Marfan Sartan: a randomized, double-blind, placebo-controlled trial

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Aims
To evaluate the benefit of adding Losartan to baseline therapy in patients with Marfan syndrome (MFS).

Methods and results
A double-blind, randomized, multi-centre, placebo-controlled, add on trial comparing Losartan (50 mg when <50 kg, 100 mg otherwise) vs. placebo in patients with MFS according to Ghent criteria, age >10 years old, and receiving standard therapy. 303 patients, mean age 29.9 years old, were randomized. The two groups were similar at baseline, 86% receiving β-blocker therapy. The median follow-up was 3.5 years. The evolution of aortic diameter at the level of the sinuses of Valsalva was not modified by the adjunction of Losartan, with a mean increase in aortic diameter at the level of the sinuses of Valsalva of 0.44 mm/year (s.e. = 0.07) (−0.043 z/year, s.e. = 0.04) in patients receiving Losartan and 0.51 mm/year (s.e. = 0.03) in those receiving placebo (P = 0.36 for the comparison on slopes in millimeter per year and P = 0.69 for the comparison on slopes on z-scores). Patients receiving Losartan had a slight but significant decrease in systolic and diastolic blood pressure throughout the study (5 mmHg). During the study period, aortic surgery was performed in 28 patients (15 Losartan, 13 placebo), death occurred in 3 patients [0 Losartan, 3 placebo, sudden death (1) suicide (1) oesophagus cancer (1)].

Conclusion
Losartan was able to decrease blood pressure in patients with MFS but not to limit aortic dilatation during a 3-year period in patients >10 years old. β-Blocker therapy alone should therefore remain the standard first line therapy in these patients.

Keywords
Aorta • Marfan • Sartan • Echocardiography

Translational perspective
Aortic dissection remains the main threat in patients with Marfan syndrome (MFS) despite current therapy. The present study evaluated whether Losartan prevents aortic dilatation in humans with MFS as it is suggested in a mouse model. Losartan was able to decrease blood pressure in patients with MFS but not to limit aortic dilatation during a 3-year period. β-Blocker therapy alone should therefore remain the standard first-line therapy in these patients.

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Introduction

Marfan syndrome (MFS) is a rare disease (with a usually reported incidence of one case per 50000) carrying a vital risk related to progressive aortic dilatation leading to dissection and rupture.¹ This genetic disease, usually associated with mutations in the FBN1 gene, has benefited from standardised care including limitation in sports, β-blocker therapy and regular echocardiographic follow-up allowing timely preventive aortic root surgery, when aortic diameter increases >50 mm.²⁻⁴ Although standardized care delays the progression of aorta diameter, the aorta still dilates, and preventive aortic surgery is required in the majority of patients during the course of the disease.⁵ Therefore, the identification of further preventive pharmacological therapy is actively sought.

In an FBN1 mutation knockin (KI) mouse model (Fbn1⁵¹⁰⁹⁵G/+), oral Losartan treatment completely prevented dilatation.⁶ It was suggested that excessive TGF-β signalling contributed to the aortic aneurysm formation of, and that TGF-β antagonism with Losartan represented a productive treatment strategy. In addition to this potential signalling effect, Losartan could also act through its vasodilator properties. Therefore, a new therapy combining β-blocker and Losartan was viewed as potentially beneficial in Marfan patients, as well as in other forms of thoracic aortic aneurysm (TAA). This elicited great expectations in Marfan patients and their national support groups.

To test this hypothesis, clinical trials were launched worldwide. Recently, the PHN trial failed to demonstrate the expected superiority of Losartan over Atenolol in young patients with MFS and dilated aorta.⁷ We report the results of the first large-scale, double-blind, placebo-controlled trial which aimed at evaluating the safety and benefit of Losartan on aortic root growth in MFS when added to standard preventive therapy.

Methods

Marfan Sartan was a prospective, randomized, double-blind, multicentre, placebo-controlled, parallel group, add-on trial comparing Losartan with placebo in patients with MFS, in addition to standard preventive therapy. The study background and design have been published.⁸ The protocol was reviewed and authorized by the Agence Française de Sécurité Sanitaire des Produits de Santé and the IRB ‘Comité de Protection des Personnes Ile-de-France XI, St Germain en Laye’. All patients provided written informed consent before study entry. This study complies with the declaration of Helsinki. This study was registered on ClinicalTrials.gov under the identifier NCT00763893.

Population

To be included, patients had to be at least 10 years old, fulﬁl diagnosis of MFS according to Original Ghent criteria⁹ and have signed informed consent. They were not included if they had prior or planned aortic root surgery, contraindication to Losartan, pregnancy or planned pregnancy. Aortic diameter was not an entry criterion into the study.

Patients were enrolled at seven sites in France (Centre National de Référence in Paris, and Centres de Compétence in Lyon, Bordeaux, Rennes, Toulouse, Dijon, Marseille).

Eligible patients were randomly assigned (1 : 1) to Losartan (Losartan 50 mg once daily if < 50 kg, 100 mg once daily if ≥ 50 kg as recommended for treatment of hypertension or heart failure) or placebo. After randomization, patients were followed up every 6 months with echocardiographic recordings. Measurement of potassium and creatinine plasma levels was performed every year. Other drugs and treatments were left to the treating physician’s discretion.

Treatment and follow-up were to be maintained 3 years, and follow-up visits were scheduled every 6 months. After 3 years into the study, the patients had the opportunity to prolong their participation until the last included patient in the study was followed up for 3 years.

Echocardiographic measurements were made in a central lab (hospital Bichat, Paris), by experienced cardiologists (O.M., F.A., G.J.) blinded to the patient’s identity and the treatment received. Measures were obtained at the level of the annulus, the sinuses of Valsalva (aortic root), the sino-tubular junction, the ascending aorta, the aortic arch immediately prior to the left subclavian artery, descending thoracic aorta (maximal diameter), and abdominal aorta (at the level of the celiac artery). As recommended by the ASE and EAE,¹⁰ measures were made using 2D imaging at end-diastole, in a strictly perpendicular plane to that of the long axis of the aorta using the leading edge to leading edge convention for aortic root, sino-tubular junction, and the ascending aorta; inner edge to inner edge convention for the annulus, aortic arch, descending aorta, and abdominal aorta.

A two-step sequencing strategy was performed to identify the molecular defect in patients in the course of diagnosis. In a first step, the FBN1 gene was investigated by combining Sanger sequencing and multiplex ligation-dependent probe amplification analysis. If no mutation was identified, other genes associated with the disease (TGFBR2, TGFBR1, SMAD3, TGFBR2, ACTA2, and FBN2) were sequenced. To assess the deleterious effect of identified sequence variants, a series of widely accepted prediction algorithms was used (for details on molecular methods and algorithms, see Ref. ¹¹).

Outcomes

The primary endpoint was the rate of change in aortic root diameter (sinuses of Valsalva), normalized to its theoretical value and expressed as mean change in z-score per year.¹²

The secondary endpoints included: mean rate of change in raw normalized aortic root diameter (expressed in millimeter per year) and aortic complications (aortic root surgery, aortic dissection, cardiac death, and death).

Sample size

Sample size calculation was based on the assumption that Losartan efficacy in patients already receiving prophylactic therapy would be half of the β-blocker efficacy reported in the study by Shores, i.e. from 0.02 to 0.01 with a SD of 0.03.¹³ Choosing a 0.80 power and a two-sided type I error of 0.05, the number of patients required by group (of similar size) would be 142. One hundred and fifty patients were required in each group.

Randomization

Randomization was made using an internet-based computerized randomization system stratified on study centre, age (< 18 or ≥ 18 years old), and treatment with baseline preventive therapy. To ensure allocation concealment, Losartan and matching placebo were supplied to study sites in masked identical packages. Drug packages were given to the patients during follow-up visits.

Blinding

All study personnel involved in the operations of the study or with any potential site contact, such as medical monitors, remained blinded to
treatment assignments from the time of randomization until after completion of statistical analysis.

Statistical methods
Statistical analyses were performed on an intention-to-treat basis. For the primary endpoint, standard linear regressions were first used to estimate individual slopes for the change of aorta diameters over time, using all available measurements of each subject. A linear model (ANOVA) with adjustment on the stratification factors (centre, protective treatment at baseline, age <18 years old at baseline), was used to compare the mean slope of aortic dilatation between groups. All calculations were performed using R software version 3.1.1.\(^\text{14}\)

Results
Population
From 24 September 2008 to 23 March 2011, 303 patients with MFS were randomized (153 to Losartan, 150 to placebo), of whom four could not be taken into account in the statistical analysis due to a regulatory issue (patients aged <18 years old for whom only one parent had signed the informed consent whereas the authorization of both parents is legally required in France). Therefore, the population of the study included 299 patients (151 receiving Losartan, 148 receiving placebo), four were lost to follow-up (two receiving placebo, five receiving Losartan): one of these patients died within the first 6 months of the study (suicide, patient receiving Losartan). The annual mean increase in aortic diameter at the level of the sinuses (two receiving placebo, five receiving Losartan) was 3.5 years (Losartan 3.5 years, placebo 3.5 years).

Additionally, three patients died, all receiving placebo. The cause of death was suicide in one case, disseminated oesophagus cancer in another, and sudden death for the last case (no autopsy was performed).

Aortic complications and mortality
(Table 3)
During the time course of the study, aortic root surgery had to be performed in 26 patients: 15 receiving Losartan (14 scheduled preventive surgeries and one emergency for type A aortic dissection) and 13 receiving placebo (11 scheduled preventive surgeries and 2 emergencies for aortic dissection).

Haemodynamic effects
Blood pressure, measured during scheduled visit for the protocol, tended to be lower in patients with Losartan than with placebo by 5 mmHg for both systolic and diastolic blood pressure, and this difference reached statistical significance from M6 to M42 (Figure 4). Mean heart rate was similar during the study period in the two groups of patients.

Outcomes and estimation
The annual mean z-score change was not significantly different [mean adjusted difference = −0.0192; 95% CI (−0.112; 0.0733), \(P = 0.68\)] between patients receiving placebo (−0.01 z/year, s.e. = 0.03) and patients receiving Losartan (−0.03 z/year, s.e. = 0.03).

The mean increase in aortic diameter at the level of the sinuses of Valsalva (aortic root) was 0.51 mm/year (s.e. = 0.06) in patients receiving placebo and 0.44 mm/year (s.e. = 0.07) in patients receiving Losartan. The mean adjusted difference between the two groups was not significant: −0.0825 mm/year [95% CI = (−0.262; 0.0968), \(P = 0.37\), Figure 3].

No interaction was found between treatment and the stratification factors (age and baseline treatment). Similar results were obtained when only patients carrying an FBN1 mutation (placebo 0.00 z/year, s.e. = 0.04; 0.51 mm/year, s.e. = 0.08; Losartan −0.038 z/year, s.e. = 0.04, 0.40 mm/year, s.e. = 0.08), or when only patients with non-dilated aorta at baseline (z-score <2) were considered (placebo 0.00 z/year, s.e. = 0.03, 0.375 mm/year, s.e. = 0.097, Losartan 0.05 z/year, s.e. = 0.03; 0.34 mm/year, s.e. = 0.18).

Similarly, average aortic diameter changes during study did not differ significantly between the two groups at the annulus (\(n = 280\), sino-tubular junction (\(n = 284\)), tubular aorta (\(n = 274\)), aortic arch (\(n = 273\)), descending aorta (\(n = 215\)), nor abdominal aorta (\(n = 275\); Table 2).

Adverse effects
None of the severe adverse events (leading to hospitalisation or death) were considered as related to therapy according to the investigator (see Supplementary material online, Table S1). However six serious adverse events observed in four patients receiving Losartan were considered by the Pharmacovigilance Department as potentially related to the trial drug because they were mentioned in the Summary of Product Characteristics of the drug. These events were pain related (lumbar, abdominal, and thoracic) or supraventricular tachycardia.

During the time course of the study the trial drug was prematurely stopped in 69 patients (41 Losartan, 28 placebo) including the patients who terminated early because of surgery (\(n = 26\)) or death (\(n = 3\)). The reasons reported more than once for treatment cessation were hypotension for three Losartan patients, and malaise with dizziness in two placebo and two Losartan patients.
Discussion

The Marfan Sartan trial was designed to evaluate the benefit of adding Losartan to baseline therapy in patients with MFS. This randomized, double-blind, multi-centre trial demonstrates that Losartan does not significantly alter the aortic root dilatation rate in this population. This result was observed despite the slight but significant decrease in both systolic and diastolic blood pressure in patients receiving Losartan, indicating that the expected haemodynamic effect of Losartan was observed.

Actually, the benefit of Losartan reported in studies of patients with Marfan appears to decrease as time progresses, and as the size of the populations studied increases: the first retrospective study of 18 children, mean age 6.5 years, selected at the time of maximal dilatation without a control group reported a 86% decrease in aortic dilatation rate; similarly, a randomized unblinded study comparing 28 children (mean age 13.1 years) receiving Losartan on top of β-blocker therapy reported a 0.79 mm/year lower aortic dilatation rate in the group receiving Losartan (0.1 mm/year vs. 0.89 mm/year), but baseline aortic diameter differed between the two groups.

Groenink reported a smaller 0.19 mm/year mean difference in aortic dilatation rate in favour of Losartan. This difference was measured in the subgroup of 137 patients (76 Losartan, 61 controls) with native
aorta at the end of the study, i.e. 62% of randomized patients (whose mean age was 37.5 years) which may have led to a bias. Actually, the baseline aortic diameter was lower in the control group than that reported previously by the same group with echocardiography in Marfan patients (0.63 vs. 0.40 mm/year). A smaller aortic dilatation rate was measured with MRI for unclear reasons. The number of prophylactic aortic surgery was similar in the two groups (10 Losartan, 8 control group).

Finally, very recently, the Pediatric Heart Network Study, designed as a superiority trial, failed to demonstrate the superiority of Losartan over Atenolol in limiting aortic dilatation in 608 children and young adults (mean age 11.5 years) with MFS. Actually, in the group of patients receiving Losartan, the number of aortic events tended to be greater (19 vs. 10), and the aortic dilatation also tended to be greater, a difference which reached statistical significance at the level of the aortic annulus.

All studies evaluating Losartan used the Ghent 1 criteria to select the population. The use of the new nosology proposed in 2010 would most likely not have modified the results: comparison of the two criteria in probands carrying an FBN1 gene mutation suggested that aortic dilatation was slightly more prevalent with the use of the recent criteria, and a high level of agreement between the two sets of criteria was reported in a Korean population with or without an FBN1 gene mutation present.

It is unlikely that our trial misleadingly concludes to the absence of any clinically relevant effect of Losartan in patients with MFS for several reasons: (i) our study is the largest reported to date directly evaluating the effect of Losartan in Marfan patients; (ii) the expected number of patients was included, although only 146 and 147 patients could be evaluated for aortic dilatation in each group; (iii) the duration of follow-up was longer than in previous studies (median 3.5 years); (iv) the severity of the population was similar to that included in other studies as indicated by a baseline z-score (3.7) similar to that in Groenink’s study (3.8) and the number of clinical events (9.5%) similar to that reported in other studies (8.5% in the Atenolol vs. Losartan trial and 8.7% in Groenink’s study); (v) our results are very consistent whatever the section of the aorta being considered, and whatever the subgroup considered as there is no interaction with age, baseline aortic diameter, presence or absence of an FBN1
and heart rate by 15–20%, and was probably much higher.\textsuperscript{6} Tapering the dosage given was tapered so as to decrease blood pressure.\textsuperscript{6} In the present study, as in the mouse model, which suggested the benefit of Losartan,\textsuperscript{21} the absence of efficacy of Losartan also questions the applicability of mouse data in human.\textsuperscript{22} The vasodilator properties of Losartan could have been expected to be beneficial in Marfan patients by producing a lowering in mean blood pressure and a decrease and delay of the rebound wave.\textsuperscript{27} However, 100 mg was also the maximal dose chosen for the other studies because it is the maximal recommended dose of the drug in humans for hypertension. Furthermore, a 5 mmHg significant decrease in blood pressure was observed in our normotensive population with few instances of symptomatic hypotension thus suggesting that the effective dosage was obtained. It is also conceivable that a beneficial effect is only possible in the very early stage,\textsuperscript{21} as in the mouse model. Losartan was given first during pregnancy and the experiment repeated with Losartan given at 7 weeks after echocardiographic documentation of aneurysm. In our study, no indication of benefit of Losartan was observed in the subgroup of patients without dilated aorta.

Figure 4 Evolution of blood pressure (systolic and diastolic) throughout the study in the two groups of patients: patients receiving Losartan (blue) and patients receiving placebo (red).

The absence of significant benefit from Losartan in patients with MFS may indicate that the dosage of Losartan was too low in our study, as in the mouse model, which suggested the benefit of Losartan, the dosage given was tapered so as to decrease blood pressure and heart rate by 15–20%, and was probably much higher.\textsuperscript{6} However, 100 mg was also the maximal dose chosen for the other studies because it is the maximal recommended dose of the drug in humans for hypertension. Furthermore, a 5 mmHg significant decrease in blood pressure was observed in our normotensive population with few instances of symptomatic hypotension thus suggesting that the effective dosage was obtained. It is also conceivable that a beneficial effect is only possible in the very early stage,\textsuperscript{21} as in the mouse model. Losartan was given first during pregnancy and the experiment repeated with Losartan given at 7 weeks after echocardiographic documentation of aneurysm. In our study, no indication of benefit of Losartan was observed in the subgroup of patients without dilated aorta.

The negative results of our trial may be attributed to the importance of complete blinding in randomized trials as recently outlined by the renal denervation studies in hypertension.\textsuperscript{22,23} Indeed, initial studies, incompletely blinded, reported a significant decrease in blood pressure associated with the procedure, whereas the last and completely blinded study demonstrated the absence of effect of denervation on blood pressure in hypertensive patients. The opposite results in the Losartan trials in MFS similarly illustrate the importance of full blinding before a definitive conclusion can be reached. The progressive decrease in the benefit observed with Losartan in previous trials also supports this hypothesis (from 3.08 mm/year in Brooke study with historical control, 0.79 mm/year in Chiu’s study and 0.19 mm/year in Groenink’s study both with a control group) and questioned the clinical significance of this effect.

The absence of efficacy of Losartan also questions the applicability in humans with MFS of the TGFβ hypothesis as proposed in the mouse model.\textsuperscript{6} The rationale for evaluating the effect of Losartan on Losartan trials in MFS patients was based on the Fbn1 KI mouse model (Fbn1\textsuperscript{C1039G/+}), in which aortic dilatation was stopped or partly prevented by Losartan treatment.\textsuperscript{6,24–26} Our negative results, the limited benefit reported by Groenink, the absence of superiority over Atenolol in the Pediatric Heart Network Study,\textsuperscript{15} would all suggest that the applicability of mouse data in human remains unclear.

The vasodilator properties of Losartan could have been expected to be beneficial in Marfan patients by producing a lowering in mean blood pressure and a decrease and delay of the rebound wave.\textsuperscript{27} Both contribute to lower central systolic blood pressure which has been associated with aortic dilatation in this population.\textsuperscript{28} Angiotensin receptor blocker appear to be more efficacious in lowering

| Table 2 | Evolution of aortic diameter at different aorta localizations in the two groups |
|----------------|-----------------|-----------------|----------------|
|               | Losartan        | Placebo         | P-value        |
| Aortic root (z-score/year) | -0.03 (0.03) | -0.01 (0.03) | 0.69 |
| Aortic root (mm/year) | 0.44 (0.07) | 0.51 (0.06) | 0.36 |
| Aortic annulus (mm/year) | 0.16 (0.09) | 0.23 (0.09) | 0.46 |
| Sino-tubular junction (mm/year) | 0.40 (0.17) | 0.28 (0.18) | 0.65 |
| Ascending aorta (mm/year) | 0.32 (0.22) | 0.45 (0.11) | 0.62 |
| Aortic arch (mm/year) | 0.34 (0.13) | 0.42 (0.12) | 0.82 |
| Descending thoracic aorta (mm/year) | 0.26 (0.31) | 0.27 (0.19) | 0.98 |
| Abdominal aorta (mm/year) | 0.16 (0.14) | 0.25 (0.10) | 0.59 |

| Table 3 | Events during the study in the two groups |
|----------------|-----------------|----------------|
|               | Losartan (n = 151) | Placebo (n = 148) |
| Any serious adverse event | 51 (33.7%) | 48 (32.4%) |
| Possibly related to drug | 6 (3.9%) | 0 |
| Death | 0 (0.0%) | 3 (2.0%) |
| Aortic surgery | 15 (9.9%) | 13 (8.8%) |
| Aortic dissection | 1 (0.7%) | 2 (1.3%) |
| Number of patients with K+ >5.5 mmol/L | 0 (0.0%) | 0 (0.0%) |
| Creatinine >ULN (120 µmol/L) | 1 (0.6%) | 0 (0.0%) |
| Creatinine increase > 26.4 µmol/L (0.3 mg/dL) | 13 (8.6%) | 11 (7.4%) |
It has been suggested that the expected haemodynamic effect was obtained.

\[\text{Beta-blocker therapy has been demonstrated to be efficacious in limiting aortic root dilation in patients with MFS in an open label randomized study including 70 patients published 20 years ago.}\]

The practical outcome of our study is that Losartan should not be systematically proposed with MFS, as there is no evidence that Losartan is effective in reducing aortic root dilatation in patients with MFS.35

In conclusion, the evolution of aortic diameter at the level of the sinuses of Valsalva in Marfan patients was not modified by the administration of Losartan to baseline therapy.

Supplementary material
Supplementary material is available at European Heart Journal online.

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Conflict of interest: none declared.

References
10. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikaan RG, Picard MH, Roman MJ, Sever J, Shawless J, Solomon SD, Spencer KT, Sutton MS, Stewart WJ; Chamber Quantification Writing Group; American Society of Echocardiography’s Guidelines and Standards Committee, European Association of Echocardiography; Recommendations for chamber quantification: a report from the American Society of Echocardiography’s Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr [Practice Guidelines]. 2005; 18: 1440–1463.


