Rationale and design of a randomized clinical trial (Marfan Sartan) of angiotensin II receptor blocker therapy versus placebo in individuals with Marfan syndrome

Étude randomisée «MarfanSartan» comparant un antagoniste de l’angiotensine II au placebo chez des patients présentant un syndrome de Marfan

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Summary
Background. — Recent studies have demonstrated that blockade of the angiotensin II type 1 receptor with losartan decreases aortic damage in an animal model of Marfan syndrome (a KI mouse model with a pathogenic mutation in the gene coding for fibrillin-1).
Aims. — To demonstrate a beneficial effect of losartan on aortic dilatation when added to optimal therapy in patients with Marfan syndrome.
Methods. — This is a multicentre, randomized, placebo-controlled, double-blind, clinical trial with a 2-year inclusion period and a 3-year follow-up period. Aortic root diameter will be measured using two-dimensional echocardiography. Secondary endpoints will include incidence of aortic dissection, aortic root surgery, death, quality of life, tolerance and compliance with treatments. We aim to enrol a total of 300 patients aged ≥ 10 years who fulfil the Ghent criteria for Marfan syndrome. Analyses will be based on intention to treat.
Conclusion. — The results of this clinical trial could lead to profound modification of the management of aortic risk and complications in patients with Marfan syndrome and possibly in patients with thoracic aortic aneurysms of other aetiologies.

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Background
Marfan syndrome: definition
Marfan syndrome (MFS) is an autosomal dominant disorder with pleiotropic features, including skeletal abnormalities, ectopia lentis and aortic root dilatation. The main causal gene for MFS is FBN1, encoding fibrillin-1, a large glycoprotein that is a main component of extracellular microfibrils. Prognosis is determined mainly by aortic complications (dissection or death), after progressive dilatation of the aortic root. Diagnosis criteria have changed over time; international criteria were proposed in 1988 and refined in 1996, to increase specificity and to integrate genetic testing [1,2]. The current nosology is based on the Ghent criteria, which define major and minor manifestations in different systems (Table [not provided]) [2]. In this setting, the diagnosis of MFS requires at least two major criteria and the involvement of at least one other body system (i.e., three criteria in total). In the presence of an FBN1 mutation or when MFS is diagnosed in a first-degree relative, only one major criterion and the involvement of another body system is required [2]. These Ghent criteria have excellent specificity for FBN1 mutation recognition, because its detection is possible in 95% of patients who fulfil these criteria [3]. However, a mutation in the FBN1 gene is not pathognomonic of MFS and may generate a large array of phenotypes that overlap with MFS (familial ectopia lentis, Shprintzen-Goldberg syndrome, other fibrillinopathies) [4]. On the other hand, some features of MFS can also be present in patients with mutations in the gene coding for transforming growth factor beta (TGFβ) receptor 2 (TGFB2), who present with MFS type 2 [5–7]. Lastly, mutations in the gene coding for TGFβ receptor 1 (TGFB1) may also lead to overlapping syndromes [3]. Therefore, defining clear frontiers for MFS, fulfilling the Ghent criteria and differentiating from other clinical conditions that overlap with MFS (MFS type 2, Loeys-Dietz syndrome, familial thoracic aortic aneurysm, Ehlers-Danlos vascular syndrome [8–10]) can sometimes be challenging. In the near future, a revised nosology will emerge, which will focus on the features and criteria that distinguish MFS from other disorders; in the meantime, however, the Ghent criteria remain the reference and will be used for classification of patients in the current study.
Current medical management

The aortic root diameter at the sinuses of Valsalva is considered to be the best predictor of the occurrence of an aortic event [11]. Patients with an absolute aortic diameter > 50 mm, an aortic ratio > 1.3 (observed/expected diameters) or a z score > 3 (the z score is the number of standard deviations above the mean) are considered at high risk for catastrophic complications, based on data obtained more than 10 years ago, before the publication of the Ghent criteria [12]. In addition, rapid growth of the aortic root (> 0.5 cm/year) and a family history of dissection are also predictors of poor outcome in patients with MFS. Therefore, routine monitoring of the aortic diameter is necessary for determining the best time for surgery.

Associated medical therapy is mandatory and includes avoidance of isometric exercises, sports limitation and preventive medical therapy with beta-blockers or calcium channel blockers, inducing bradycardia [13]. Indeed, the use of beta-adrenergic blockade to decrease the haemodynamic stress on the ascending aorta has been suggested since the 1970s [14]. The first randomized, open-label trial was published in 1994 [15] (Fig. 1). In this study, the rate of change of aortic root diameter and clinical outcomes (aortic regurgitation, aortic dissection, surgery, heart failure and death) were compared between 32 patients assigned randomly to receive propanolol and 38 controls. At baseline, the absolute aortic diameters were larger in the treated group than in the non-treated group (34.6 vs 30.2 mm) but the aortic ratios (observed/expected diameters) were similar (1.4 vs 1.3, not significant). After a decade of follow-up, the mean change in aortic ratios over time was significantly lower in the treatment group (0.023/year) than in the control group (0.084/year). No statistically significant difference was observed between groups in event-free survival, but the rate of clinical events was higher in the control group than in the treatment group. Retrospective studies in children have demonstrated the beneficial role of beta-blockers [16,17], with a reduction in the rate of change in aortic diameter of 0.16 mm/year and a decrease in the number of aortic complications [16].

From a theoretical point of view, the efficacy of beta-blockers relies on their haemodynamic properties, reducing the force of left ventricular ejection (i.e., dp/dt) by negative inotropy and the number of impulses due to bradycardia, particularly during stress and exercise. Most series also demonstrate an increase in indexes of arterial wall compliance (which is basically decreased in patients with MFS [18]) with beta-blockers [19–21]. Nevertheless, the absence of an increase in aortic compliance with beta-blocker therapy has been observed in patients with marked aortic enlargement or increased weight, stressing the importance of starting treatment early in the course of the disease, with dose optimization [22–24].

Marfan syndrome in 2010: physiopathology

In MFS, histological observations of the aortic wall classically show medial degeneration, with smooth muscle cell disappearance, disorganization of elastic fibres and accumulation of mucopolysaccharides. These abnormalities are not specific to MFS, as they can be observed in the aortic wall of aneurysms with other aetiologies, such as those related to bicuspid aortic valves or even degenerative aneurysms [25–27].

These histological abnormalities led to hypotheses being generated on the pathogenesis of MFS aortic disease. Mutations in the FBN1 gene encoding fibrillin-1 result in an abnormal protein and enhanced proteolytic degradation of fibrillin-1. This protein is an essential component of the microfibrils that play a role in extracellular matrix structure and regulation, elastic fibre organization and cell adhesion [28]. Hence, the presence of structurally abnormal microfibrils would weaken the extracellular matrix, allowing progressive aortic dilatation. In this hypothesis, all features associated with MFS are secondary to the weakening of the extracellular matrix in different tissues, leading
Figure 2. Relationship between transforming growth factor beta (TGFβ)-binding protein and extracellular microfibrils (from Isogai et al. [31]). TGFβ is synthesized as a precursor molecule containing a propeptide region. After it is synthesized, the TGFβ homodimer interacts with a latency-associated peptide (LAP; a protein derived from the N-terminal region of the TGFβ gene product), forming a complex called "small latent complex". This complex remains in the cell until it is bound by another protein called latent TGFβ-binding protein (LTBP), forming a larger complex called "large latent complex". This complex is secreted to the extracellular matrix.

to increased growth of bones, hernia, cutaneous striaes, etc.

Alternative hypothesis have been proposed, however, based on observations made in a mouse model of MFS, which carries a pathogenic mutation in FBN1 [29]. In this model, markers for activation of the TGFβ pathway (i.e., p-smad-2) were present in smooth muscle cells, suggesting activation. TGFβ molecules are cytokines, synthesized and secreted by smooth muscle cells as inactive precursors in the form of a large latent complex (that includes a pro-TGFβ molecule and the "large TGFβ-binding protein"), which is stored in the extracellular matrix [30]. One proposed hypothesis is that abnormal fibrillin causes failure of the latent complex sequestration and excessive activation [31,32] (Fig. 2). Excessive TGFβ signalling would then cause increased smad-2 phosphorylation and nuclear localization, resulting in altered gene expression (Fig. 3).

Experimental therapy

Experimental studies in the KI mouse have shown an absence of development of aortic aneurysm or myxomatous mitral valve in animals treated with TGFβ antibodies [33,34]. Interestingly, a similar effect has been observed with the use of losartan, a blocker of the angiotensin II type 1 receptor (AT1) [33]. Indeed, AT1 stimulation with angiotensin II activates the process of fibrosis and cell proliferation. These effects are mediated by TGFβ activation.

In the experimental study by Habashi et al. [33], comparison of aortic diameter growth between Marfan mice treated with placebo, propranolol or losartan showed that both beta-blockers and AT1 blockers (angiotensin II type 1 receptor blockers [ARBs]) reduced the rate of change in aortic diameter compared with placebo. However, regarding the histological abnormalities, only treatment with losartan reduced the disarray of the extracellular matrix significantly.

The primary results of non-randomized and small studies in humans are encouraging. Brooke et al. [35] compared the progression of aortic root diameter in 18 children with MFS, before and after treatment with ARBs. After treatment with ARBs in addition to beta-blockers over 2 years, the rate of change of the Valsalva and sinotubular junction decreased significantly from 3.5 mm/year to 0.46 mm/year. However, this study had many limitations, including its retrospective nature, the selection of children at the time of maximal aortic growth, the absence of a control group of any kind and the normalization of the aortic diameter using unusual rules.

Although this hypothesis is very appealing, some unexplained results have been observed. Firstly, blocking only AT1, ARBs allow beneficial effects (fibrosis and cell proliferation inhibition) via signalling through the angiotensin II type 2 receptor (AT2), whereas angiotensin-converting enzyme inhibitors (ACEIs) reduce both AT1 and AT2 signalling. Ahismatos et al. [36] randomized 17 adults with MFS to receive either an ACEI (perindopril) or placebo in association with beta-blocker therapy. The study showed that, after 6 months, the stiffness of the aorta and the aortic root diameters had decreased significantly in patients receiving the ACEI and the aortic diameter became smaller compared with placebo. Levels of TGFβ were reduced by
ACEI therapy, suggesting that ACEIs, like ARBs, target the underlying tissue pathology, in addition to reducing haemodynamic stress. These results, obtained in only one centre in a very small number of patients (10 receiving placebo, seven receiving perindopril) will have to be reproduced by others before being accepted widely. However, they are supported by non-randomized data [37]. Secondly, the presence of p-samd-2 in smooth muscle cells has been observed in the aortic wall of patients with a TGFBR2 mutation blocking transmission of the signal and in aneurysms of various aetiologies; this is compatible with the release of TGFβ by the matrix when it is destroyed, regardless of the aetiology of the extracellular matrix alteration [27]. Hence the benefit of blockade of the renin-angiotensin system seems promising in MFS, but has not been demonstrated in humans. Multicentre trials are needed to address this question. A trial designed to test the efficacy of losartan vs atenolol is ongoing in the USA [38] and aims to include 604 patients (children and adults). Another three-arm Italian trial is ongoing to evaluate the effect of losartan vs nebivolol vs a combination of both on the progression of aortic root dilatation [39].

Rationale for this trial
We have initiated this trial to evaluate the safety and benefit of losartan on aortic root growth in MFS when added to accepted standard therapy. Beta-blocker therapy has limitations (merely a haemodynamic effect, lack of target in the underlying tissue pathology, side effects), but remains the standard of medical care in MFS. Therefore, considering a trial without allowing beta-blocker therapy might be perceived to be unethical. Besides, while beta-blockers are exerting their haemodynamic effects, losartan should modify the physiology within the aortic wall; in other words, the pathophysiology indicates that their effects should be additive.

Methods
Study design
Hypothesis and overview
This trial is designed to test the hypothesis that the addition of ARB therapy (losartan) to optimal standard therapy will reduce the rate of aortic dilatation compared with placebo in MFS patients aged ≥ 10 years. The decision to include younger patients rather than just adults derives from the more rapid aortic root dilatation progression during growth and the fact that demonstration of benefit in a mouse model was obtained in young individuals.

Population
Inclusion criteria
The inclusion criteria for this study are as follows: age ≥ 10 years; diagnosis of MFS according to Ghent criteria, with or without known FBN1 mutation; informed consent and assent of participant, and parent(s) or legal guardian, as applicable. Patients will be excluded from the study for the following reasons: prior or planned aortic root surgery; inability to obtain accurate measurement of aortic root due to poor acoustic windows with transthoracic echocardiography; contraindication to losartan (bilateral renal artery stenosis, history of angio-oedema while taking ARB therapy); lactose intolerance, galactosaemia, or glucose or galactose malabsorption; pregnancy or planned pregnancy within 36 months of enrolment; absence of medical insurance (Securité sociale or Couverture maladie universelle).

Randomization and stratification
Eligible subjects will continue their standard prophylactic therapy (beta-blocker [or calcium channel blocker if beta-blocker therapy is not tolerated]) and will be assigned randomly to receive either losartan or placebo. Randomization will assign losartan or placebo in a 1:1 ratio and will be stratified according to centre, age at inclusion (< or ≥ 18 years) and existence (or not) of ongoing prophylactic therapy (beta-blocker or calcium channel blocker). The randomization code will not be available to the investigators by any means, ensuring double-blind assignment.
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Follow-up visits are scheduled every 6 months (Table 1) and include: clinical examination; questions about adverse drug reactions; SF-36 quality of life questionnaire; echocardiography; laboratory testing, including creatinine plasma concentration, uraemia and kalaemia (annually).

The aortic root diameter will be measured using transthoracic echocardiographic pictures or sequences recorded by trained echocardiographers according to recommendations [40] (Fig. 4).

Endpoints

The primary endpoint is the rate of change in aortic root diameter (sinuses of Valsalva), normalized to its theoretical value (expressed as z-score per year). The secondary endpoints include: rate of change in aortic root diameter with absolute dimension (expressed in mm per year); aortic complications (aortic root surgery, aortic dissection, cardiac death, death); compliance with treatment (incidence of adverse drug reactions reported during routine surveillance [hypotension, syncope]; compliance with losartan [proportion of patients taking at least 80% of pills given]; compliance with beta-blockers based upon patient’s declaration); evaluation of quality of life based upon the SF-36 questionnaire.

Statistical considerations

Sample size

The sample necessary to verify the hypothesis has been calculated using data obtained from Shores et al. [15]. In this study, which compared the rate of aortic root dilatation in patients receiving beta-blockers vs no treatment, the rate of aortic root dilatation was 0.084/year without treatment vs 0.0253/year with beta-blockers (around four times lower) and the maximum standard deviation was 0.03.

The sample size calculation was based on the assumption that losartan efficacy in patients already receiving prophylactic therapy will be half of the beta-blocker efficacy (0.01), and on the comparison of losartan with placebo with 0.80 power and a two-sided α of 0.05. To account for potential patient dropout, a total of 150 patients will be required in each group.

Analyses

Analyses will be performed on an intention-to-treat basis. Baseline characteristics will be summarized as means ± standard deviations and percentages, and compared between the two arms of the trial using the chi-square test or analysis of variance. As a supplementary analysis, the mortality rate or event-free survival will be compared using the log-rank test. The significance level will be 0.05. For the primary endpoint, the final analysis will compare the mean slope of aortic dilatation between groups with covariate-adjusted analysis and after 3 years of follow-up.

Trial organization and timeline

The Assistance publique—Hôpitaux de Paris and the département de recherche clinique et du développement will be
Marfan Sartan study recently published new nomograms for children [41]. However, we chose to use, like most teams, standardization according to the aortic root diameter (our second endpoint). We recognize that differences in body size between groups, reducing differences in body size between groups and therefore increase the power of the study to detect a difference. Randomization should result in two similar groups, reducing differences in body size between groups and allowing comparison of the changes in mean absolute diameter (our second endpoint). We recognize that different normalizations exist in the literature, and we planned to use, like most teams, standardization according to the nomogram publication by Roman et al. [40]. However, we recently published new nomograms for children [41].

Discussion

Choice of primary endpoint

Our primary endpoint is the rate of change in aortic root diameter normalized to its theoretical value. This choice is based on the relationship between aortic root dimensions and outcome, namely aortic complication, and therefore prophylactic intervention. Aortic root diameters are also easily measured by transthoracic echocardiography, with good reproducibility. Furthermore, from a statistical point of view, the primary endpoint is a continuous variable, which is more powerful than a quantitative variable for identifying a potential difference between groups. Moreover, it was used previously for the evaluation of beta-blocker therapy in 1994 [15] and, more recently, for the evaluation of losartan [33,35].

Our decision to compare normalized diameters and not absolute diameters was due to the relationship between aortic diameter and body surface area, which is very variable. Using the normalized diameter should decrease variability and therefore increase the power of the study to detect a difference. Randomization should result in two similar groups, reducing differences in body size between groups and allowing comparison of the changes in mean absolute diameter (our second endpoint). We recognize that different normalizations exist in the literature, and we planned to use, like most teams, standardization according to the nomogram publication by Roman et al. [40]. However, we recently published new nomograms for children [41].

Implication for future management of Marfan syndrome

If demonstration of safety and efficacy of losartan in association with beta-blocker therapy can be obtained in this double-blind, randomized trial, standard care of these patients will be modified. Until the results are obtained, however, the standard care remains beta-blocker therapy, as a few differences can be identified between a KI mouse model with one FBN1 mutation and humans with MFS. Firstly, in the mouse model, only one mutation has been evaluated, and this mutation has been associated with only one genetic background (all the mice have the same genotype). In contrast, in humans, each family carries its own mutation or almost so (private mutations), and the genetic background differs from one individual to another, which is responsible for the unique aspect of each human. It is not clear that all mutations will act through the same mechanism (negative dominance vs haploinsufficiency), and the effect of a given mutation is probably highly dependent on the genetic background, as suggested by the great clinical variability of MFS severity within one family (modifier genes).

Besides, compliance may be an issue for a drug given long-term; while tap water is an absolute necessity for mice, taking an additional pill is optional for patients. Side effects may occur that may further limit the compliance of patients (including hypotension, which gives an increased perception of fatigue). These factors are crucial for drugs that have to be taken for life.

It is impossible, therefore, to conclude before the results of the ongoing randomized trials are available, whether the prescription of losartan is useful in patients with mutations in the FBN1 gene.

Comparison of ongoing trials

The USA trial is comparing beta-blocker therapy (atenolol) directly with losartan in an open-label, randomized trial [38]. This study evaluates the advantages of two different first-line therapies but not the benefit of combining the two drugs compared with up-to-date standard therapy. Besides, in this protocol, the criteria chosen for optimizing beta-blocker therapy are not in keeping with usual care in European countries, including France (up-titration based on 24-hour electrocardiogram and mean dose of beta-blockade higher than that used in France).

The Italian trial is comparing three different approaches directly: beta-blocker or losartan or both [39]. The beta-blocker being used (nebivolol) carries theoretical advantages over the non-selective propanolol used in the landmark study of Shores et al. [15], and over the beta-blocker used in the USA trial (atenolol): its vasodilatory properties could decrease the rebound wave and therefore the stress applied on the proximal aorta and enhance the haemodynamic benefit of the drug; its beta-1 selectivity should increase its tolerance and therefore compliance. The ARB being used also allows 1-day administration and optimal receptor blockade. Lastly, the relative benefits of the two classes of drug and their combination are ideal. The drawback of having three groups is the necessity for a high number of patients to obtain the statistical power to be able to recognize differences between groups.

Lastly, the University of Ghent has also started a randomized trial with a design similar to ours, but also evaluating the evolution of aortic stiffness over time. The similar design may allow secondary combination of the populations to increase statistical power, which is obviously an issue when the protocol aims to include such a selected population.

Which population may benefit from the results?

In current medical practice, beta-blocker therapies are proposed for patients with ascending aorta aneurysms of various aetiologies, based on haemodynamic concept and the randomized trial completed in the population with MFS. We reported recently that similarities in the pathogenesis of aortic aneurysms of varying aetiologies, including increased p-smad-2 (a marker for TGFβ activation), have been observed [27]. Therefore, if slowing of aortic dilatation can be demonstrated in patients with MFS with AT1 blockade, the question of the applicability of the findings to a
larger population (i.e., all aortic aneurysms) will obviously arise. Similarly, AT1 blockade may become the first-line medical therapy in hypertension associated with conditions known to be at risk of development of aneurysm of the ascending aorta (e.g., bicuspid aortic valve, coarctation of the aorta).

Limitations

Our study will not be able to evaluate the superiority of one therapy over the other. However beta-blocker therapy is the standard of care for patients with MFS and the current study will evaluate the efficacy of losartan in addition to this prophylactic standard therapy.

The study results may not be extrapolated to children aged younger than 10 years and to variants of MFS who do not meet the Ghent criteria.

Conclusions

The rationale behind a trial of losartan therapy in addition to standard beta-blocker therapy in MFS is strong and is based on recent advances in the understanding of the disease pathogenesis. The results should establish if the benefits demonstrated in the mouse model are applicable to humans or if other strategies should be looked for. If strong evidence of a treatment benefit for ARBs in patients with MFS can be obtained, the natural history of MFS and aortic aneurysms with other aetiologies may change dramatically in the future.

Conflict of interest statement

No conflict of interest to report.

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