Losartan in Marfan Syndrome—Beyond Blood Pressure Lowering


Marfan syndrome is a disease resulting from a mutation of the gene coding for fibrillin-1 (1), a protein that forms fibrils in the extracellular matrix, thus depleting elastic tissue of a key building block. As a result, the ascending aorta of these patients weakens and enlarges progressively, ending in dissection and fatal rupture, unless prophylactic surgical correction is performed (2). Because microfibrils are also constituents of the suspensory ligament of the lens, the aortic abnormality is typically associated with dislocation of the lens. With considerable phenotypic variation, further features of the syndrome are long, thin, spider-like fingers (arachnodactyly), elevated height with disproportionately long limbs relative to the trunk, hypermobility, and occasionally spontaneous pneumothorax. Untreated aortic dilation is a ticking time bomb: Without surgical replacement of the aortic root (2), the median life expectancy is reduced by half. The only prophylaxis available today are β blockers—without definite proof of their efficacy, but with the underlying rationale that apart from absolute pressure values in the aorta it is also the rise in pressure (dp/dt) that has a deleterious impact on progressive aortic dilation, both parameters of aortic pressure are reduced in parallel by β blockers.

The disease is catastrophic, but rare. Why does the nice piece of translational work by Habashi *et al.* command wider interest and exhibit relevance even for nephrologists? The original concept is certainly too simple, *i.e.*, that the primary defect, loss of function mutation of the gene *FBN-1* in humans (or the corresponding *Fbn-1* in mice) coding for fibrillin-1 (1), accounts fully for the observed clinical abnormalities. Previous studies (3,4) had documented overactivity of TGF-β. Dietz (4) had hypothesized that fibrillin-1 normally binds TGF-β in the aorta, thus inactivating the cytokine. He speculated that in mice with a mutation of the *Fbn-1* gene the absence of fibrillin-1 permitted unbridled activity of TGF-β. This hypothesis prompted him to administer in the perinatal period TGF-β antibodies to the mutant mice, with the rationale that blocking the overshooting TGF-β activity would attenuate tissue damage (4). The experiment actually showed that progressive lung damage with impaired pulmonary alveolar septation as well as myxomatous thickening of the cardiac atrioventricular valves were prevented (5). Further findings also suggested that fibrillin-1 did not only play a structural role in connective tissue, but also an additional role as a regulator of the cytokine TGF-β. The working hypothesis was that TGF-β overactivity played a causal role in the aortic abnormality with aberrant thickening of the aortic media, fragmentation and disarray of elastic fibers, as well as increased collagen deposition (6,7). The hypothesis of a causal role of TGF-β was supported by increased nuclear translocation of factor pSmad2, an indicator of TGF-β signaling (8–12), in the aorta of mutant mice (13) as well as Marfan patients.

In various models of organ fibrosis, losartan had previously been shown to interfere with TGF-β signaling. To test the working hypothesis, the authors administered the angiotensin receptor blocker losartan to Fbn1/C1039G/+ mice. Pregnant mice heterozygous for the *Fbn-1* allele with a cystein/glycine exchange in position 1039 received losartan in their drinking water, beginning at 2 wk of gestation (in humans this would be contraindicated because of the impact of renin-angiotensin system blockade on renal development). This was continued throughout lactation and administration to the pups was also continued after weaning. Controls received placebo or propranolol (β blockers are currently the agent of choice in Marfan syndrome).

What were the results? The authors looked at:

- elastic fiber fragmentation in the aorta
- aortic wall thickness
- aortic dilation
- nuclear accumulation of pSmad2 as a marker of TGF-β action

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The long and the short of it is that losartan improved all parameters relative to placebo and also relative to propranol.

Improvement, although not complete normalization, of aortic geometry and elastic fiber fragmentation was also seen when losartan treatment was started postnatally (as would be necessary in Marfan patients). It is unlikely that blunting of the hemodynamic stress accounts for the superiority of losartan over propranol, among others, because (1) benefit was also seen after administration of TGF-β antibodies; (2) losartan but not propranol antagonized TGF-β signaling as evaluated by pSmad2 nuclear staining; and (3) improvement of disease manifestations was also seen in the lungs, organs not subject to hemodynamic stress.

The finding is mechanistically plausible, since signaling through the AT1 receptor increases the expression of TGF-β ligand and receptor. It further leads to thrombospondin-1 upregulation, which activates TGF-β.

The results of this nice piece of translational research has led to the design of a National Institutes of Health–sponsored trial in young patients (6 mo to 25 yr old) with Marfan syndrome to compare the effects of treatment with losartan to those with a β blockers (14).

For several reasons this study is of interest to nephrologists. First, TGF-β signaling plays a key role in the genesis of renal and cardiac fibrosis, and this is one more piece of evidence that inhibition of the AT1 receptor has antifibrotic effects (15–19).

A second, broader implication is this: Despite justified hope in the long-term success of gene transfer techniques, this approach will certainly not be a panacea and will not be around the corner soon. The above study exemplifies that monocular fixation on genetic strategies may not be the best way to go. Better insight into pathomechanisms of genetic disease may lead to clinically useful interventions even before the hypothetical genetic manipulations will eventually become available, if at all.

Thorough understanding of pathomechanisms in hereditary diseases has already led in some spectacular successes without so much as touching the gene. Examples include regression of astrocytoma by rapamycin in tuberous sclerosis (20), treatment of cystinosis with cysteamine (21,22), pharmacologic inhibition of tyrosine catabolism in hereditary tyrosinemia type 1 (23), and vitamin E substitution in pseudo-Friedreich disease (24), to mention just a few to illustrate the principle.

The potential power of this approach in nephrology is illustrated by recent work in a model of Alport’s disease (25). Mutations or deletions of the α3(IV), α4(IV), or 5(IV) chains of type IV collagen in the glomerular basement membrane (GBM) lead to abnormal GBM material, which is more susceptible to proteolytic removal by the three isotypes of metalloproteinases (MMP)-2, -3, and -9. Interestingly, genetic ablation of either or both MMP-2 or MMP-9 causes compensatory upregulation of other MMP. In contrast, in the model of the α3(IV) knockout mouse, if started early on, pharmacologic inhibition of a broad spectrum of multiple GBM-degrading MMP by a cocktail of MMP inhibitors attenuates the progression of GBM abnormalities. It also impressively delays progression of proteinuria and increase of serum creatinine. (For reasons that are not completely understood, once proteinuria has set in, inhibition of MMP aggravates progression and worsens tubulointerstitial fibrosis, suggesting that there is a “time window of opportunity”). Underscoring the potential clinical relevance of this finding, MMP-2, MMP-3, and MMP-9 levels by immunoblotting were also increased in the kidneys of patients with cross-linked Alport’s disease.

An ancient saying states that there is more than one way leading to Rome. Although genetic manipulation is certainly the king’s highway, it pays off to also consider the more modest pharmacologic interference with pathomechanisms of genetic diseases.

References


Bridgham J.T., Carroll S.M., Thornton J.W.

Sidore Edelman (1) had difficulty publishing his groundbreaking paper on isolation and characterization of the mineralocorticoid receptor (2), because reviewers argued—not without good reasons—that he might have gotten the wrong receptor. They objected that its affinity was admittedly greatest for aldosterone, but considering the fact that the intracellular concentration of glucocorticoids was higher by a factor of $10^3$, the receptor should be stimulated in humans mainly by cortisol in vivo. When the human mineralocorticoid receptor was cloned, the structural and functional kinship with the glucocorticoid receptor was readily apparent (3).

Today we know that one trick to resolve the dilemma of shared affinities is preceptor metabolism, i.e., local conversion of cortisol to inactive 11-keto congeners, cortisone, and 11-dehydrocorticosterone by the 11β hydroxysteroid dehydrogenase isoenzyme type 2. These products have markedly lower affinity to the mineralocorticoid receptor. This is the main, potentially not the only, mechanism that protects the promiscuous receptor from stimulation by the “wrong” ligand and guarantees relative ligand specificity, at least in the classic sodium transporting target organs (4–6).

Nature obviously has difficulty keeping apart within the cell the ligands that trigger mineralocorticoid and glucocorticoid actions respectively (7,8). Part of the answer may be found in the evolution. Recently the hypothesis had been proposed that the mineralocorticoid and the glucocorticoid receptors are the result of a gene duplication step in the earliest stages of the evolution of the vertebrate lineage (9). Hidden behind this curiosity is a more fundamental question. It was addressed in the above paper Bridgham et al. (10) and goes to the heart of Darwin’s hypothesis of selection: In systems comprising a corresponding specific ligand (hormones) and receptor (ligand-activated regulators) respectively, how can selection drive the selectivity of the receptor’s affinity if the hormone is not yet present? Can a ligand, e.g., a hormone, contribute to the evolution of a receptor “in absentia”? This question is pertinent since the mineralocorticoid receptor existed (10) well before aldosterone synthase, i.e., CYP11B2, was present (8).

In the evolution of vertebrates, the ancestral corticoid receptor (from which both the glucocorticoid and the mineralocorticoid receptors are thought to have descended by gene duplication) is found only in the most primitive species, i.e., jawless fish (Petromyzon marinus) and hagfish (Myxina gelatinosa). Even in the relatively primitive cartilaginous fish (elasmobranch), e.g., Raja erinacea, distinct glucocorticoid and mineralocorticoid receptors already exist. Phylogenetic analysis indicated that the duplication which separated the two receptors occurred approximately 450 million years ago, the time when cartilaginous fish had separated from more primitive jawless fish and hagfish, but before the split between cartilaginous fish (elasmobranch) and bony fish (teleosts) had occurred.
In a next step, the investigators assessed the hormone specificities of the receptors. The data showed that in higher species, *i.e.*, teleosts (bony fish) and tetrapods (quadripeds), the “basal” receptors were activated by low concentrations of both aldosterone (mineralocorticoids) and cortisol (glucocorticoids) or 11-deoxycorticosterone. In contrast in higher species, *i.e.*, teleosts and tetrapods, the only receptor which is insensitive to aldosterone is the glucocorticoid receptor. Applying Occam’s razor (*i.e.*, the postulate by the 14th century friar that for the explanation of a phenomenon one should make as few assumptions as possible), the authors concluded that the ancient receptor was activated by aldosterone as well as by glucocorticoids, while in the higher species of bony vertebrates the aldosterone sensitivity was restricted to the remaining ancient steroid receptor, this sensitivity having been lost by the novel glucocorticoid receptor.

This sounds like a nice hypothesis, but how can this postulate be proven? In a methodologic tour de force, the authors resurrected the ancestral receptor with its broad glucocorticoid and mineralocorticoid specificity, aligning extant receptor sequences (full discussion of which is beyond the scope of this communication) and inferring the most likely amino acid sequence of the ancient ligand binding domain (maximum likelihood approach). To provide further evidence they expressed in cultured cells the molecule with the sequence they had thus inferred. In a next step, they monitored the effect of ligand binding using the reporter assay technique. The resurrected ancient receptor was activated by low doses of desoxycorticosterone (DOC) and, to a lesser extent, by cortisol. Using Bayesian phylogenetics, plausible ancestral trees were reconstructed and the plausibility of the constructs was tested by mathematic modelling.

Nature is parsimonious and the same or modified molecules are used over and over again—for related or completely different purposes. A telling example is a remarkable finding in Argentina: The cause of a serious problem of farmers, entque secco, *i.e.*, hypercalcemia of cows which then fail to produce milk, results from the synthesis of $\text{1,25(OH)}_2\text{D}_3$ by the plant solanum malacoxylon (11,12)—obviously, as a plant, without bones, parathyroids, or need of calcemia control. Other examples are ligands interacting with the mammalian estrogen receptor in plants (13,14), although plants obviously have no female reproductive organs akin to the mammalian ones.

Why should nephrologists now care about steroid receptors in a lamprey? The complexity of the evolution of this system may help explain the enormous complexity of the mammalian mineralocorticoid system which, beyond the classic role in vectorial transepithelial sodium transport, plays an increasingly important pathogenetic role in nonepithelial tissues such as heart (15), vessels (16,17), or kidney (18). Such insight has recently led to novel treatment strategies in cardiac (19,20) and renal (21–23) diseases. What is interesting, and still unresolved, is the issue of why in nonepithelial tissue where $11\beta$ hydroxysteroid dehydrogenase isoenzyme type 2 is not coexpressed with the mineralocorticoid receptor, glucocorticoids ordinarily fail to mimic the effect of aldosterone (7). And even more puzzling is why the deleterious effect of aldosterone on nonepithelial tissues is dependent upon, and provoked by, high salt intake, although high salt suppresses endogenous aldosterone. A recent study suggests that the missing link is generation of reactive oxygen species under high salt intake and stimulation of the (unprotected) promiscuous mineralocorticoid receptor by glucocorticoids, despite the low aldosterone concentration (24). Against this background some more simple paradigms of the past may require revision.

The Nestor of nephrology, Homer W. Smith, would be delighted to see how studying issues “from fish to philosopher” helps us better understand human pathophysiology.

References