Pharmacological Research 107 (2016) 205–210

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Perspective

RAAS inhibition and the course of Alport syndrome

Isavella Savva[a], Alkis Pierides[a,b], Constantinos Deltas[a,*]

[a] Molecular Medicine Research Center and Laboratory of Molecular and Medical Genetics, Department of Biological Sciences, University of Cyprus, Cyprus
[b] Department of Nephrology, Hippocrateon Hospital, Nicosia, Cyprus

ARTICLE INFO

Article history:
Received 18 November 2015
Received in revised form 4 March 2016
Accepted 14 March 2016
Available online 16 March 2016

Keywords:
Renin angiotensin aldosterone system
Angiotensin converting enzyme inhibitors
Angiotensin receptor blockers
Aldosterone blockade
Alport syndrome
Thin basement membrane nephropathy

ABSTRACT

Alport syndrome (AS) is a hereditary progressive glomerulonephritis with a high life-time risk for end-stage renal disease (ESRD). Most patients will reach ESRD before the age of 30 years, while a subset of them with milder mutations will do so at older ages, even after 50 years. Frequent extrarenal manifestations are hearing loss and ocular abnormalities. AS is a genetically heterogeneous collagen IV nephropathy, with 85% of the cases caused by mutations in the X-linked *COL4A5* gene and the rest by homozygous or compound heterozygous mutations in either the *COL4A3* or the *COL4A4* gene on chromosome 2q36-37. There is no radical cure for the disease and attempts to use various stem cell therapies in animal models have been met with ambiguous success. However, effective treatment has been accomplished with pharmacological intervention at the renin-angiotensin-aldosterone system (RAAS), first in animal models of AS and more recently in humans. Angiotensin converting enzyme inhibitors (ACEis) and angiotensin receptor blockers (ARBs) have been shown to significantly delay the progression of chronic kidney disease and the onset of ESRD. Also, renin inhibitors and aldosterone blockade were used with positive results, while the combination of ACEis and ARBs was met with mixed success. An important study, the EARLY-PROTECT, aims at evaluating the efficacy of ACEis when administered very early on in children with AS. Novel therapies are also tested experimentally or are under design in animal models by several groups, including the use of amniotic fluid stem cells and synthetic chaperones.

1. Introduction

Alport syndrome (AS) is a hereditary progressive nephritis, characterized by persistent microscopic hematuria since childhood and the subsequent onset of proteinuria and renal impairment. Sensorineural hearing loss and typical ocular changes may also be present. It is caused by mutations in *COL4A3*, *COL4A4* or *COL4A5* genes encoding the type IV collagen α3, α4 and α5 chains, which are major components of the glomerular basement membrane (GBM) [1,2]. Approximately 85% of patients inherit the X-linked form of the disease (XLAS), whereas 15% the autosomal recessive (ARAS) one [3,4]. Currently, there is no curative treatment for AS, so all males with the X-linked disease and all male and female patients with the autosomal recessive disease will eventually develop end-stage renal failure. Subjects who are heterozygous for a mutation in the *COL4A3* or *COL4A4* gene are considered carriers of ARAS and at the same time express an autosomal dominant form of microscopic hematuria due to thin basement membrane nephropathy (TBMN). A subset of these heterozygous patients, the exact percentage of which varies according to the populations studied, progress to renal impairment and even end-stage renal disease (ESRD) on long follow-up. Several authors refer to this condition as an autosomal dominant form of AS, some of whom may or may not have mutations in the *COL4A3/COL4A4* genes [5–9]. Over the past several years, it has become increasingly evident that more patients reach ESRD due to TBMN than due to classical X-linked or ARAS, even though this occurs at a much older age. This is not surprising, as TBMN is a much more frequent condition, with an estimated prevalence of about 0.3–1% in the general population [10–12]. Therefore, it is important to note that ideally any future treatments should aim to prevent altogether the onset of ESRD. However, in view of the difficulties encountered in achieving this goal, any treatment resulting in delaying the onset of ESRD is a significant progress.

Large scale studies in adults with diabetic and non-diabetic kidney disease have shown that renin angiotensin aldosterone system (RAAS) inhibitors significantly delay the progression of kidney disease [13–17]. Specifically, angiotensin-converting enzyme

Abbreviations: RAAS, renin angiotensin aldosterone system; ACEi(s), angiotensin converting enzyme inhibitor(s); ARB(s), angiotensin receptor blocker(s); AS, Alport syndrome; ARAS, autosomal recessive AS; XLAS, X-linked AS; TBMN, thin basement membrane nephropathy; ESRD, end-stage renal disease; CKD, chronic kidney disease; SP, spironolactone.

* Corresponding author at: Molecular Medicine Research Center Department of Biological Sciences University of Cyprus 1, University Avenue, 2109 Nicosia, Cyprus.
E-mail address: Deltas@ucy.ac.cy (C. Deltas).

http://dx.doi.org/10.1016/j.phrs.2016.03.017
1043-6618/

Table 1
Summary of animal and human studies on RAAS inhibition in Alport syndrome.

Reference	Pharmaceutical agent	Study	Adverse effects	Main Results
[18]	Enalapril	XLAS samoyed dog	no	increased survival, improved renal histological and biochemical profile
[19]	Ramipril	ARAS mouse	no	increased survival, beneficial effects of preemptive treatment, ramipril as an antifibrotic agent
[20]	Ramipril vs Candesartan	ARAS mouse	no	greater increase of survival with ramipril, stronger antifibrotic effect
[21]	Aliskiren	ARAS mouse	no	increased survival
[22]	Enalapril	7 AS children (5–14 years)	orthostatic hypotension, transient reduction of creatinine clearance	significant variation regarding proteinuria reduction
[23]	Enalapril	10 AS children (3–14 years)	no	significant variation regarding proteinuria reduction, escape phenomenon
[24]	Enalapril	11 AS children	no	without effect on albuminuria or TGF-β1 and nitrite urine levels
[25]	Losartan vs Placebo/Amlodipine	30 AS patients (1–17 years)	no	greater proteinuria reduction in the losartan group
[26]	Losartan vs Enalapril	27 AS patients (1–17 years)	Renal function impairment, hypotension, hyperkalemia (low incidence rates)	further reduction of proteinuria in the enalapril group
[27]	ACEis	238 XLAS and ARAS patients	Hyperkalemia, dry cough, symptomatic hypotension/fatigue (low incidence rates)	delayed age at onset of ESRD in a time-dependent manner
[32]	ACEis and/or ARBs	189 carriers of XLAS and 29 carriers of ARAS	no	delayed age at onset of ESRD
[27]	Ramipril	Early stage XLAS and ARAS patients (2–18 years)	?	? ongoing trial aiming to evaluate the safety and efficacy of ramipril in pediatric patients
[34]	Spironolactone (additionally to lisinopril or lisinopril/candesartan)	5 AS patients (11–19 years)	no	proteinuria reduction
[35]	Spironolactone	10 AS patients (9–24 years)	Gynecomastia (1/10 patients)	proteinuria reduction, urinary TGF-β1 levels reduction

inhibitors (ACEis) and angiotensin II type I receptor blockers (ARBs) exert antiproteinuric and nephroprotective effects, in addition to their antihypertensive action. Based on these findings, it is not surprising that these therapeutic agents were also used as treatment for AS patients, even when interventional clinical trials had not yet been conducted ensuring their safety and efficacy in AS pediatric patients (Table 1).

2. The use of RAAS inhibitors in Alport syndrome dogs

The first evidence of ACEis' beneficial effect in AS was in 1997, when a Samoyed dog model with X-linked hereditary nephritis was treated with enalapril [18]. Affected pups in this study carried a mutation in the *col4a5* gene and the male ones developed proteinuria by 12–16 weeks of age and died due to renal failure around the age of 7 months. Male pups were divided in 4 groups: normal-treated with enalapril, normal-untreated, affected-treated with enalapril and affected-untreated. Enalapril was initiated at 4 weeks of age and treatment was continued until dogs reached 40 weeks or developed uremic symptoms. Affected dogs treated with enalapril showed less GBM splitting, delayed onset of serum creatinine elevation, slower rate of proteinuria increase and delayed onset of weight loss. Most importantly, there was a 36% increase of survival. It is important to note that all the above beneficial effects of ACEis were not followed by a parallel blood pressure reduction. No adverse effects were noted, even though the enalapril dose used was four times higher than the one used for the treatment of congestive heart failure in dogs.

3. The use of RAAS inhibitors in Alport syndrome mice

Recognizing the potential benefits of ACEis, Gross et al. proceeded to the treatment of 122 homozygous *col4a3*-knockout mice on the 129/SvJ background with ramipril [19]. This model of AS represents a non-hypertensive model of progressive renal fibrosis. Mice were categorized in four groups: (A) No treatment; (B) Late therapy starting at week 7 (proteinuria > 3gr/L); C) Early short therapy, starting at week 4 until week 10 (before the onset of proteinuria until uremia development); and (D) Early long therapy, starting at week 4 until death. No obvious side effects of the treatment were reported. In group B, urine protein and serum urea levels were significantly reduced, but the onset of urea elevation was not delayed and lifespan was not significantly increased. Early treatment resulted in a delayed onset of proteinuria by 2 weeks and in a more than 80% reduction of urine protein levels in week 9. A slight increase of proteinuria levels was noted in group C, after ramipril was discontinued. Urea elevation was also postponed in the early treatment groups by 3 weeks. Remarkably, lifespan was increased by almost 50% and more than 100% in groups C and D respectively, highlighting the importance of preemptive intervention. Unlike the dog model with X-linked AS, GBM abnormalities were noted in all mice groups, treated or not. However, effacement of podocyte foot processes was delayed. Experiments focusing on the renal matrix were also performed. Immunohistochemistry, western blot, as well as light and electron microscopy studies, indicated the additional nephroprotective effects of ramipril as an antifibrotic agent. Moreover, the role of TGF-β1 in AS progression was emphasized, as it was noted that TGF-β1 levels were increased in the *col4a3*-knockout mice and early ramipril therapy resulted in reduced TGF-β1 expression.

One year later, the effects of ACEis vs Angiotensin II receptor (AT1) antagonists on the same *col4a3*-knockout mouse model were evaluated [20]. A cohort of 114 homozygous *col4a3*-knockout mice were categorized in 3 groups: (A) Untreated mice; (B) Mice on ramipril treatment starting at week 4; (C) Mice on candesartan treatment, also starting at week 4. No adverse effects were noted in either of the treated groups. Both pharmaceutical agents reduced proteinuria levels by more than 50%, having only a minor effect