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AT1 receptor antagonism to reduce aortic expansion in Marfan's: Lost in translation or in need of different interpretation?

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Marfan's syndrome is an inherited autosomal dominant disorder of the connective tissue caused by mutations in the gene encoding fibrillin-1 (*FBN1*).[1,2] This disease has impacted the ATVB community with the loss of one of our highly respected members, David Williams.[3] The most devastating health issue for Marfan's patients is dissection and rupture of the proximal aorta. Aortic dimensions are routinely monitored in Marfan's patients and surgical graft imposition is performed to sustain life. However, surgical intervention is a formidable process. To abrogate surgical repair, there is a desperate need for a medical approach to attenuating aortic expansion. Presently, Marfan's patients are routinely provided with β adrenoceptor blockade. This standard of care is derived from the study of Shores and colleagues.[4] However, the benefit derived from administration of β-adrenoceptor blockade is far from established.

Although FBN1 is a component of extracellular matrix, mutations in this protein do not directly promote the structural fragility that characterizes specific aortic regions of Marfan's patients. Instead, a concept has developed that *Fbn1* mutations lead to enhanced TGF-β activation that is the mechanistic basis of Marfan pathology. This concept originated from the demonstration that *Fbn1* deficiency led to excessive activation of TGF-β signaling due to reduced binding, sequestration, and inactivation of TGF-β.[5] Activation of TGF-β in *Fbn1* deficient mice was associated with a lung pathology similar to that observed in some Marfan's patients. This paradigm of FBN1 regulation of TGF-β activation was extended to aortic disease by generating heterozygous mice that express a C1039G mutation.[6] These mice develop pronounced dilation of the ascending aorta associated with thickened media containing disrupted elastin fibers. In this model, the major proof of concept for the role of TGF-β was the attenuation of ascending aortic dilation by administration of a neutralizing antibody to all isoforms.[6] Losartan was also highly effective in attenuating aortic dilation and media pathology. Losartan was the first of the class of AT1 receptor antagonists that block the effects of AngII on the major receptor type responsible for most of the major physiological and pathological effects. Losartan has known effects beyond antagonism of AT1 receptors, although this does not extend to antagonism of TGF-β on any of its receptors.[7] However, since AngII stimulates release of TGF-β, losartan could indirectly reduce TGF-β mediated responses.[8] Therefore, the benefits of losartan administration infer

that AT1 receptors are involved in these *Fbn1* C1039G mice. Conversely, chronic infusion of AngII into mice leads to ascending aortic dilation and medial pathology that bears strong similarities to tissue characteristics of *Fbn1* C1039G mouse.[9,10] Collectively, these are convincing preclinical data that antagonism of AT1 receptors would be a beneficial approach to treating aortic disease in Marfan's patients. The translational potential of the preclinical results was greatly reinforced by the reported superiority of losartan over β-adrenoceptor antagonism in *Fbn1* C1039G mice, with losartan providing significantly better protection against aortic dilation and structural remodeling compared to propranolol.[6]

The excitement of this preclinical demonstration of losartan effects provoked a retrospective analysis of Marfan's children whose aortic dimensions were sequentially monitored during administration of atenolol, and subsequently treated with an AT1 receptor antagonist. Losartan was administered to all but one of the children, who received irbesartan.[11] This small cohort study had the provocative conclusion that AT1 receptor antagonism reduced the rate of aortic expansion. This report noted the requirement for randomized trials to confirm the observations of this retrospective analysis.

The combined data from animal studies and the retrospective analysis stimulated the initiation of several trials throughout the world.[12] These trial designs have many differences, including patient age at study initiation, the group used to compare losartan treated individuals, and the sartan used. A few preliminary reports of small open-label clinical trials have subsequently provided support for this concept in Marfan's patients and suggested that losartan treatment, either alone or in combination with β-adrenoceptor antagonists, was associated with reduced rate of aortic dilatation.[11,13]

One of the most anticipated trials of AT1 receptor antagonism on aortic root expansion was designed by the Marfan Trial Subcommittee of the Pediatric Heart Network.[14] The results of this trial were eagerly awaited and many expected it would definitely validate the superiority of losartan over atenolol in providing aortic protection through reductions of aortic growth rate. However, Lacro et al.[15] reported no benefit of losartan when compared to atenolol over a 3-year period. There was even a tendency towards less decrease in (baseline-adjusted) aortic-root Z-score over time ($P=0.08$) in the losartan group, which was statistically significant when considering the aortic-annulus Z-score ($P<0.001$), and a trend towards increased risk (1.9 fold, $P=0.10$) for clinical events (aortic-root surgery, aortic dissection, and death) in losartan compared to atenolol-treated patients.

So why did losartan fall so far short of its promise in this clinical trial? Several shortcomings were noted in the trial design that need to be accounted for in the evaluation of the data. One of the principal issues was the lack of a placebo group. This will be addressed in some of the completed and ongoing trials.[16,17] Another critical issue was the optimization of losartan dosing. There was no indication of the extent of AT1 receptor antagonism.

From a perspective of preclinical studies providing insight into aortic dilation in Marfan's patients, an important question is whether the study of Lacro et al.[15] invalidates the use of mouse studies that indicated benefit of AT1 receptor antagonism in proximal aortic dilation. Prior to making such a damming conclusion, several facets of experimental design should be

considered that may impact the extrapolation of effects from the preclinical to the clinical arena.

1. **Hemodynamics.** Losartan was superior to β-adrenoceptor antagonism with propranolol in attenuating aortic dilation in *Fbn1* C1039G mice when both drugs "were titrated to achieve comparable hemodynamic effects in vivo, including a 15 to 20% decrease in heart rate and a 10 to 20% decrease in blood pressure in both groups".[6] However, hemodynamic data were not provided to assess the extent of blood pressure reduction in losartan versus propranolol administered mice. Small, but sustained, differences in blood pressure over time may prove significant effects, and could substantially impact disease progression. Although losartan has reproducibly been shown to induce significant reduction of blood pressure in mice,[18] β-adrenoceptor antagonism is less effective at altering blood pressure, despite significant reductions of heart rate.[19,20] If this were the case in *Fbn1* C1039G mice,[6] differences in blood pressure response to β-adrenoceptor antagonism between species might have accounted, at least in part, for the superiority of losartan over propranolol in *Fbn1* C1039G mice, and for the absence of any such advantage of losartan over atenolol in Marfan's patients. Another important difference is the substantial negative chronotropic effect of losartan seen in Marfan mice,[6] which has not been reported in humans.

2. **Dose.** While the definition of dose response characteristics of drugs is a basic principle of pharmacology, its application to in vivo studies with chronic end points presents practical impediments. Despite these impediments, future studies need to execute pivotal studies on dose response. While such studies would not be conceptually innovative, they would be critical to meaningful interpretation.

3. **Mode of administration.** In Marfan's syndrome, the stimulus for aortic dilation is likely to be persistent throughout the duration of a day. Maintenance of adequate inhibition of AT1 receptor throughout the day is reliant on mode of administration and pharmacokinetics of the drug. In the case of mouse studies, delivery systems such as osmotic minipumps can be used to provide consistent AT1a receptor inhibition throughout the day.[21] Losartan was administered in drinking water in the Marfan mice.[6] Mice imbibe throughout the day. Therefore, although plasma drug levels fluctuated more than administration via osmotic mini-pumps, it is likely that effective AT1 receptor antagonism occurred for major duration of the day.[6] In contrast, the humans study used a single dose per day of losartan. This mode of delivery probably led to inconsistent degrees of AT1 receptor inhibition throughout the day.

4. **Receptor affinity and pharmacokinetics of losartan.** While the sartan class of drugs has the common feature of preventing AngII stimulating the AT1 receptor, there are major divergences within the class in achieving this result. At the receptor level, there are marked differences in affinity, with telmisartan, irbesartan, and candesartan being at the upper end of the spectrum, whereas losartan is one of the least efficacious in the class. Also, half lives of drugs within this class differ

widely, with telmisartan being at the upper end of approximately 24 hours, while losartan's half life is approximately 2 hours, the shortest half life of this class.

5. **Stage of disease.** The initial studies demonstrated that aortic expansion was attenuated in *Fbn1* C1039G mice when losartan was administered during gestation. Clearly, treatment at this stage of disease could not be mimicked in human studies. It was also demonstrated that losartan attenuated aortic expansion when administered post gestation. However, even in these post gestational experiments, losartan administration was started when mice were only 7 weeks of age. Indeed, in the clinical trial, it was inferred that more benefit was present in younger patients. Clearly, more studies are required to determine whether the beneficial effects of losartan are influenced by the stage of disease advancement.

With the disappointment of this clinical trial outcome, there is the potential for switching focus to direct TGF-β inhibition. However, this switch should be approached with considerable caution. While TGF-β neutralization has been shown to decrease aortic expansion in *Fbn1* C1039G mice, this promoted fatal abdominal and thoracic aortic rupture in AngII-infused mice, even within the ascending aorta.[22,23] Moreover, loss of function mutations of TGF-β receptors lead to marked acceleration of aortic root expansion and to high incidence of early death due to dissection and rupture of the thoracic aorta,[24] even in *Fbn1* C1039G mice.[24] Therefore, additional mechanistic work in this area needs to solve these controversies before any recommendation could be made for translational studies to block TGF-β signaling in Marfan's patients.

What is the next step in pursuing whether AT1 receptor antagonism is helpful for the devastating effect of aortic expansion and rupture in Marfan's patients? More clinical data will be forthcoming when the other 8 major trials are completed.[12] It will be of interest to determine whether some of the variances in designs will provide insights when other studies are completed. Of particular interest, one of the studies will use irbesartan,[25] which is a much more effective inhibitor of AT1 receptors than losartan that has been used in all other trials. Also, the affinity and pharmacokinetic properties of candesartan and telmisartan make them attractive candidates to test. In preclinical studies, more in-depth knowledge is required for the extent and duration of AT1 receptor antagonism needed to attenuate diseases to determine the optimal mode of antagonism in the clinical world. Additionally, beyond AT1 antagonism, it is important to determine whether progressive aortic dilation and rupture have distinct mechanisms that may require different approaches to therapy. Overall, we are still far away from completely understanding the mechanisms of aortic aneurysms to assist Marfan's patients by providing optimal medications. Enhanced research funds invested in this area would profoundly benefit this dire need.

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