Histology

Serial cross-sections (7 μm, 10 series) were made after paraffin embedding (Microm HM360; Thermo Scientific, Walldorf, Germany). Hematoxylin and eosin as well as Verhoeff–Van Gieson (VVG) staining were performed. Images of the ascending aorta were obtained at the level of the sinotubular junction at ×40 magnification using a Zeiss Axioplan microscope and an Axiocam MRc5 camera. Two blinded observers (D.V. and M.C.) performed measurements using ImageJ software and results were averaged. Media thickness, the average of 8 measurements (2 per quadrant), was obtained using hematoxylin and eosin–stained samples. VVG was used to evaluate wall architecture at 4 different locations, using an arbitrary scale based on the number of elastin breaks per cross-section. A "break" was defined as an isolated area where an elastic fiber was fragmented with interposed excessive connective tissue matrix. A scale of (1) indicated no breaks, (2) 1 to 2 breaks, (3) 3 to 4 breaks, (4) 5 to 6 breaks, and (5) 7 or more breaks.[12] Dividing the area of elastic fibers by the total area of the media determined the elastin fiber content.[22]

Plasma Concentration

Eight-week-old WT mice were administered NaCl (0.9%), normal dose C21 (0.5 $mg \cdot kg^{-1} \cdot d^{-1}$), or high dose

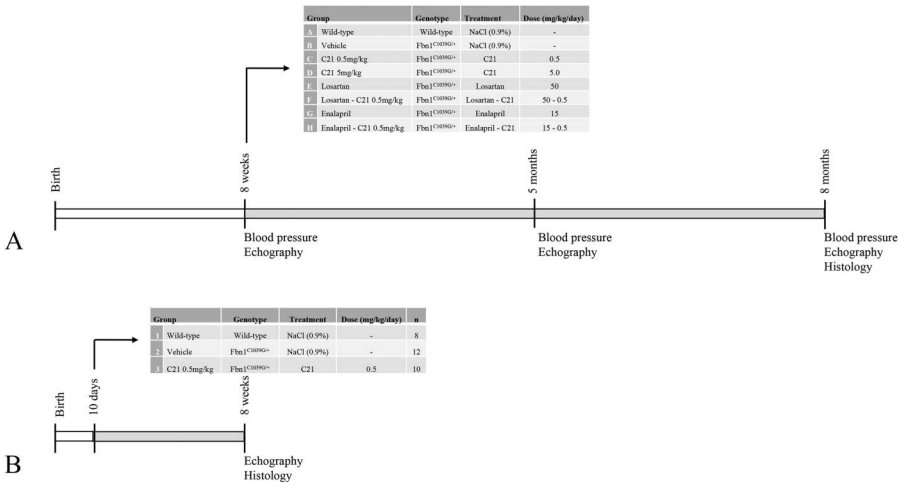

FIGURE 1. Protocol showing timing, initiation, and duration of therapy for the different groups, based on genotype and treatment. This for the long-term study (A) and the early-start study (B).

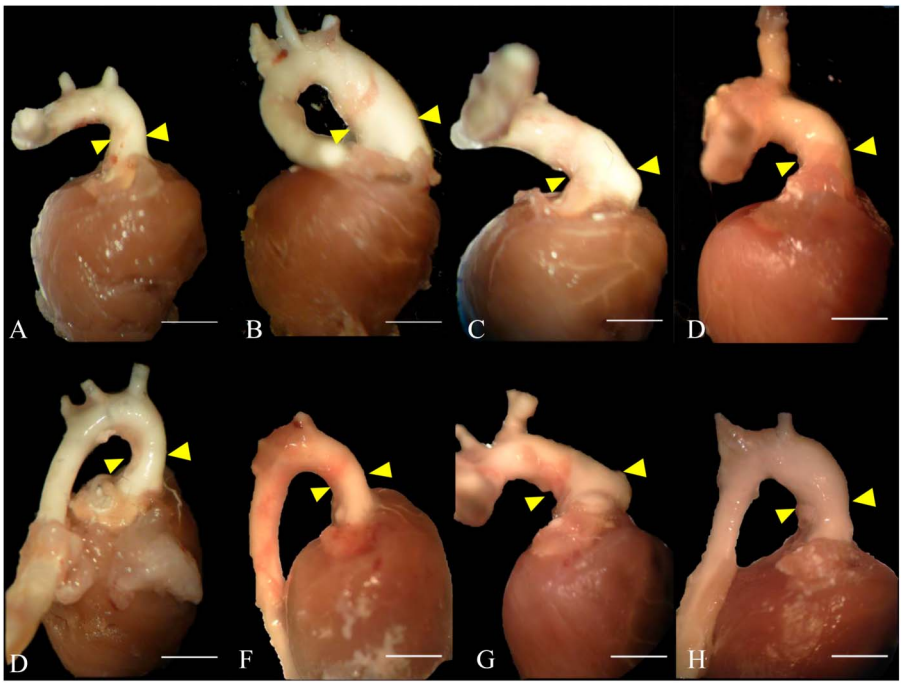

FIGURE 2. Gross inspection of ascending aortic dimension after latex injection in representative WT (A), and Fbn1$^{C1039G/+}$ mice treated with vehicle (B), C21 0.5 mg·kg^{-1}·d^{-1} (C), C21 5 mg·kg^{-1}·d^{-1} (D), losartan (E), losartan-C21 (F), enalapril (G), or enalapril-C21 (H). Arrowheads indicate the dimension of the ascending aorta (scale bar: 2 mm).

C21 (5.0 mg·kg^{-1}·d^{-1}) for 7 consecutive days intraperitoneally. Thereafter, the animals were euthanized 1, 6, 12, and 24 hours after the past injections (n = 3 per time and dose). Blood was sampled from the right atrium using ethylenediaminetetraacetic acid solution. The blood was centrifuged (2500*g* for 10 minutes). Plasma was stored at −80°C. Determination of the plasma concentration was provided by Vicore Pharma (Data on file; Vicore Pharma, Mölndal, Sweden). In general, C21 was extracted from mouse plasma by addition of acetonitrile and determined using ultra performance liquid chromatography. Based on the exponential best-fit line, plasma half-time was calculated.

Statistics

Statistical analysis was conducted using SPSS (IBM SPSS statistics; IBM Corporation, Armonk, NY). The Kolmogorov–Smirnov test evaluated normal distribution and the Levene's test evaluated the homogeneity of variance. One-way analysis of variance (ANOVA) with post hoc Scheffe's correction was used to compare mean values. A *P*-value of ≤0.05 was set for

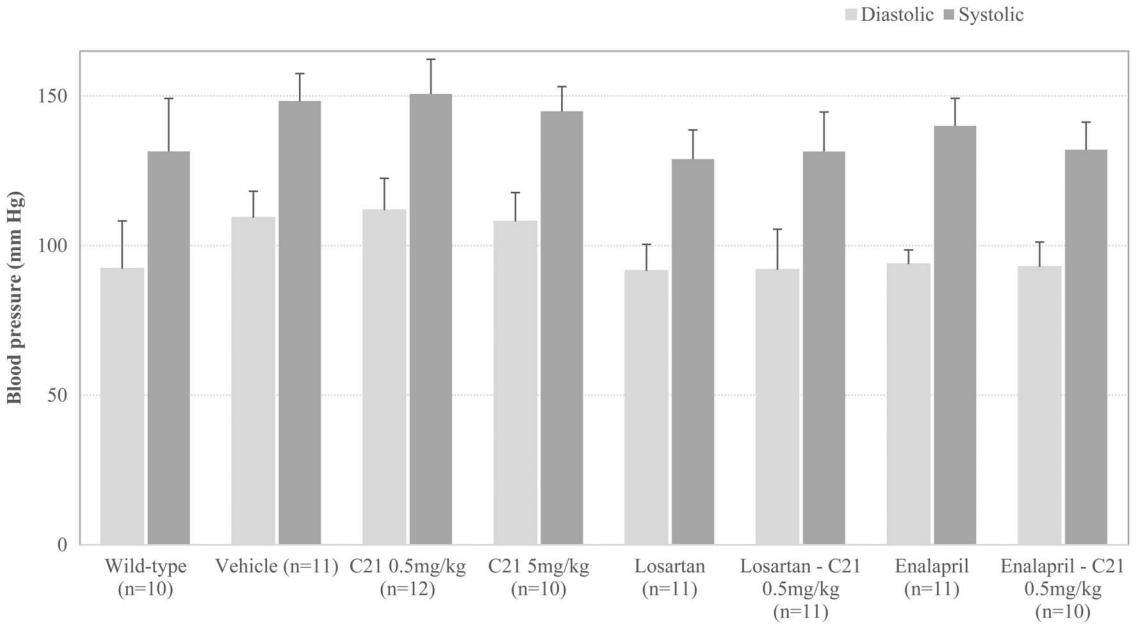

FIGURE 3. Average diastolic and systolic blood pressures (mean ± 2 SEM) after 6 months of treatment.

TABLE 1. Mean Aortic Sinus Diameter, 2 SEM, Number of Measured Animals per Group, and *P*-value Compared With the Start of the Experiment

	Month 0			Month 3				Month 6			
Group	**Mean**	**2 SEM**	**n**	**Mean**	**2 SEM**	**n**	***P* (M3-M0)**	**Mean**	**2 SEM**	**n**	***P* (M6-M0)**
(A) WT	1.68	0.08	12	1.76	0.06	12	0.240	1.73	0.08	12	0.567
(B) Vehicle	1.84	0.12	11	2.17	0.14	11	0.079	2.27	0.28	11	0.015
(C) C21 0.5 mg/kg	1.83	0.09	14	2.17	0.18	14	0.009	2.19	0.16	14	0.006
(D) C21 5 mg/kg	1.77	0.08	13	1.92	0.18	12	0.210	2.20	0.09	10	0.0002
(E) Losartan	1.85	0.09	13	1.85	0.11	11	0.995	1.99	0.10	10	0.172
(F) Losartan—C21 0.5 mg/kg	1.86	0.15	13	1.93	0.10	12	0.790	2.09	0.13	12	0.054
(G) Enalapril	1.90	0.06	14	1.89	0.12	14	0.988	2.23	0.14	12	0.0005
(H) Enalapril—C21 0.5 mg/kg	1.95	0.05	14	1.99	0.11	14	0.845	2.18	0.18	11	0.032

statistical significance. Data are presented as mean ± 2 SEM. Measurements were not corrected for weight of the mice because there were no differences between the groups after 3 and 6 months of treatment.

RESULTS

Murine Model

Ten mice (9%) died before the end of the experiment. Two in the WT group, the others were Fbn1$^{C1039G/+}$ distributed over different treatment groups (no significant difference). Autopsy revealed a retroperitoneal hematoma in one of the losartan-treated animals, but without dissection or dilatation of the thoracic aorta. In all other animals, there were no signs of aortic dissection or rupture. Figure 2 shows macroscopic images of the heart and ascending aorta of the different groups.

Blood Pressure

Diastolic and systolic blood pressures at baseline, after 3 months, and 6 months of treatment were comparable in all groups. Figure 3 shows the diastolic and systolic blood pressures at the end of the experiment.

Echographic Evaluation

There was a significant increase in aortic sinus diameter at 6 months in all groups except in WT and losartan-treated mice (Table 1). In the C21 0.5 mg/kg treated mice, the sinuses of Valsalva were already significantly larger after 3 months of treatment.

At the age of 8 weeks, the aortic root in mutant mice is larger than in WT mice: 1.69 ± 0.09 mm versus 1.909 ± 0.032, respectively ($P < 0.001$). After 3 months, the diameter in vehicle and C21 0.5 mg/kg treated Fbn1$^{C1039G/+}$ mice was already significantly larger than in WT mice (Figure 4). Another 3 months later, the diameter of the C21 5 mg/kg, enalapril, and enalapril-C21–treated mice was also significantly larger. At this point in time, there was no difference between WT and Fbn1$^{C1039G/+}$ mice treated with losartan, with or without C21 associated.

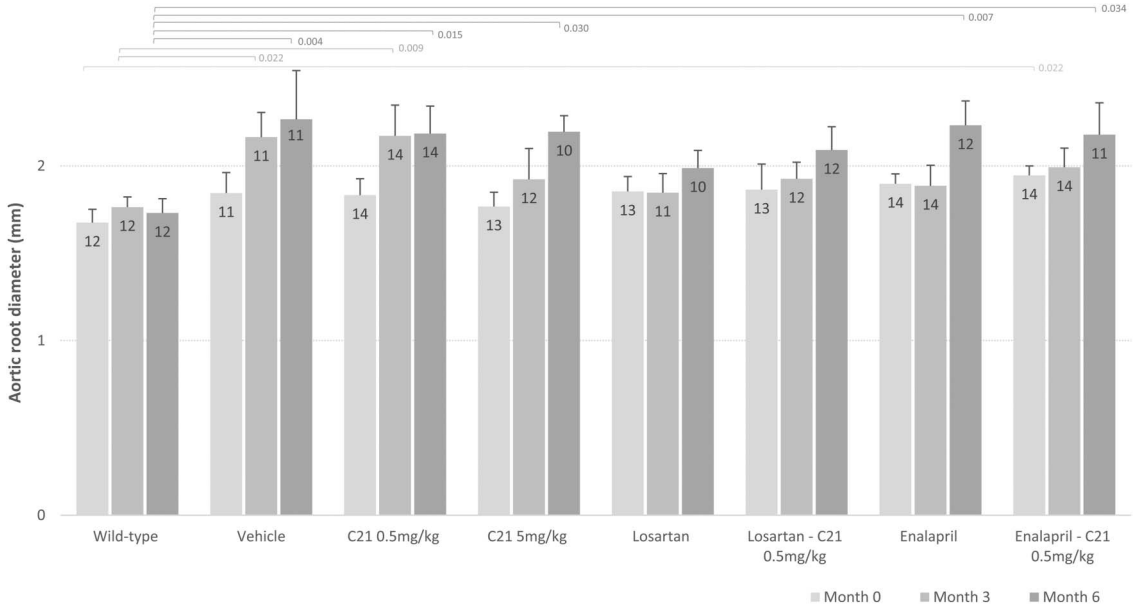

FIGURE 4. Average aortic root diameter (±2 SEM) at start, after 3 months, and 6 months of treatment. Number of animals at the top of each column. Horizontal line indicates significance (*P*-value at the end of each line).

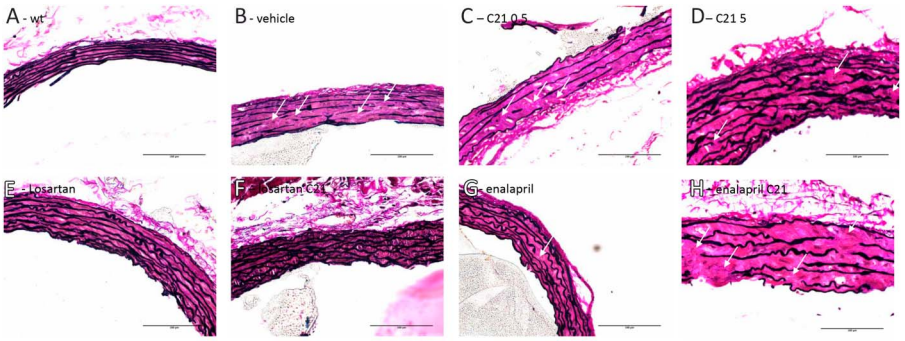

FIGURE 5. VVG stain reveals disruption of elastic fiber architecture and moderate thickening of the aortic media in Fbn1$^{C1039G/+}$ mice, especially in vehicle (B), C21 0.5 mg·kg^{-1}·d^{-1} (C), C21 5 mg·kg^{-1}·d^{-1} (D), enalapril (G), and enalapril-C21 (H) treated mice; relative to the normal elastic fiber architecture observed in WT mice (A), losartan (E), and losartan-C21 (F) mice (scale bar: 100 μm) (arrow: Elastin break).

Histology

A representative sample of VVG staining for each group is depicted in Figure 5. The media of the WT, losartan, and enalapril-treated mice were thinner although not significantly (Table 2). Quantification of the elastin fragmentation could not reveal any difference between the groups, nor the elastin fiber content.

Plasma Concentration

The plasma concentration of C21 in the control group (NaCl 0.9%) was 0 ng/mL. The plasma concentrations after injection of C21 is depicted in Figure 6. The plasma half-time is 2.5 and 2.2 hours for 0.5 and 5.0 mg·kg^{-1}·d^{-1}, respectively.

Early Start

At the age of 8 weeks, the echographic diameter of the aortic sinuses was significantly larger in the Fbn1$^{C1039G/+}$ mice independent of treatment (Table 3). Histological evaluation showed a significant increase in media thickness and elastin breaks in the mutated mice. The elastic fiber content was comparable in all groups.

DISCUSSION

It is believed that AT_1R is primarily involved in pathophysiological actions, whereas AT_2R signaling antagonizes many of the effects of AT_1R signaling.[23] Studies suggest that sartan-mediated effects occur at least in part because of the unopposed action of the AT_2R.[12,17,24] This highlights the potential of the AT_2R as a therapeutic target for blunting aortic aneurysm formation, the key idea of this study.

Serial blood pressure measurements were performed because blood pressure lowering has a beneficial effect on aortic aneurysm growth. There were no significant differences between the treatment groups in systolic, diastolic, or mean blood pressure at the start nor after 3 and 6 months of treatment. The administered doses of losartan and enalapril have a comparable blood pressure lowering effect, as described previously.[12,20] In this study, lower systolic and diastolic blood pressures, although not statistically significant, occurred in those 2 groups. Data on hemodynamic effects of C21 are controversial. Most of the ex vivo studies report vasodilatation and thus potentially a fall in blood pressure.[25,26] By contrast, as in this study, C21 did not decrease blood pressure in vivo.[14–16,27–31] Evaluation of the growth of the aortic root showed a significant increase in diameter in all groups except in the WT and losartan-treated animals, independently of C21 association. Comparing echographic measurements between groups at the age of 8 months confirmed that treatment with losartan was the only effective one. Evaluation of media thickness, architecture score, and elastic fiber content could not reveal any difference between groups. The reason for the lack of histological effect of any treatment, even losartan, is unclear. One of the principal findings of Habashi et al[6] was that losartan achieved full correction of the phenotypic abnormalities in the aortic wall of Fbn1$^{C1039G/+}$ mice. The age at the start, duration of the treatment, and sample size were comparable. In this study, cross-sections were taken at the level of the sinotubular junction and not the aortic root, this might attribute to differences in histological results. Another reason might be the difference in losartan intake. The objective was to have an intake of 50

TABLE 2. Average, 2 SEM, and Number (n) of Wall Thickness, Elastin Break, and Elastin Fiber Content After 6 Months of Treatment

	Wall Thickness (μm)			Elastin Break			Elastin Fiber Content (%)		
Group	Mean	2 SEM	n	Mean	2 SEM	n	Mean	2 SEM	n
WT	55.51	6.91	8	1.2	0.1	8	54.09	4.06	8
Vehicle	71.03	13.85	8	1.8	0.3	8	43.94	3.17	8
C21 0.5 mg/kg	80.41	8.16	10	1.9	0.4	12	49.49	5.59	12
C21 5 mg/kg	67.15	16.85	7	1.8	0.5	8	51.42	7.59	8
Losartan	57.18	7.61	7	1.9	0.3	8	45.66	4.47	8
Losartan—C21 0.5 mg/kg	78.07	20.84	5	1.9	0.9	7	53.80	6.50	7
Enalapril	58.99	15.06	8	2.0	0.5	8	48.10	4.54	8
Enalapril—C21 0.5 mg/kg	70.55	14.74	9	2.2	0.6	9	47.48	8.04	9
P	0.056			0.177			0.206		

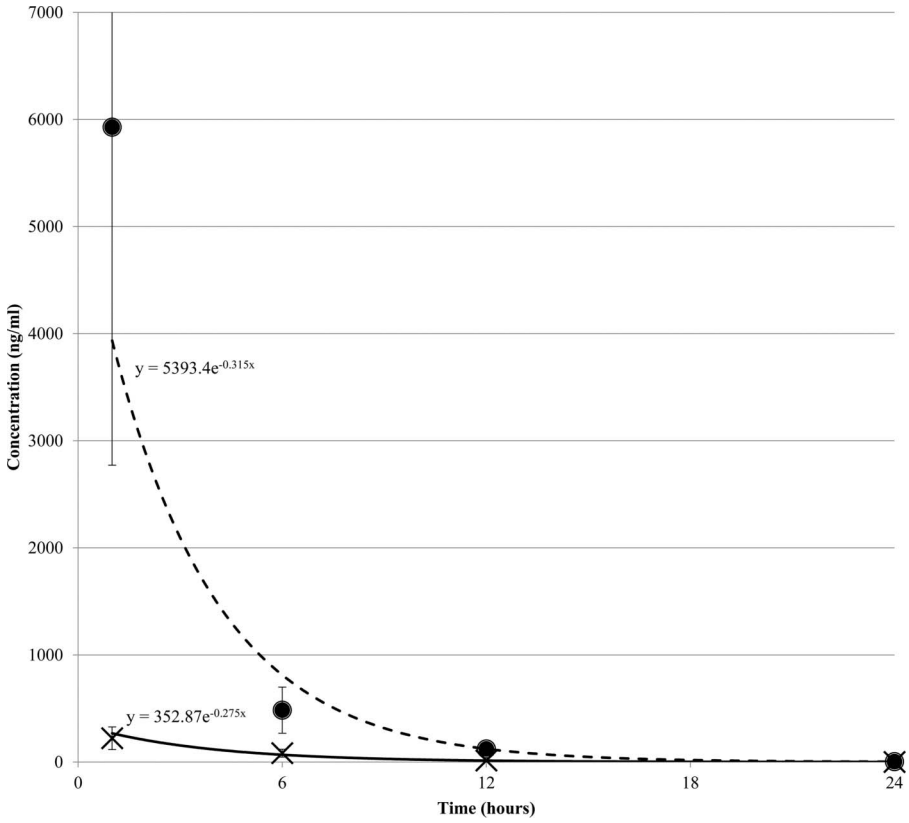

FIGURE 6. Mean plasma concentrations of C21 (ng/mL) (±2 SEM) 1, 6, 12, and 24 hours after IP injection of 2 doses (0.5 $mg \cdot kg^{-1} \cdot d^{-1}$: ×, full line and 5.0 $mg \cdot kg^{-1} \cdot d^{-1}$: •, dashed line). The exponential best-fit line model was used to calculate the intercept.

$mg \cdot kg^{-1} \cdot d^{-1}$.[12] Based on the used losartan concentration in the drinking water of 250 mg/L, average monitored water intake per animal of 5.2 mL/d, and average mice weight throughout the experiment of 26.8 g, there was an average losartan intake of 49 $mg \cdot kg^{-1} \cdot d^{-1}$. In the described studies, the losartan concentration in the drinking water was more than double, at 600 mg/L.[6,12,22] This means that the water intake of these mice was much lower, or that the real losartan intake was higher than the targeted 50 $mg \cdot kg^{-1} \cdot d^{-1}$. Higher losartan intake might have caused beneficial histological changes and lower blood pressures[12,22]; 2 things which could not be validated in this study.

There are several possible reasons for the lack of benefit of C21 on aneurysm growth in this study. The first two which are discussed below, the timing of initiation of therapy and the doses used, are also the main limitations of this study. First, the treatment with C21, started at the age of 8 weeks, might be too late in the pathogenesis of aortic root dilatation in MFS. This timing was based on previous studies evaluating drug treatment in this mice model.[6,12] However, the aortic root in $Fbn1^{C1039G/+}$ mice undergoes progressive dilatation, evident as early as 2 weeks of age. In this study, at the age of 8 weeks, before the start of any treatment, the diameter of the aortic root was significantly larger in mutant compared with WT mice. Comparable differences have been described by Habashi et al.[6] To overcome this, C21 was started at the age of 10 days in a subgroup of mice. This early treatment could not show any beneficial effect. At the age of 8 weeks, $Fbn1^{C1039G/+}$ mice had a significantly larger aortic root diameter in comparison with WT mice, independently of treatment. This was confirmed with histological evaluation, showing aberrant thickening of the aortic media as well as fragmentation and disarray of elastic fibers. In these young mice, only the lower dose (0.5 $mg \cdot kg^{-1} \cdot d^{-1}$) was tested because this is best within the range of used doses of C21 in other models. Despite the lack of effect of C21 even in these young mice, a potential benefit of AT_2R agonism cannot be ruled out. This because, second, the used doses and administration route of C21 may not be ideal. C21 has been applied IP or orally at doses ranging from 0.01 to 10.0 mg/kg per day in different species.[13,14,16,17,28,31,32] Because very little is known about the pharmacodynamics and pharmacokinetics of the studied compound, 2 doses (0.5 and 5 $mg \cdot kg^{-1} \cdot d^{-1}$) and 1 IP injection per day were chosen. Plasma concentrations were determined at specific time intervals after IP injection. The average calculated plasma half-time was 2.4 hours, which is in the range of previously described results after oral or intravenous administration in rats: 3–6 hours, and 0.5–2.5 hours, respectively.[13,33] Alternatively, the compound could have been administered orally, generating a more constant plasma concentration. Then, the oral bioavailability of 20%–30% should be considered.[33] Contrary to Ang II, which binds with similar affinity to the AT_1R and AT_2R,[34] C21 is a highly specific AT_2R agonist. The affinity of C21 for the AT_2R is 25,000-fold higher than for the AT_1R.[13] However, increasing the dose will not always enhance beneficial effects because high doses might stimulate the AT_1R.[33,35] Therefore, lower doses of C21 might have

TABLE 3. Average Aortic Root Diameter, Wall Thickness, Architecture Score, and Elastic Fiber Content of Early Start Experiment

	WT			Vehicle				C21 0.5 mg/kg			
Group	Mean	2 SEM	n	Mean	2 SEM	n	*P*	Mean	2 SEM	n	*P*
Aortic root diameter (mm)	1.63	0.06	8	1.90	0.06	12	<0.001	1.90	0.10	10	<0.001
Wall thickness (μm)	50.2	8.3	7	68.5	7.3	12	0.049	70.9	10.4	10	0.027
Architecture score	1.25	0.25	7	1.82	0.29	12	0.032	1.92	0.23	10	0.013
Elastic fiber content (%)	59.9	7.4	7	51.2	4.2	12	0.121	51.6	3.8	10	0.177

been needed to selectively stimulate AT_2R.[14] Stimulation of the AT_1R effect can potentially be blocked by combining C21 with losartan,[33] although this created no additional benefit in this study. Also, Chow et al[17] found that coadministration of C21 and candesartan had no additive effect. However, administration of enalapril reduces the availability of Ang II for the AT_1R and AT_2R. Theoretically, adding C21 to enalapril treatment would have a comparable effect, blocking AT_1R and stimulation of AT_2R signaling, as isolated losartan treatment. Nevertheless, the aortic root diameter after 6 months' treatment in the C21-enalapril group was significantly larger in comparison with the WT group. In addition to the capability of C21 to activate AT_1R, the drug has also proven to have effects in an angiotensin-receptor–independent manner, such as vasorelaxation due to calcium entry blockade[33] and antagonism of the thromboxane receptor.[36] Thus, the absence of a beneficial effect of C21 on aneurysm growth might be due to the used doses, the administration route or non-AT_2R mediated effects. Third, the lack of effect of C21 might be related to the unclear contribution of the RAA system, and more specifically of AT_1R and AT_2R, to aortic aneurysm progression. Initial studies characterizing thoracic aortic enlargement in MFS identified excessive TGF-β signaling as a driver of aortic disease.[6] Because the AT_1R antagonist losartan had been shown to inhibit TGF-β signaling, it was used to blunt TGF-β hyperactivity in the medial layer of $Fbn1^{C1039G/+}$ mice. In this way, losartan showed to be more effective in mice than β-blockers and as effective as TGF-β-Nab in preventing aortic root enlargement and pathologic changes. On the contrary, clinical trials failed to validate these animal data.[37,38] This puts in question whether TGF-β activation is truly the primary driver of aortic disease and whether losartan effect is exclusively the result of TGF-β inhibition.[4,39] Some suggest that disruption of mechanosensing through the elastin-contractile complex can lead to aberrant AT_1R activity and that this is the mode of action of losartan.[2,39] The pathophysiological role of the AT_2R on aortic aneurysm growth is probably even more complex. The presumed protective effect is based on blockade of the AT_1R rather than direct stimulation of AT_2R.[12] Moreover, there is accumulating evidence to suggest that the role of AT_2R is more than just the opposed action of the AT_1R.[11,33,40] Furthermore, the expression of AT_2R varies a lot under physiological and pathophysiological conditions.[41] One example is that expression and activity of the AT_2R is highly enhanced in female animals.[31,42,43] This means that there is probably a sex-specific role for the AT_2R and its interfering drugs. It should be stated that this study was exclusively performed in male animals, in accordance with most other studies describing the effect of C21.[14,16,17,27,32,35,44] To make things even more complex, angiotensin receptors might interfere with one another, like dimerization, affecting the consecutive signal transduction.[40,45] To elucidate the role of the AT_2R in aneurysm formation in MFS, the creation of a double knockout mice model combining an MFS model with a model with aortic overexpression of the AT_2R might be interesting. A disadvantage of the used $Fbn1^{C1039G/+}$ model is that long-term experiments are needed. This mouse model produces equal amounts of normal and abnormal fibrillin-1 and replicates the less commonly observed form of mild MFS.[33] It was reported that by 6 months of age, more than 90% of these mice developed TAA of variable diameters, but only 5% died of a ruptured aneurysm by 8 months of age.[46] In this study, there was no evidence of aortic aneurysm related mortality. The retroperitoneal hematoma in one losartan treated mouse was not associated with an aneurysm and might be related to a traumatic IP injection. The use of another mouse model, $Fbn1^{mgR/mgR}$, which replicated the more frequently diagnosed form of progressively severe MFS, might be more valuable.[47] These mice only produce approximately 20% of normal fibrillin-1, and aortic rupture is a fully penetrant manifestation within the first year of life. This has the additional advantage of including mouse survival as a more informative clinical endpoint.[4] Moreover, there is evidence that the protective effect of losartan is not as clear in $Fbn1^{mgR/mgR}$ mice, compared with $Fbn1^{C1039G/+}$ mice.[48,49], which reflects the disappointing results of the clinical trials in MFS evaluating the effect of losartan.[37,38]

This study reveals that C21, a selective nonpeptide AT_2R agonist, is ineffective—at the doses studied—to attenuate aneurysm growth in an MFS mouse model. Simultaneously, it was confirmed that specific AT_1R antagonism is more effective when it comes to aortic root dimensions than the dual AT_1R/AT_2R blockade. Further studies are warranted to elucidate the exact role of the RAA system, and more specifically the role of the AT_2R in aneurysm formation in MFS.

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