Treatment of Alport syndrome: beyond animal models

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Alport syndrome (AS) is a hereditary glomerulopathy due to abnormal composition of the glomerular basement membrane, leading to end-stage renal disease (ESRD). Studies of animal models of AS have suggested a variety of potentially effective therapies, but none of these has been definitely shown to prevent or delay ESRD in human AS. Studies in Alport mice suggest that angiotensin inhibition not only has antiproteinuric effects but suppresses cytokine and collagen production as well as tubulointerstitial fibrogenesis and inflammation. For these reasons, many Alport patients are treated empirically with angiotensin antagonists. Cyclosporine may reduce proteinuria in AS, but the risk of nephrotoxic side effects complicates long-term therapy in children. Current data on the role of HMG-CoA reductase inhibition are sparse, so therapy should be limited to adults with dyslipoproteinemia. Results of some, but not all, studies suggest that bone marrow–derived cells may ameliorate disease in Alport mice. However, until experimental doubts concerning the superiority of bone-marrow transplantation over other treatments are resolved by additional investigation, human research subjects should not be exposed to cell-based therapies that may carry substantial risks. In summary, all potential therapies are off-label use in children. As a consequence, initial therapeutic trials should focus on the safety and efficiency of medical treatment, as well as the optimal timing of therapy.

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Alport syndrome (AS) is an inherited basement membrane disorder characterized by a progressive hereditary nephropathy leading to end-stage renal disease (ESRD) in all affected males and many affected females, hearing loss in 60–80% and ocular lesions in 25–40% of affected males. Numerous mutations in the COL4A5 gene encoding the α5-chain of type IV collagen have been described in families with X-linked AS.[1] Autosomal recessive AS arises from mutations in both alleles of COL4A3, which encodes the α3(IV) chain, or COL4A4, which codes for the α4(IV) chain. Heterozygous mutations in COL4A3 and COL4A4 cause thin basement membrane nephropathy.[2] Hematuria is the cardinal symptom of thin basement membrane nephropathy and AS. Almost 1% of the population are heterozygous carriers for mutations in the autosomal Alport genes and have thin basement membrane nephropathy, progressing to renal failure in 5–20%.[2]

Mutations in COL4A5 that severely alter the α5(IV) chain (such as large rearrangements, premature stop, or frameshift mutations) result in an early-onset of ESRD at a mean age of 20 years and increased prevalence of extrarenal symptoms. In contrast, mutations altering the quaternary structure of type IV collagen (such as small in-frame mutations or glycine missense mutations) cause a later onset of ESRD (mean age about 30 years).[2,3]

Mutation analysis in patients and families with suspected AS adds significant information for genetic counseling and can replace diagnostic renal biopsy in many cases. Further, molecular genetic diagnosis before the onset of proteinuria would theoretically allow early effective medical intervention. Preemptive therapy with the ACE inhibitor ramipril in an Alport mouse model prolonged lifespan until death from renal failure by more than 100%.[4] These encouraging results raise the possibility that early therapy before the onset of proteinuria might also delay renal failure in humans with AS. As a consequence, mutation analysis of the very large collagen genes has begun to be offered on a professional basis.[5] The chain of events in AS leading from a defective basement membrane to progressive renal fibrosis, however, remains unclear and is a subject of further studies.

Although animal studies have presented us with a variety of potentially effective therapies for Alport kidney disease,[2,6] none of these potential treatments has been formally tested in

Alport populations, and no pharmacological or biological agent has been definitely shown to prevent or delay the development of terminal renal failure in people with AS. The purpose of this brief review is to assess the opportunities for clinical trials in AS and the challenges such trials will face.

Alport nephropathy is essentially cured by renal transplantation. The success of renal transplantation in Alport patients is similar – if not superior – to outcomes in patients with structural renal disease.[3,7] AS does not recur in the allograft as long as the organ donor does not have the disease. Antiglomerular basement membrane nephritis in the allograft, though devastating in individual patients, is fortunately a rare complication of transplantation for AS.[3] While remaining conscious of the morbidities of chronic renal failure and the limitations on the availability of donor organs, we should be cognizant of the high success rate of transplantation when we consider the potential risks of intervention for patients and the possible impacts on transplantation.

This review will discuss data generated by studies of pharmacological and biological interventions in animal and human AS. As the majority of animal studies involve transgenic mice, it is important to be aware of the strain effects on the Alport phenotype in mice.[8,9] The discussion of pharmacological intervention is divided into three categories – angiotensin antagonism, cyclosporine, and other treatments – because most of the 'other' treatments involve drugs with which there is relatively little clinical experience, particularly in children. The discussion of biological interventions will focus on stem cell therapy.

PHARMACOLOGICAL THERAPY

Angiotensin antagonism

Both angiotensin-converting enzyme inhibition (ACEI) and angiotensin receptor blockade (ARB) have been shown to suppress proteinuria, delay loss of renal function, and prolong survival in *Col4a3*$^{-/-}$ mice with autosomal recessive AS, raised on a 129 genetic background (Figures 1 and 2).[4,10] The most impressive results were observed in mice in which continuous treatment with ramipril was initiated before the onset of proteinuria; duration of survival was doubled in these mice.[4] The beneficial effects on urine protein excretion, renal function, and survival were associated with reduced glomerular and tubulointerstitial fibrosis. In addition, therapy resulted in the downregulation of proteins thought to be key players in human Alport pathogenesis, such as transforming growth factor-β1, connective tissue growth factor, matrix metalloproteinases 2 and 9, and type I collagen.[10] Studies of angiotensin antagonism in autosomal recessive AS mice on the B6 background or in mice with X-linked AS (XLAS) have not been reported. ACEI therapy slowed the decline of renal function in canine XLAS.[11]

To date, only uncontrolled, relatively short-term studies of the effect of angiotensin antagonism on established proteinuria have been reported in human AS.[12–14] ACEI appears capable of transiently reducing protein excretion,[12] an effect that may be augmented by aldosterone inhibition.[15] Although the mechanisms by which angiotensin antagonism ameliorates Alport kidney disease remain uncertain, evidence from animal studies suggest that the suppression of cytokine and collagen production by podocytes and the antifibrotic and anti-inflammatory effects in the tubulointerstitial compartment may be more important than the antiproteinuric and antihypertensive effects.

Cyclosporine

Cyclosporine therapy slowed the progression of the glomerular basement membrane changes and the deterioration of renal function in canine XLAS.[16] Although there have been no randomized controlled trials of cyclosporine therapy in human AS, uncontrolled studies have shown that cyclosporine can reduce urine protein levels in Alport patients.[17,18] A study of eight Alport males who received cyclosporine for 7–10 years suggested slower progression to ESRD in comparison with related affected males who did not receive such treatment, without development of histological evidence of cyclosporine nephrotoxicity after 5 years of treatment.[17] However, another group of investigators found that cyclosporine therapy in similar doses resulted in reduced proteinuria and decreased inulin clearances in children with AS.[18] Four of five patients who underwent renal biopsy after 20–27 months of therapy exhibited cyclosporine nephrotoxicity.[18]

The mechanisms by which cyclosporine might have a beneficial or harmful effect on Alport kidney disease remain uncertain. Cyclosporine may interfere with alterations in the podocyte cytoskeleton or type IV collagen turnover.

Other pharmacological therapies

Studies of *Col4a3*$^{-/-}$ mice with autosomal recessive AS, raised on a 129 genetic background, have shown the beneficial effects of a variety of pharmacological approaches, including anti-transforming growth factor-β1 antibody,[19] inhibition of matrix metalloproteinases,[20] vasopeptidase inhibition,[21] chemokine receptor 1 blockade,[22] HMG-CoA reductase inhibition, [23] and bone morphogenetic protein-7.[24] There are no reported studies of these approaches in other murine or canine models of AS.

BIOLOGICAL INTERVENTION

Gene- and cell-based therapies aim at repairing the underlying defect in AS: the defective assembly of α3/4/5(IV) collagen. Replacement of the defective genes by gene therapy in models of AS has not been successful thus far. Delivery of sufficient copies of the normal collagen genes to the appropriate glomerular location is one of the difficult challenges that will need to be overcome to make gene therapy of AS successful.[25] Although new gene vectors (and their efficacy and safety) still have to be established, two research groups have reported that wild-type bone marrow-derived cells can ameliorate disease in Alport mice by differentiation of stem cells into podocytes secreting the missing collagen α3/4/5(IV) chains [26,27] – basically a curative