Alport syndrome—is there a genotype–phenotype relationship?

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Alport syndrome (AS) is a relatively frequent monogenic human disease (estimated prevalence of 1:5000), affecting type IV collagens in specialized basement membranes, and featuring progressive nephritis, sensorineural hearing loss, ocular lesions, and typical changes of the glomerular basement membrane (GBM). Interestingly, AS shows a wide range of phenotypic expression, including purely renal forms, as well as extrarenal features such as hearing loss, ocular and haematological findings, and diffuse smooth muscle cell benign tumours (see below). AS can be further associated with juvenile (<31 years) or adult onset (>31 years) of end-stage renal disease (ESRD). The variability of the clinical phenotype can be partially explained by genetic and molecular heterogeneity. AS is in fact caused by mutations in any of several genes encoding various type IV collagen chains. The culprit AS genes are COL4A3 and COL4A4 on chromosome 2q35–37, and COL4A5 on chromosome Xq22. The great majority of families show ‘classic’ X-linked dominant inheritance, but rarer autosomal forms have been increasingly reported. Furthermore, within a given gene, many different mutations can be responsible for disease, so called allelic (or molecular) heterogeneity, which is also the rule in most human genetic diseases (for a comprehensive review on AS see reference [1]).

Molecular basis for AS classification

Before the availability of molecular genetic diagnosis, the classification of AS was based mainly on clinical parameters. Now, based on molecular genetic parameters, AS can be classified in at least four different nosological entities, and if we also take into account familial benign haematuria (FBH), the ‘AS phenotype’ can be included in the larger group of the ‘type IV collagen disease phenotypes’ (Table 1).

Spectrum of COL4A5 mutations in large AS population screenings

Recent studies from large AS populations (more than 640 patients extensively screened) have described over 140 small mutations in the COL4A5 gene (Table 2) [2–5]. The mutations are spread all over the gene, with no evidence of mutational hot spots. All types of changes were detected, including missense, frameshifts, nonsense, small deletions and insertions, and splicing mutations. Importantly, even in selected subpopulations of patients with clear AS diagnosis and X-linked inheritance pattern, the mutation detection rate was less than 50%. Several reasons might account for this discrepancy: low sensitivity of the screening methods,

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Gene(s)</th>
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<tbody>
<tr>
<td>X-linked AS with deafness</td>
<td>COL4A5</td>
</tr>
<tr>
<td>X-linked AS without deafness</td>
<td>COL4A5</td>
</tr>
<tr>
<td>X-linked AS with leiomyomatosis</td>
<td>COL4A5 and A6</td>
</tr>
<tr>
<td>Autosomal recessive AS</td>
<td>COL4A3 or A4</td>
</tr>
<tr>
<td>Autosomal dominant AS</td>
<td>COL4A3 or A4 (?)</td>
</tr>
<tr>
<td>Autosomal dominant BFH*</td>
<td>COL4A3 or A4</td>
</tr>
</tbody>
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*Benign familial haematuria.

Table 2. COL4A5 mutation screening results obtained in some recent large AS population surveys

<table>
<thead>
<tr>
<th>AS patients studied (n)</th>
<th>Mutations identified (n)</th>
<th>COL4A5 exons screened (n)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>201</td>
<td>48</td>
<td>51</td>
<td>2</td>
</tr>
<tr>
<td>250</td>
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<td>10</td>
<td>3</td>
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<td>131</td>
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<td>60</td>
<td>22</td>
<td>51</td>
<td>5</td>
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<tr>
<td>642</td>
<td>141</td>
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and mutations in non-coding areas, such as introns or regulatory regions; mutations within the adjacent COL4A6 gene appear not likely to cause disease [3]. Finally, no particular phenotypic features distinguished the families where mutations were found from the other families.

**Autosomal inherited AS and benign familial haematuria (BFH)**

The rarer autosomal recessive AS phenotype has been demonstrated by the identification of pathogenic mutations in COL4A3 and COL4A4 genes [6]. Typically, these families lack a positive history, females appear as severely affected as males, and the asymptomatic parents are often consanguineous. Patients normally reach ESRD before the age of 30. The correct molecular identification of this type of families can bear crucial implications for genetic counselling [7].

Autosomal dominant AS has been sporadically reported, often in association with haematological (platelet and granulocyte) alterations. Recently this very rare form was also linked to the COL4A3/A4 region on chromosome 2q35–37, but no mutations have been found (Jefferson et al., abstract, Third International Workshop on Alport Syndrome, Erlangen, Germany, September 1994).

BFH is an autosomal dominant condition characterized by GBM thinning and normal renal function, sometimes difficult to differentiate from the initial stages of AS. Interestingly, BFH has been recently linked to a mutation in the COL4A4 gene [8] and possibly COL4A3 [9], raising the attractive suggestion that BFH patients might be manifesting heterozygous carriers of autosomal recessive AS. However, further studies are needed to unravel the molecular mechanisms by which the BFH phenotype does not progress towards ESRD. It's also tempting to speculate that the severe autosomal recessive AS and the milder BFH phenotypes represent two extremes, due to different ‘dosages’ of COL4A3/A4 mutations, homozygous and heterozygous respectively.

**AS and diffuse leiomyomatosis: a novel contiguous gene syndrome?**

At least 35 cases have been now described of AS associated with diffuse oesophageal leiomyomatosis, a phenotype also belonging to the AS spectrum (see [1]). Both COL4A5 and COL4A6, which lie just 450 bp apart in a head-to-head fashion, are affected, usually by large deletions removing the 5’ ends of the two genes. These findings indicate still unknown but important role(s) of the two genes in the normal processes of morphogenesis, differentiation and proliferation in smooth-muscle cells. The proliferative changes are probably induced by aberrant and inappropriate alpha 6 (IV) chain synthesis triggered by an alternative distal promoter, spared by the deletion [10].

**Genotype/phenotype correlations**

One of the most relevant clinical benefits of molecular studies is the significant correlation between the age of onset of ESRD in AS and the nature of the underlying COL4A5 mutation. In fact, mutations predicted to lead to truncated or absent alpha 5 (IV) chain, such as severe inactivating (null) mutations (frameshifts, nonsense, and large rearrangements), consistently cause juvenile-type disease, usually with deafness. The lack of the C-terminal NC (non-collagenous) domain of the molecule might prevent the critical step of triple helical formation, with dramatic alterations of the protein and thus severe derangement of the GBM structures. Conversely, amino acid substitutions (missense changes) can lead to either juvenile or adult-onset AS, and virtually all adult cases bear either splice-site mutations or missense changes involving glycine substitutions, which affect the proper folding of the molecule. Nonetheless, these mutations might allow some type IV collagen processing and assembly.

**Conclusions and future perspectives**

Why can such diverse clinical phenotypes in AS arise from different mutations in the same gene? Explaining the underlying mechanism(s) of this phenomenon, which has also been referred to as ‘phenotypic diversity from allelic series’ [11], is not easy. One reason could be the different functional domains involved by the mutations, causing different consequences. Other factors might involve interactions of additional mutations and/or ‘benign’ polymorphisms in the same gene, with modification of clinical expression. A family with very severe AS has been described bearing two missense mutations on the same COL4A5 allele [4]. Finally, interactions among mutations in different, ‘modifier’ genes, commonly referred to as our ‘genetic background’, might also cause phenotypic diversity. This in turn could explain the common phenomena of incomplete penetrance and variable expression, so puzzling to geneticists. Known examples of quantitative and qualitative phenotypic diversity due to allelic heterogeneity are Duchenne and Becker muscular dystrophies (dystrophin gene in Xp21.2), achondroplasia and hypochondroplasia (FGFR3 gene in 4p16.3), cystic fibrosis and bilateral absence of vas deferens (CFTR gene in 7g31.2), and several more [11]. In AS, however, the phenotypic variability appears mainly quantitative, reminiscent of the wide spectrum of disease in CFTR mutation carriers, associated with different levels of mRNA transcript [12].

The ‘phenotype’ results from complex ontogenic and developmentally regulated steps, where genes and non-genetic factors interact. In this scenario the genotype appears as a necessary but not sufficient component. Thus, even ‘simple’ monogenic traits should be considered multifactorially determined, and the genotype/phenotype relationship expected to be irregular and variable.
The recent spectacular and dramatic achievements in DNA-based technology and molecular genetics have greatly increased our understanding in many aspects of human genetic disorders, and have shed a little more light on the molecular mechanisms underlying genetic heterogeneity, with relevant clinical consequences in the management of AS families, such as adequate genetic counselling and identification of asymptomatic carrier females. For example, detection of a COL4A5n (n=3, 4, or 5) mutation is now another accepted criterion for the molecular diagnosis of AS, both prenatally and postnatally. As for the possibility of providing a ‘molecular prognosis’ to our AS patients, we might be able to predict early or late onset disease, whether or not a severe inactivating mutation is found. However, no predictions can be made about extrarenal symptoms. Molecular genetic advances also allowed new disease classification based on genotype, rather than phenotype, thus changing our very clinical and medical attitudes. Finally, new hopes and promise for new specific molecular therapeutic strategies can now be envisaged [13]. Although much is still to be learned, we might soon be able to dissect the basis of different clinical phenotypes at the molecular level, particularly the interplay between different genes and their products. And this of course will be a daunting but challenging task.

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References


Renal function in the elderly—is the dogma of an inexorable decline of renal function correct?

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Senectus ipse morbus (Seneca, AD 65)

Renal function

Past literature apparently took a cue from the above motto that age in itself is a disease and propagated the view that with senescence various indices of renal function, most notably glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), decrease inexorably. In retrospect, this concept has arisen for several reasons: (i) use of inadequate measures of GFR, i.e. serum creatinine concentration and endogenous creatinine clearance, which are known to be confounded by changes in muscle mass and problems in urine collection, (ii) cross-sectional studies examining patients with comorbid conditions which confound the effect of age, particularly hypertension, atherosclerotic vascular disease, heart failure, diuretic treatment, malnutrition, and (iii) secular changes, e.g.
today the elderly tend to be, on average, more healthy than in the past. Because of the important effect of confounding variables it is of note that only one longitudinal study has been published that extended over more than 20 years. Despite the use of serial measurements of creatinine clearance as a less than ideal marker of GFR, the ‘Baltimore Longitudinal Study on Aging’ is a milestone insofar as it revealed two important facts: (i) in no less than one-third of apparently healthy elderly subjects there was no decrease of GFR at all, and (ii) a decrease in GFR was found to be associated primarily with cardiovascular diseases (e.g. hypertension, oedema of unknown origin) [1]. Recent studies showed that in the healthy elderly GFR (by inulin clearance) is mostly within the normal range, although lower than in younger individuals [2,3]. An exception is the elderly with consuming diseases and heart failure. In elderly patients with mild compensated heart failure (NYHA I-II) GFR is significantly lower than in normotensive healthy elderly subjects [3]. We conclude that in the elderly a major decrease of GFR is uncommon in the absence of disease. Apparently, the population of elderly is heterogenous. It is of considerable interest that centenarians are, on average, more healthy than the average octagenarian [4]. This observation is compatible with the view that a subgroup of healthy elderly stay alive, while the more frail ones die off (healthy survivor effect). This underlines again how important it is to take care in selecting subjects for studies.

How about more subtle indices of renal function? Effective renal plasma flow is reduced proportionally more than GFR and vasodilatation in response to agonists such as acetylcholine or amino acids is diminished [2,5]. As a consequence, even in the healthy elderly, filtration fraction (FF) is increased [3]. The kidney is vasoconstricted similarly to what also occurs in extrarenal vascular beds. These subtle age-related abnormalities of renal haemodynamics are more marked in patients with cardiovascular disease. They are accompanied by, or predispose to, changes in renal electrolyte handling and hormonal status, i.e. increased proximal tubular sodium reabsorption, decreased plasma renin activity, low calcitriol, and elevated parathyroid hormone concentration [3].

Renal structure

It is well known that ageing is associated with some loss of renal mass, but not all renal structures are affected in a similar fashion. Involution of renal tissue and glomerulosclerosis occurs primarily in the renal cortex with relative sparing of the renal medulla [5,6]. Selective loss of cortical and preservation of juxtamedullary nephrons with higher FF may explain, at least in part, the rise in FF with age. Not only does one see sclerosis of glomeruli, but also sclerosis of larger renal vessels. In addition, a high prevalence of abnormalities was reported in the renal vasculature, e.g. tapering of interlobular arteries and increased tortuosity of intralobular arteries [7]. The pattern of glomerular and vascular changes differs according to renal zones: whereas in the cortex hyalinization and collapse of the glomerular tufts goes in parallel with obliteration of preglomerular arterioles, anatomical shunts between afferent and efferent arterioles are commonly seen in the juxtaglomerular region [7]. If one evaluates studies examining age-related changes in renal structure it is again important to exclude the elderly with disease. This includes not only the elderly with hypertension, but also the elderly with atherosclerosis of the abdominal aorta [8]. In the past elevated mean arterial blood pressure has been recognized as one factor affecting renal function. It may be equally important to take into consideration diminished aortic elasticity, which causes an increase in pulse pressure with important consequences for the pulse profile and the mechanical stress to which peripheral vessels are exposed. More careful exclusion of patients with comorbid conditions may explain, at least in part, why more recent post-mortem (and imaging) studies find a less severe decrease in renal size and less glomerulosclerosis with ageing than did studies in the past [8,9].

Missing information

Consideration of the above methodological points will help in the future to ask more intelligent questions and to analyse the data in a more sophisticated fashion. In-depth analysis of the effect of ageing is hampered by the fact that the general mechanisms of ageing are poorly understood. Accumulation of mutations in somatic DNA, particularly in mitochondrial DNA, irreversible commitment of stem cells to terminally differentiated cells, accumulation of oxidative damage and advanced glycosylation end-products have all been proposed as playing a role in ageing [4]. None of the proposed mechanisms has so far provided a satisfactory explanation for age-related changes in renal function.

What are the clinical consequences?

Better understanding of the effects of ageing of the kidney may have implications for (i) use of kidney from elderly donors for organ donation, (ii) pharmacokinetics of drugs cleared by the kidney, and (iii) understanding of the age-specific prevalence of different renal disease in the elderly. The incidence of renal failure from various renal diseases increases proportionally with age. This may be due to one or more of the following factors: (i) a higher prevalence of the conditions leading to renal disease in the elderly, particularly hypertension, type 2 diabetes mellitus, atherosclerosis, and vasculitis, (ii) increased susceptibility of the kidney of the elderly to the renal complications of the above diseases, and (iii) accelerated
progression, since some studies indicate that in the elderly renal prognosis is more adverse, particularly in postinfectious glomerulonephritis, IgA glomerulonephritis, and vasculitis [10].

Conclusion

There is good news and bad news. The bad news is that we do not completely understand the mechanisms of change of renal function with age (although the important role of hypertension implicates a novel approach in prevention). The good news is that the healthy elderly apparently does not experience serious deterioration of renal function, in contrast to what was thought in the past.

References


Diabetes and renal failure in Indo-Asians in the UK—a paradigm for the study of disease susceptibility

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Introduction—disease susceptibility in Indo-Asians

There are large Indo-Asian communities in several cities in the United Kingdom. In Leicester for example there are 60,000 Indo-Asians, 20% of the population of the city. They originate from the Indian subcontinent and the term Indo-Asian is preferred to avoid confusion with many studies of Asians from North America which usually refer to populations originating mainly from Japan and South East Asia. Patterns of disease in Indo-Asians contrast markedly with those in indigenous White populations; in particular Indo-Asians have a strikingly increased prevalence of coronary heart disease, type II diabetes, and renal disease, as well as susceptibility to tuberculosis [1]. The type II diabetes has a relatively young age of onset and prevalence increases with age: 30% of the Indo-Asian population aged greater than 60 years are diabetic.

The population at risk for the development of ESRD due to diabetic nephropathy is therefore increased in an Indo-Asian community. But the Indo-Asian diabetic also has an increased risk of developing nephropathy compared to a White diabetic. This seen as a greater prevalence of proteinuria among Indo-Asians in a diabetic clinic, a susceptibility independent of glycaemic control or hypertension [1]. But in Leicester the risk of an Indo-Asian diabetic developing ESRD is also increased to more than 10 times that of a White diabetic [2]. This very powerful effect appears to reflect susceptibility rather than risk of progression once diabetic nephropathy has occurred—neither rates of progression nor control of blood pressure differ between Indo-Asian and White diabetics.

The Indo-Asian susceptibility to renal disease is not restricted to diabetic nephropathy. Indo-Asians have an increased incidence of immune renal disease, which may not progress to renal failure—steroid sensitive nephrotic syndrome in children and lupus nephritis [1]. ESRD due to non-diabetic renal disease is also three to five times as common in Indo-Asians; this includes ESRD due to biopsy-proven glomerulonephritis, chronic pyelonephritis and renal failure of uncertain cause presenting with shrunken kidneys [3]. It has been suggested that tuberculosis may play a role in the renal failure of undetermined cause, but this is unproven [3].

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Other high-risk populations

Are there comparable populations elsewhere in the world? African Americans have an excess of type II diabetes but also have severe hypertension, which is not seen in Indo-Asians. Similar increases in diabetes and renal failure are also seen in Polynesian populations in Australasia. The closely studied Pima Indians in Arizona, USA have the world’s highest incidence of type II diabetes. The vast majority of ESRD in the Pimas occurs in diabetics, and of that virtually all is attributable to diabetic nephropathy [4]. In contrast, another group of American Indians, the Zunis of New Mexico, carry a very high risk of ESRD due to glomerulonephritis, with little diabetic nephropathy [5].

The Indo-Asian paradigm

The Indo-Asian therefore carries risks seen in both the Pimas and the Zunis, and has a most unfortunate ‘double jeopardy’—susceptibility to type II diabetes and to renal disease. This unhappy coincidence provides a challenge for health care provision for ESRD in areas with large Indo-Asian communities; but also an opportunity to pursue mechanisms of renal disease.

Indo-Asians have come to Britain either directly from the Indian subcontinent, or via stable migrant communities in sub-Saharan Africa. This migration occurred very rapidly over 20 years from the late 1960s. This rapid move of a large population in a short time offers an unrivalled opportunity to compare the pathogenic influence of genetic and environmental factors, including the prenatal environment at which time it is thought that individual susceptibility to disease later in life may be ‘programmed’

Genetic factors

Familial clustering of nephropathy is well described in type I diabetes. There is some evidence that this susceptibility is inherited as a familial predisposition to hypertension marked by increased velocity of the red-cell sodium–lithium countertransport system. However no role for abnormalities in this system have been found in type II diabetes. Nor is there any proven influence of ACE gene polymorphisms in type II diabetes despite some evidence that this polymorphism may be linked to risk of progression of renal failure in other disease groups. Nevertheless there is familial susceptibility to nephropathy in African Americans with type II diabetes and in Pima Indians there is also clustering in families. Our own observations suggest a similar familial susceptibility to nephropathy in Indo-Asian diabetes but this has not been formally studied. There have been no studies of the sodium–lithium countertransporter nor of ACE gene polymorphisms in Indo-Asians; but studies of these and other candidate genes may soon prove informative.

Environmental and in utero environmental factors

In migrant populations all over the world, including Indo-Asians, there has been an ‘epidemic’ of type II diabetes, which may be explained in part by environmental influences—the consumption of an energy-rich processed ‘western’ diet combined with a reduction in physical exercise. Neel [6] proposed a ‘thrifty genotype’ (rapid insulin release promoting fat storage and reduced calorie wastage), which would confer survival advantage during times of fasting but during prolonged feasting (urbanization) would confer risk of insulin resistance, central obesity, and diabetes. Barker et al. [7] has suggested that maternal undernutrition during pregnancy results in an increased susceptibility to type II diabetes; thus the ‘thrifty genotype’ should be renamed the ‘thrifty phenotype’ and syndrome X be called the ‘small-baby syndrome’. He proposed that the fetus is ‘programmed’ in utero by irreversible changes in response to the fetal environment. Mackenzie and Brenner [8] have combined the concept of ‘programming’ with that of ‘nephron dose’ suggesting that nephron number at birth may be the missing link between birthweight and hypertension. Continuing organogenesis of the kidney into the third trimester, with induction of some 60% of the normal complement of nephrons during this period, could make the kidney uniquely susceptible to maternal malnutrition, which would reduce nephron number. There is emerging evidence that fetal kidney growth is disproportionately restricted in the small-for-gestational-age fetus [9] and evidence that women at risk of diabetic nephropathy is doubled between those with lowest and highest birthweights, although no such relationship in men was found [10].

As yet assessment of birthweight and subsequent risk has not yet been undertaken in Indo-Asians. Nevertheless low birthweight would be an attractive explanation for the increased prevalence of type II diabetes, and might also explain, because of reduced nephron dose, the increased incidence of all forms of renal disease in Indo-Asians. (However, Mackenzie and Brenner’s hypotheses do not fit the Indo-Asian population in their entirety, since there is susceptibility to renal disease and diabetes without hypertension and renal failure is so common despite a vegetarian low-protein diet.)

The great majority of Indo-Asians in Britain who are now developing ESRD due to diabetic nephropathy were born in India or Africa, and reliable birthweight data are not available. However, birthweights among Indo-Asians in Leicester are lower than in the equivalent White population, although any influence of changing birthweight on the prevalence of type II diabetes and renal failure will take some generations to be detected with certainty.

Conclusion

The geopolitics which resulted in the Indo-Asian migration to Leicester may afford a unique opportunity to
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Hypertension in haemodialysis patients: is it only hypervolaemia?

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Cardiovascular complications remain the leading cause of death in haemodialysis (HD) patients [1]. Hypertension predisposes to structural abnormalities of the cardiovascular system and is identified as an important risk factor for cardiovascular disease in this population [1,2]. Therefore, adequate diagnosis and treatment of hypertension in HD patients is a logical step in the prevention of cardiovascular morbidity and mortality in this population.

Definition of hypertension in HD patients: Which is the biologically most relevant blood pressure value?

Some problems arise with the diagnosis and definition of hypertension in HD patients. Pre-dialytic values may overestimate inter-dialytic blood pressure because of factors such as volume overload or the accumulation of vasoactive factors in the inter-dialytic period (to be discussed later), whereas post-dialytic blood pressure may be influenced by the dialysis procedure itself. The fact that refill of plasma volume from the interstitial volume is not yet completed after dialysis, a phenomenon that is most pronounced after short and rapid ultrafiltration [3], and at underhydration, may lead to an underestimation of inter-dialytic blood pressure by post-dialytic values. In consequence, intravascular underfilling during dialysis may be followed by a rapid rise of blood pressure in the first hours after dialysis. This could explain the discrepancy between the findings of Cheigh et al. [4], who observed a return to predialytic values within 12 hours after rapid and short ultrafiltration and the results of Battistella et al. [5] and our group [6], in which blood pressure values gradually returned to pre-dialytic values during the inter-dialytic period. In the latter studies, post-dialytic values appeared to be more representative for inter-dialytic values [5,6]. Therefore, the sole use of pre-dialytic values may lead to overestimation of the incidence of hypertension, whereas post-dialytic blood pressure may lead to an underestimation of intradialytic blood pressure because of intravascular underfilling. In doubtful cases, inter-dialytic ambulatory blood pressure measurements may be helpful. Ambulatory blood pressure measurements also informs us about the day–night blood pressure rhythm, which is often disturbed in uraemic patients [6]. In patients with essential hypertension, it was found that ambulatory blood pressure measurements correlated better with end-organ damage compared with office blood pressure [7]. If this also holds true for dialysis patients remains to be elucidated.

What is the role of volume overload?

Undoubtedly, a major role in the pathogenesis of hypertension in HD patients is played by fluid retention, as the majority of HD patients become normotensive after dialysis treatment is initiated and the excess volume is removed. However, the relation between

References


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volume overload and hypertension is certainly not cle... First, when the volume status of patients treated... in the pathogenesis of hypertension in dialysis patients [26]. Using micro-electrodes to assess sympathetic nerve discharge, they observed an increased sympathetic activity in haemodialysis patients (most of them being hypertensive) in comparison with healthy subjects. This sympathetic overactivity appears to be related to the diseased kidney, as in dialysis patients who had undergone bilateral nephrectomy, sympathetic nerve discharge equalled that of healthy controls. The mechanism behind the increased sympathetic nerve activity in dialysis patients remains as yet speculative. Possibly, uraemic toxins or ischaemic metabolites stimulate renal afferent nerves, either directly or via renal chemoreceptors, leading to an increase in sympathetic nerve activity through central nervous pathways [27]. Indeed, in rats with experimentally induced renal failure, an increased turnover of norepinephrine was observed in the locus coeruleus and posterior hypothalamic nuclei, which play an important role in the regulation of sympathetic nerve activity [28]. All these data prompt further research towards the role of an increased sympathetic activity in the pathogenesis of hypertension in HD patients. For instance, it would be very interesting to investigate whether differences in blood pressure between HD patients (at equal fluid status) are related to differences in sympathetic activity.

How does haemodialysis treatment affect long-term control of blood pressure?

An adequate blood pressure control with an incidence of hypertension below 5% was achieved by long treatment time dialysis (three times a week for 8 h), as performed in the Tassin centre [29]. Blood pressure levels in these patients are even lower compared with the healthy population [30]! Although these results might suggest that volume overload is the only factor responsible for hypertension in dialysis patients, there are arguments that suggest that other factors play a role. First, when the volume status of patients treated in Tassin was compared with that of patients treated with shorter haemodialysis (three times a week for 4 h), only small and non-significant differences in volume status (assessed by echocardiography, alfa-ANP and echography of the inferior caval vein) were observed, whereas the incidence of hypertension was manifold higher in the group treated with shorter dialysis. The main haemodynamic finding in the patients treated with long dialysis was a reduced peripheral vascular resistance [30]. In a recent prospective study, the influence of both extra volume removal and...
increase in dialysis time was studied independently. It appeared that both a decrease in dry weight without a change in dialysis time and an increase in dialysis time without a change in dry weight, lead to a significant improvement in blood pressure control [31]. Could indeed an increased removal of uremic toxins, implicated in sympathetic overactivity, play a role in the blood pressure lowering effect of long dialysis? A role for middle molecules is suggested by the decreased incidence of hypertension with the use of haemofiltration, as found by Quellhorst et al. [32]. It certainly appears worthwhile to address these important pathophysiologic questions in further research.

**Treatment of hypertension in HD patients**

What should be our target blood pressure in the treatment of hypertension in dialysis patients? Until now, this cannot be elucidated from present studies. However, it is probably advisable to be aggressive in the treatment of hypertension in dialysis patients, as in a recent study it was shown that patients with a mean arterial pressure below 99 mmHg had a significantly better survival compared with patients with higher blood pressure levels [29].

Furthermore, we think that it is desirable to look at the relationship between hypertension and the impact of antihypertensive treatment on end-organ damage, as a clear relation exists, e.g. between echocardiographic abnormalities (left ventricular mass and volume) and long-term prognosis of dialysis patients [33]. In our clinic, we aim to perform echocardiography once a year in our dialysis population.

How should hypertension be treated in HD patients? First, fluid and salt restriction is of utmost importance. The patients should be ultrafiltrated until optimal dry weight, preferably assessed with the help of an objective method, such as echography of the inferior caval vein (not to be performed immediately after dialysis) or bio-impedance measurements. Short and rapid ultrafiltration should be avoided, as this may lead to blood pressure drops and therefore, inadequate volume removal. In patients in whom ultrafiltration is difficult because of massive inter-dialytic weight gain or cardiac problems, certain manoeuvres may be of value, such as fluid removal by isolated ultrafiltration, sodium modelling or the use of intra-dialytic blood volume measurements. One should be very careful with excessive lowering of dry weight, as this may result in serious intra-dialytic morbidity and even in cerebrovascular and coronary ischaemia.

When patients remain hypertensive, adjunctive treatment with antihypertensive agents should be initiated. Until now, there are few controlled data that suggest the use of any agent as first line treatment in dialysis patients. However, converting-enzyme inhibitors are often very effective, which is in line with the activated renin-angiotensin system in uremic patients. Moreover, in a recent randomized study in patients with end-stage renal disease it was shown that, in contrast to a dihydropyridine calcium antagonist, the use of an ACE-inhibitor resulted in a significant decrease in left ventricular dimensions [34]. Whether this will result in decreased cardiovascular morbidity or mortality remains to be elucidated. When despite multi-drug antihypertensive therapy the patient still remains hypertensive, increasing dialysis time might be an option.

In conclusion, hypertension in the dialysis population is not a simple phenomenon, but has a complex and intriguing pathophysiology. Further research should provide more insight into the complex role of volume and vasopressor systems in the pathogenesis of hypertension in dialysis patients, and should address the role of blood pressure control in reduction of cardiovascular morbidity and mortality in dialysis patients.

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Role of growth factors in acute renal failure

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Key words: acute renal failure; EGF; HGF; IGF-I; peptide growth factors

Introduction

Endogenous peptide growth factors play important roles during the recovery of the kidney from acute renal failure (ARF). Moreover, in laboratory animals with experimentally induced acute renal injury, exogenous administration of recombinant human peptide growth factors, such as epidermal growth factor (EGF), insulin-like growth factor I (IGF-I), and hepatocyte growth factor (HGF), all have been shown to accelerate the recovery of renal function and histological architecture. These observations have generated great enthusiasm for the use of these agents in elucidating biological mechanisms of growth and healing and as new potential therapeutic avenues.

Acute renal failure and tubular epithelial cell cycling

In acute tubular necrosis in humans and laboratory animals most of the tubular damage and subsequent regeneration occurs in the S2/S3 segments of proximal convoluted tubules and in the thick ascending limb of the loop of Henle (TALH). Tubular epithelial cells are highly differentiated, metabolically very active cells, which are normally quiescent, and cells reside in G0 of the cell cycle. However, within minutes to hours after acute, transient injury, such as a period of ischaemia, epithelial cells primarily in the proximal tubule and the TALH re-enter the cell cycle, and the injured nephron segments become mitogenically highly active. The mitosis index increases by several orders of magnitude as compared to the baseline state.

The effect of EGF, IGF-I and HGF on injured tubular cells

This cell cycle re-entry of tubular cells is orchestrated by many genes, including immediate early genes. Several peptide growth factors participate further
downstream in the chain of events. Epidermal growth factor (EGF), insulin-like growth factor I (IGF-I), and hepatocyte growth factor (HGF) are among the best-studied peptides. These growth factors participate in the cell transition into G1 of the cell cycle. Growth-factor-induced accelerated cell cycle transition of tubular cells during repair after acute injury is an important function of these peptides. However, it is not the sole mechanism of function through which these peptides contribute to accelerated recovery after acute ischaemic renal injury. Growth factors may also reduce the incidence of apoptosis of injured cells. Although apoptosis is commonly observed in the experimental settings of in vitro injury to cultured tubular cells or in vivo in rats with acute ischaemic tubular injury, its role in human ARF is unclear. There may be several other metabolic actions of these growth factor peptides which contribute to repair of tissue integrity.

The role of EGF

EGF and EGF receptors are normally expressed in TALH and distal tubules, but EGF receptors may also be present in the basolateral membrane of proximal tubule cells. After ischaemic injury, distal tubular EGF expression and the urinary excretion of EGF decrease and both virtually disappear transiently, and EGF and its receptor become expressed in proximal tubules at sites of injury [1]. The decrease in distal tubular EGF expression is caused by disease events that interrupt the function of an upstream region in the preproEGF gene promoter [2].

Exogenous administration of EGF in rats with nephrotoxic ARF accelerates the recovery of renal function [3]. This report was the first demonstration of in vivo activity of a recombinant growth factor peptide to accelerate the recovery from ARF. This effect of EGF results, at least in part, from direct mitogenic action of the peptide on proximal tubular cells as suggested by series of studies in cultured cells.

The role of IGF-I

IGF-I is more widely expressed along the nephron compared to other growth factor peptides. However, in normal rats and man IGF-I is not, or is only minimally, expressed in proximal tubules. In contrast, IGF-I receptors are found abundantly in basolateral as well as apical membranes of proximal tubules. After ischaemic ARF in the rat, IGF-I is transiently expressed in some proximal tubular cells and possibly by migrating macrophages suggesting a paracrine action of IGF-I in the natural repair after tubular injury [4]. Several investigators have demonstrated that the exogenous administration of human recombinant (rh) IGF-I in rats with ischaemic ARF accelerates the recovery of renal function, the restoration of anatomic integrity and reduces mortality [5,6] (for review see also [7]). Cell culture studies suggest that IGF-I is mitogenic in proximal tubular cells, but also reduces the incidence of apoptosis [8]. The importance of the latter finding is unclear. Other actions of IGF-I that may contribute to improved outcome in rats with experimental ARF include the induction of EGF receptors allowing for further augmentation of paracrine/autocrine actions of EGF [9]. IGF-I also activates inducible nitric oxide synthase (iNOS) generating increased amounts of nitric oxide (NO). Since iNOS isoenzyme is also expressed in proximal tubular cells [10], increased tubular NO activity may help to regulate mitosis, but its precise function is unclear. Inhibitors of NOS reduce the IGF-I-accelerated rate of recovery from ARF in rats [11]. Whether this results from the reduction of IGF-I-induced microvascular or tubular NO activity or both is unknown. In any event, these findings give rise to the possibility that the IGF-I-induced activation of iNOS contributes significantly to the recovery from acute renal injury in the rat.

Effects of IGF-I on catabolism and nephron haemodynamics

In patients with chronic renal failure and in rats with acute renal failure rhIGF-I reduces muscle protein catabolism and promotes protein synthesis [5]. Since rats and patients with ARF are often severely catabolic, this effect of rhIGF-I may also contribute to accelerated recovery. In addition, rhIGF-I has been shown to raise renal blood flow and GFR in normal rats and humans and in patients with chronic renal failure [7]. The biological activities that may all contribute to mechanisms through which IGF-I accelerates the recovery from ischaemic ARF in the rat are summarized in Figure 1. These multiple benefits suggest that among different recombinant growth factors rhIGF-I may be particularly useful in the treatment of patients with ARF.

Lack of conclusive clinical evidence of a therapeutic role of rhIGF-I in acute renal failure

To date two clinical trials have been performed to examine the efficacy of rhIGF-I to accelerate the recovery from acute renal failure. In a single-centre trial, patients undergoing surgical repair of abdominal aortic aneurysms received rhIGF-I or placebo for 3 consecutive days [12]. Treatments were commenced shortly after conclusion of the surgical procedure. Hence, at the time of initiation of therapy it was unknown whether ARF was present in a given patient since serum creatinine may not have yet risen. In this study the incidence of ARF was remarkably low, the study was underpowered, and the effect of rhIGF-I on the incidence or recovery of ARF could not be assessed. However, there was a trend for rhIGF-I to improve GFR in these patients.

In another multicentre, placebo-controlled, randomized clinical trial, 72 patients with established ARF...
secondary to transient shock, sepsis and other causes were treated with placebo or rhIGF-I, 100 μg subcutaneously twice daily, for up to 2 weeks. However, rhIGF-1 did not accelerate the recovery of renal function as measured by iothalamate clearance, creatinine clearance or urine flow rate and there was no benefit in mortality rate in the rhIGF-I-treated subjects [13]. Thus, in contrast to many (but not all) *in vivo* studies in laboratory animals, both clinical trials in humans thus far have failed to demonstrate that rhIGF-I accelerates the recovery from ARF. This apparent discrepancy may be explained by the underlying diseases causing ARF. In the rat models either transient ischaemia or defined nephrotoxic events were used to induce limited and well defined renal injury. In contrast, in the multicentre clinical trial, most patients had severe underlying or associated diseases instead of a single transient event.

**Role of HGF**

HGF is expressed in the normal kidney primarily in interstitial cells, and proximal tubules express c-met, the HGF receptor. In laboratory animals with ischaemic or tubulotoxic ARF there is increased expression of HGF and c-met occurring within 12 h of injury, suggesting involvement of this growth factor in the repair process in a paracrine mode [14]. In mice and rats with ARF and recently in a dog model of renal allograft acute tubular necrosis, several investigators demonstrated that exogenous rhHGF accelerates the recovery of renal function and tubular integrity [15,16]. In the latter dog model of renal allograft acute tubular necrosis, a single injection of HGF resulted in better recovery of renal function than six injections of rhIGF-I given over a period of 3 consecutive days [16]. At present there is no data on the efficacy of HGF or any other growth factor peptide to reduce the incidence of delayed renal allograft function in patients.

**Role of TGF-β**

EGF, IGF-I, and HGF (and possibly several other growth-factor peptides) are transiently expressed or overexpressed at sites of nephron injury in experimental or human ARF and seem to contribute to G<sub>1</sub> transition and cell cycle progression resulting in mitogenic recovery of injured tubules. TGF-β<sub>1</sub> expression is also increased in regenerating renal tubules in rats with ischaemic ARF [17]. Since TGF-β<sub>1</sub> in many biological settings causes cell cycle arrest, its antimitogenic activity may help to offset the mitogenicity caused by several other factors and events. However, this is at present hypothetical, and there may be other functions of TGF-β<sub>1</sub> in ARF that are not yet identified.

**Summary and conclusions**

EGF, IGF-I, and HGF are involved in the endogenous tissue repair after acute renal injury. All three growth factors accelerate the recovery of renal function and the anatomical restoration of tubular integrity when given exogenously to laboratory animals with experimental ARF. However, clinical study of the therapeutic efficacy of recombinant peptide growth factors in ARF is limited to rhIGF-I. Both clinical trials of rhIGF-I in patients with ARF have been indeterminant or
negative. Thus, the therapeutic use of peptide growth factors may not be the magic bullet for the cure of a disease that contributes significantly to morbidity and mortality in severely ill patients. However, there is ample experimental basis for further clinical study of recombinant human peptide growth factors in ARF.

References


Biologically active peptides in acute renal failure: recent clinical trials

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Key words: atrial natriuretic peptide; insulin-like growth factor I; clinical trials

In acute renal failure (ARF), proven therapies that have passed the test of rigorous state-of-the-art clinical trials are not available. Unfortunately, this list of failed trials just has become longer. Recently, two clinical trials examined the efficacy of insulin-like growth factor I (IGF-I) to improve outcome in patients with ARF [1,2]. One of these studies has been published in a full-length manuscript [2], and data from the other trial has been reported in abstract form [1]. Both studies had been initiated following several years of experimental research indicating that IGF-I accelerates the recovery of renal function and reduces mortality in rats with ischaemic or toxic ARF [3–9]. The experimental studies suggest that recombinant human IGF-I (rhIGF-I) accelerates the recovery of renal function due to the renal haemodynamic effects, its mitogenic properties, other metabolic actions, and possibly due to its effect of preventing apoptosis of tubular cells.

Atrial natriuretic peptide (ANP) also has been demonstrated to accelerate the recovery of renal function in rats with ARF. ANP appears to induce arteriolar dilatation and reduce the rate of NaCl absorption resulting in improved supply and reduced demand of oxygen and substrate. Moreover, in a single centre, open label clinical study, Rahman et al. had demonstrated that infusions of ANP raise the creatinine clearance and reduce the need for haemodialysis in patients with ARF [10].

These results gave rise to the hypothesis that treatment with each of the two peptides would improve outcome in patients with ARF and clinical trials of both rhIGF-I and a 25-amino acid synthetic form of...
ANP in patients where ARF had been initiated independently.

**A single centre clinical trial of rhIGF-I after renal cross-clamping**

Franklin et al. recently reported their findings in 54 patients who underwent surgery of the suprarenal aorta or the renal arteries requiring periods of renal ischaemia of 60–70 min [2], similar to the period of renal ischaemia that is used in most experimental studies in rats. In this study patients were randomized to receive either rhIGF-I, 100 μg/kg s.c., or placebo every 12 h for a total of 72 h beginning shortly after the surgery. Patients underwent measurements of the creatinine clearance before surgery and at 24, 48, and 72 h after the operation. None of the subjects developed severe ARF requiring dialysis therapy. The creatinine clearance tended to decline below baseline in the control group. In the rhIGF-I treated patients, the creatinine clearance tended to increase, and was on average ~7 ml/min greater than the pre-surgery values at 72 h (Table 1). There was no difference with regard to several other secondary outcome measures (Table 1). The authors conclude that their ‘findings establish the feasibility and potential utility for the use of IGF-I to reduce the incidence of postoperative renal dysfunction in high risk patients’ [2].

This study, although well intended, failed to answer clinically important questions. This author is not sure what the possible benefit might be to reduce the incidence of transient ‘renal dysfunction’ when this is not associated with any improved clinical outcome. Moreover, neither creatinine clearance nor serum creatinine concentrations were different at any given time during the study or at hospital discharge. It would be difficult to justify the clinical use of an expensive treatment based on this data. The study failed to examine the important question as to the efficacy of rhIGF-I to prevent the incidence or improve the outcome of ARF in high risk patients. Simply, the study was underpowered to answer this question, since sub-scribing to the surgeons’ skills, the incidence of severe ARF requiring renal replacement therapy was nil.

**A multicentre placebo-controlled, randomized clinical trial of rhIGF-I in ARF**

Recently, data from a multicentre, placebo-controlled clinical trial of rhIGF-I to improve outcome in patients with ARF was published in abstract form [1]. Seventy-two patients with ARF had been enrolled and were randomized to receive either rhIGF-I, 100 μg/kg, or placebo s.c. twice daily for up to 14 days. In this study, treatments were initiated after ARF was established. Hence, the study evaluated whether the experimental treatment would accelerate recovery of renal function, improve diuresis, reduce the need for renal replacement therapy, or reduce mortality in patients with ARF rather than preventing ARF after potential injury. At a pre-determined interim analysis which included 72 subjects the study was terminated due to a lack of any trend that would suggest that patients with ARF may benefit from treatment with rhIGF-I (Table 2).

**A multicentre, randomized, placebo-controlled trial of a synthetic ANP derivative in ARF**

Allgren et al. studied the efficacy of a synthetic ANP-derivative (Anaritide) in patients with ARF [11]. Five-hundred-and four critically ill patients with ATN received either continuous i.v. infusions of anaritide, 0.2 μg/kg/min, or placebo for 24 h. As primary outcome measurement, the investigators examined the incidence of the dialysis-free survival rate at 21 days. Secondary end-points included the need for renal replacement therapy, changes in serum creatinine, and mortality. Analyses were also performed in prospectively-defined subgroups of patients with oligo-anuria versus normal or high urine output at baseline. There was no difference in the primary outcome, the dialysis-free 21 day survival, between placebo and ANP-treated subjects (Table 3). The subgroup analysis indicated that oliguric patients receiving ANP had a significantly

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**Table 1. RhIGF-I in patients with transient acute ischaemic renal injury**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>rhIGF-I</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>12/15</td>
<td>15/12</td>
<td>ns</td>
</tr>
<tr>
<td>Age (years)</td>
<td>66.9</td>
<td>66.6</td>
<td>ns</td>
</tr>
<tr>
<td>Type of surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abd. aortic aneurysm</td>
<td>12</td>
<td>9</td>
<td>ns</td>
</tr>
<tr>
<td>renal revascularization</td>
<td>13</td>
<td>16</td>
<td>ns</td>
</tr>
<tr>
<td>both</td>
<td>2</td>
<td>2</td>
<td>ns</td>
</tr>
<tr>
<td>Renal ischaemia time (min)</td>
<td>64.3</td>
<td>64.8</td>
<td>ns</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min) before surgery</td>
<td>52.7±5.3</td>
<td>64.6±12.5</td>
<td>ns</td>
</tr>
<tr>
<td>Change in creatinine clearance by 72 h (ml/min)</td>
<td>≃−5</td>
<td>≃±7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Serum creatinine at discharge (mg/dl)</td>
<td>1.5±0.1</td>
<td>1.5±0.2</td>
<td>ns</td>
</tr>
<tr>
<td>Length of hospital stay (days)</td>
<td>13.9</td>
<td>11.4</td>
<td>ns</td>
</tr>
<tr>
<td>Length of stay in intensive care unit (days)</td>
<td>5.4</td>
<td>4.3</td>
<td>ns</td>
</tr>
</tbody>
</table>

Data from Franklin et al. [2]; ns = not significant.
Table 2. Multicentre clinical trial of rhIGF-I in patients with established ARF

<table>
<thead>
<tr>
<th>Placebo</th>
<th>rhIGF-I</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>37</td>
<td>35</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>59%</td>
<td>46%</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>GFR* at baseline (ml/min)</td>
<td>$7.5 \pm 6.2$</td>
<td>$5.6 \pm 4.7$</td>
</tr>
<tr>
<td>Renal replacement therapy (% of patients)</td>
<td>46%</td>
<td>57%</td>
</tr>
<tr>
<td>Mortality (% of patients)</td>
<td>32%</td>
<td>34%</td>
</tr>
</tbody>
</table>

* Clearance of non-radioactive iothalamate; ns = not significant; data from Kopple et al. [1].

Improved dialysis-free survival rate (due to reduced need for dialysis, not reduced mortality) compared to placebo treated oliguric patients. In contrast, in ARF-patients with normal diuresis, ANP-treatment worsened dialysis-free survival (Table 3). There was no benefit from ANP-treatment with regard to other secondary outcome measures (Table 3).

What are reasonable conclusions?

Unfortunately, these three recent studies of bioactive peptides in patients with ARF only add to the (long) list of disappointing clinical trials. RhIGF-I appears to have no benefit in severely ill patients with ARF, and there may be a benefit of rather questionable clinical significance in patients with less severe renal injury not requiring renal replacement therapy. The findings with Anaritide are even more puzzling. Although a benefit was found in the subgroup of oliguric patients, it is disturbing to note that the outcome was worse in subjects with normal diuresis. Although statisticians accept subgroup analyses as valid if they were prospectively defined, such analyses are always more likely to provide statistically positive results. This subgroup analysis demonstrated that a greater portion of oliguric patients receiving ANP did not need dialysis therapy compared to placebo-treated individuals. This benefit may be of some clinical importance assuming that renal replacement therapy is intrinsically worse compared to intravenous administration of ANP. Anaritide is not available for clinical use and a cost-benefit analysis is impossible at this time, since Anaritide has at present no commercial price tag.

What are suitable outcome measures for clinical trials in ARF?

These recent clinical trials also raise questions regarding the optimal design of therapeutic trials in patients with ARF. The use of the primary outcome variable mortality, although the most convincing parameter, is unrealistic. ARF per se is not a lethal disease, at least in societies where continuous renal replacement therapies or haemodialysis are routinely applied. Mortality rates in severely ill patients with ARF have been reported in the range of $\sim 25\text{--}85\%$. However, these data reflect the mortality due to underlying or associated severe diseases or multi-organ failure. In short, patients die with but not of ARF. Hence, mortality is not a useful outcome parameter in therapeutic studies of ARF. Need for dialysis is also of questionable clinical value. It suggests that renal replacement therapies are intrinsically bad compared to other therapies, which may or may not be true if examined by unbiased observers. A better outcome parameter for clinical trials in ARF might be the incidence of non-recovering renal failure that requires chronic maintenance dialysis in surviving patients. In most settings, a large number of patients would have to be enrolled since only patients with long-term survival could be included in the analysis. The cost of such a trial might be prohibitive. Alternatively, to test the efficacy of an experimental therapy to improve the rate of recovery of renal function in ARF, only patients with ARF but without other severe associated diseases should be enrolled. Clinical experience indicates that outcomes in this group of patients is usually good and expensive drug therapy may not be necessary.

In summary, none of the three trials discussed above promotes enthusiasm for the use of rhIGF-I or Anaritide in patients with ARF in the clinical setting. In this author’s opinion all three studies show either

Table 3. Multicentre clinical trial of Anaritide in patients with ARF

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Anaritide</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>256</td>
<td>248</td>
</tr>
<tr>
<td>dialysis-free survival by day 21</td>
<td>121 (47%)</td>
<td>107 (43%)</td>
</tr>
<tr>
<td>mortality by day 21</td>
<td>67 (26%)</td>
<td>73 (29%)</td>
</tr>
<tr>
<td>serum creatinine concentration on day 21</td>
<td>$3.0 \pm 2.2$</td>
<td>$2.8 \pm 2.0$</td>
</tr>
<tr>
<td>Oliguric patients at baseline (n)</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>mortality by day 21 in oliguric patients</td>
<td>5 (8%)</td>
<td>16 (27%)</td>
</tr>
<tr>
<td>Non-oliguric patients at baseline (n)</td>
<td>195</td>
<td>183</td>
</tr>
<tr>
<td>dialysis-free survival by day 21 in non-oliguric patients</td>
<td>116 (59%)</td>
<td>88 (48%)</td>
</tr>
<tr>
<td>mortality by day 21 in non-oliguric patients</td>
<td>39 (20%)</td>
<td>48 (26%)</td>
</tr>
</tbody>
</table>

Data from Allgren et al. [11]; ns = not significant.
no clinical benefit [1] or statistical benefits that are of questionable clinical value [2,11]. Thus, there continues to be a need to conduct clinical research on treatments particularly for severely ill patients with ARF to define new therapeutic strategies and approaches. Any ideas?

References


Cyclosporin or tacrolimus: which agent to choose?

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Introduction

Kidney transplantation became the preferred modality for the treatment of chronic renal failure in the early 1980s when, in many centres, graft survival results approached 90% and mortality fell to less than 5% at 1 year post-transplant. There is little question that much of the credit for these results can be ascribed to the introduction of cyclosporin, which became the backbone of solid organ transplant immunosuppression. The protocols chosen at different centres; using induction antibody therapy, various steroid regimens, adding azathioprine or mycophenolate mofetil, are all essentially variations on the theme of cyclosporin-based immunosuppression.

The decade of primacy of cyclosporin is over, however, and a powerful challenger has stepped into the ring, while others are awaiting their turn. Tacrolimus (the affection for the ‘FK506’ designation is hard to undermine), despite its chemical dissimilarity to cyclosporin, is remarkably similar to it in its mode of action, clinical effectiveness, and side-effect profile [1]. There are, however, some important differences between these two agents. It is the responsibility of the transplant community to assess their relative benefits and those of the new generation of immunosuppressive drugs so as to improve on the achievements of the last decade without awakening the ever-present enemies of immunosuppression; opportunistic infection, malignancy, and other toxic complications that have the capacity to undermine the marvellous rehabilitative potential of successful transplantation.

Mode of action of cyclosporin and tacrolimus

The discovery of these remarkable drugs and the parallel efforts to understand their immunosuppressive mechanism of action have led to impressive advances in the understanding of the mechanisms of T-cell activation. Though tacrolimus is structurally unrelated to cyclosporin, its mode of action is very similar. Both bind competitively to an intracytoplasmic binding protein (FKBP for tacrolimus and cyclophilin for cyclosporin) and the combination of the drugs and their specific binding protein block the action of calcineurin phosphatase probably by impairing access to its phosphatase site [2]. Calcineurin normally serves to dephosphorylate nuclear activating factors and thereby permits their passage through the nuclear membrane. The blockade of calcineurin inhibits T-cell

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function by impairing the gene transcription of interleukin-2 and other cytokines. In addition to the central role of calcineurin inhibition in the immunosuppressive action of these drugs calcineurin inhibition may account for their remarkably similar nephrotoxic profiles. Calcineurin may regulate Na\(^+\)/K\(^+\)-ATPase activity and thereby, directly or indirectly, account for the effect of cyclosporin and tacrolimus on GFR and tubular electrolyte transport [3]. In this respect it is instructive to note that the new immunosuppressive agent sirolimus (rapamycin), which binds to FKBP but does not inhibit calcineurin, appears to be less nephrotoxic.

Tacrolimus and cyclosporin should not be administered simultaneously because of the potential for synergistic toxicities. Cyclosporin and sirolimus can be administered simultaneously since they occupy different receptors (phase 3 clinical trials of this combination are in progress). It has been suggested that it may be unwise to administer tacrolimus and sirolimus simultaneously since they both compete for FKBP. Since the degree of saturation of FKBP is incomplete, however, this concern may prove to be unfounded.

Despite the similar immunosuppressive mode of action of cyclosporin and tacrolimus there may be differences in their physiological effects. Cyclosporin has been shown, both in vivo and in vitro to have a greater impact on peripheral vascular endothelial function and release of mediators of vascular resistance [4]. This could account for the greater tendency to systemic hypertension in patients receiving cyclosporin.

**Comparative clinical effectiveness**

The preponderance of evidence would suggest that, in the dose ranges typically employed clinically, tacrolimus is a somewhat more potent immunosuppressant than cyclosporin. Tacrolimus was first introduced into clinical transplantation in recipients of liver transplants. In the multicentre randomized trials in Europe and the USA comparing its use to cyclosporin, patient and graft survival were similar, the incidence of episodes of acute rejection was less in the tacrolimus groups, while the incidence of side-effects was less in the cyclosporin group [5,6]. This pattern of results has repeated itself with remarkable consistency in equivalent studies in renal transplant recipients. In the USA phase II multicentre trial comparing tacrolimus to cyclosporin, patient and graft survival were not significantly different at 1 year post-transplant, while the incidence of acute rejection episodes in the early post-transplant period was significantly less in the tacrolimus group and the side-effect profile was more favourable in the cyclosporin group [7]. Evaluation of the tacrolimus doses administered in this study showed that the incidence of rejection and toxicity was related to trough blood levels in a manner similar to that of cyclosporin.

This latter study, like others in progress, suffers from a new problem in current transplantation clinical trials—the ‘moving goalposts’. Whereas tacrolimus-based immunosuppressive protocols may, indeed, reduce the incidence of acute rejection episodes compared to standard cyclosporin protocols, when cyclosporin is used with mycophenolate mofetil the incidence of episodes of acute rejection is not different from that achieved with tacrolimus [8–10]. The cyclosporin/mycophenolate combination has become routine therapy in many transplant centres and the results achieved set a new standard against which competing therapies need to be measured. To complicate matters further, the studies referred to above used the standard formulation of cyclosporin, which has since been replaced in most centres by the microemulsion formulation. To address the rapidly changing therapeutic environment new multicentre studies are now in progress to compare the relative benefits of varying combinations of tacrolimus, neoral cyclosporin, and mycophenolate mofetil. These studies will, in turn, need to be compared to the results of the sirolimus trials and trials with new monoclonal antibody preparations.

In all the multicentre trials noted above therapeutic efficacy has been measured in terms of the capacity to reduce the incidence of episodes of acute rejection. Though such a reduction, and with it a reduction in the use of high-dose corticosteroids and anti-lymphocytic agents, is clearly a worthy and achievable goal, it is sobering to note that none of the studies have yet shown a statistically significant improvement in short- or long-term graft survival.

It has been claimed that, compared to cyclosporin, tacrolimus is ‘steroid sparing’ in that its use safely permits a more rapid reduction or discontinuation of post-transplant corticosteroid dose. Unfortunately this claim has not been rigorously tested and it remains in the realm of ‘clinical impression’. Multicentre, placebo-controlled trials of steroid withdrawal in patients receiving the cyclosporin/mycophenolate combination are in progress in Europe and the USA and it will be instructive to compare the results to equivalent studies using tacrolimus. Either switching from cyclosporin to tacrolimus [11] or the addition of mycophenolate to cyclosporin [12] have been shown to be effective therapies for the treatment of refractory rejection, though these two manoeuvres have not been directly compared in randomized trials.

**Comparative side-effect profile of cyclosporin and tacrolimus**

Though the toxicity profile of tacrolimus is remarkably similar to that of cyclosporin there may be differences in the severity of adverse events that need to be considered when therapeutic choices are made. Both drugs are nephrotoxic and may produce acute, reversible deterioration in renal function as well as a chronic interstitial fibrosis [1]. The pattern of nephrotoxicity is essentially indistinguishable both clinically and pathologically. A haemolytic–uraemic syndrome has been described with both drugs. Hyperkalaemia and hypomagnesaemia of a similar degree and frequency are common with both [1,3].
Tacrolimus is more neurotoxic than cyclosporin. Headaches, insomnia, paraesthesias, and tremor are all more common in patients receiving tacrolimus, and dose adjustment is frequently required [7]. Coma was described when the drug was first introduced but is rarely reported now as experience with the drug has grown. Gastrointestinal adverse events manifesting as nausea, vomiting, diarrhoea are also more common in patients receiving tacrolimus. Hirsutism, gingival hypertrophy, and so-called ‘facial brutalisation’, all bothersome complications of cyclosporin use, are relatively uncommon with tacrolimus, and this difference between the drugs may be an important consideration for teenagers and other ‘cosmetically sensitive’ patients [13]. Tacrolimus may have a less unfavourable impact on the lipid profile than cyclosporin and this difference may prove to be important in long-term follow-up [14].

Both cyclosporin and tacrolimus are islet-cell toxic, though tacrolimus is more so. In the USA randomized tacrolimus trial, the incidence of new-onset insulin requirement during the first post-transplant year was 25% in patients receiving tacrolimus compared to 5% in those receiving cyclosporin, though the incidence tended to fall with time [7]. The problem was particularly notable in Black patients receiving tacrolimus, close to 40% of whom required insulin for the first time at some stage during the first year. In children receiving tacrolimus the incidence of post-transplant lymphoproliferative disease (PTLD) may reach 10%, and this may reflect both the immunosuppressive potency of the drug and their lack of prior exposure to Epstein–Barr virus [13]. In adults no clear-cut difference in the incidence of PTLD has been reported in published trials.

The annual cost of standard dose regimens of cyclosporin and tacrolimus are currently approximately equal. The increasingly popular cyclosporin/mycophenolate combination is considerably more expensive, though in the first year post-transplant both tacrolimus and the cyclosporin/mycophenolate combination may reduce the costs incurred by the requirement for OKT3 treatment of steroid-resistant rejection. The anticipated availability of generic forms of cyclosporin may well alter these financial considerations.

Which agent to choose?

Clinicians tend to be intrinsically conservative in their approach to new agents and protocols and demand persuasive arguments to change effective therapeutic practice. After more than a decade of familiarity with cyclosporin, the attitude of many transplant clinicians when reviewing the currently available data on tacrolimus may well be a matter of ‘the devil they know compared to the one that they don’t!’ This attitude may well change as new information becomes available. For the moment cyclosporin will probably remain the backbone of immunosuppressive therapy in most kidney transplant centres. Current evidence supporting the somewhat greater immunosuppressive potency of tacrolimus has already been enough to persuade many programmes performing simultaneous kidney/pancreas transplant to base their protocols on tacrolimus, and a similar policy has been applied to patients deemed to be at high immunological risk. Switching from cyclosporin to tacrolimus in patients with severe, recurrent, or refractory rejection has become common practice, though the addition of mycophenolate to cyclosporin is a rational option. In the absence of clear-cut data supporting the differential long-term benefit of any of the newly available and soon-to-be-available immunosuppressive agents and protocols, clinicians will need to continue to make their best judgements based on their individual experience and reading of the literature. Plus ça change, plus c’est la même chose.

References