Common pathophysiological mechanisms of chronic kidney disease: Therapeutic perspectives

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ABSTRACT

It is estimated that over 10% of the adult population in developed countries have some degree of chronic kidney disease (CKD). CKD is a progressive and irreversible deterioration of the renal excretory function that results in implementation of renal replacement therapy in the form of dialysis or renal transplant, which may also lead to death. CKD poses a growing problem to society as the incidence of the disease increases at an annual rate of 8%, and consumes up to 2% of the global health expenditure. CKD is caused by a variety of factors including diabetes, hypertension, infection, reduced blood supply to the kidneys, obstruction of the urinary tract and genetic alterations. The nephropathies associated with some of these conditions have been modeled in animals, this being crucial to understanding their pathophysiological mechanism and assessing prospective treatments at the preclinical level. This article reviews and updates the pathophysiological knowledge acquired primarily from experimental models and human studies of CKD. It also highlights the common mechanism(s) underlying the most relevant chronic nephropathies which lead to the appearance of a progressive, common renal phenotype regardless of aetiology. Based on this knowledge, a therapeutic horizon for the treatment of CKD is described. Present therapy primarily based upon renin–angiotensin inhibition, future diagnostics and therapeutic perspectives based upon anti-inflammatory, anti-fibrotic and hemodynamic approaches, new drugs targeting specific signaling pathways, and advances in gene and cell therapies, are all elaborated.

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waste products eventually results in the implementation of dialysis (or kidney transplant) in order to prevent azotemia, systemic organ damage and death. Due to its high prevalence and associated mortality, CKD is an important human and social burden. It is estimated that over 10% of adults in developed countries suffer some degree of CKD (De Zeeuw et al., 2005; U.S. Renal Data System, 2005). Direct cost derived from the disease consumes up to ~2% of health care system budgets, the majority of which is consumed by only ~0.1% of the population receiving dialysis in developed countries (Excerpts from the United States Renal Data System, 2000; Xue et al., 2001; Winkelmayer et al., 2002; Szczec & Lazar, 2004; U.S. Renal Data System, 2009). CKD can result from a variety of etiologically distinct causes. Presently, diabetes and hypertension are the two leading causes of CKD, although infectious glomerulonephritis, renal vasculitis, ureteral obstruction, genetic alterations, autoimmune diseases and others are also common causes of CKD. However, as the disease progresses, a common renal phenotype develops regardless of the cause. In addition to addressing the cause, a greater knowledge of the pathophysiological mechanisms underlying the common progression of CKD may unravel new targets for pharmacological intervention. In this sense, animal models have emerged as important tools for understanding the mechanisms implicated in the pathogenic process, and also for the assay of prospective therapies.

2. Hypertensive nephropathy

Hypertension is the second leading cause of end-stage renal disease (ESRD). As an example, according to the United States Renal Data System (U.S. Renal Data System, 2009), about 51–63% of all patients with CKD are hypertensive. This number grows to 90% in patients over 65 years. In the corresponding general population the incidence of hypertension is 11–13% and 50%, respectively. Hypertension causes a nephrosclerotic glomerulopathy characterized by (i) renal vasculopathy affecting preglomerular arteries and arterioles, resulting mainly from atherosclerosis, endothelial dysfunction, wall thickening and fibrosis; (ii) microvascular disease of the glomerular tuft capillaries; (iii) diffuse glomerulosclerosis and, less often, focal and segmental glomerulosclerosis (FSGS), involving damage to the filtration barrier constituents (podocytes, mesangial cells and basement membranes); and (iv) interstitial fibrosis (Rosario & Wesson, 2006). Overall renal blood flow decreases as a consequence of arteriolar vasculopathy, vascular obstruction and decreased vascular density. However, GFR initially stays relatively constant. This is due to (i) increased glomerular capillary pressure resulting from deficit or upwardly reset renal autoregulation; and (ii) damage to the filtration barrier resulting in greater permeability. Subsequently, GFR decreases as a consequence of a progressive loss of surface area, mesangial hypertrophy and increasing glomerular and peritubular fibrosis. Concomitantly, basement membrane alterations produce albuminuria and protein hyperfiltration.

2.1. The hypertension-renal damage loop

Hypertension is a common outcome of CKD regardless of etiology, which contributes to the progression of renal damage. Approximately 40% of patients with stage 2 CKD (glomerular filtration rate, GFR: 60–90 ml/min per 1.73 m² of body surface), and virtually all in stage 4 (GFR: 15–29) or 5 (GFR: <15 ml/min per 1.73 m² of body surface) are hypertensive (Rosario & Wesson, 2006). Similarly, chronically hypertensive animals and humans develop CKD as a consequence of high blood pressure, which mechanically damages renal glomeruli and renal vessels (Wiederkehr et al., 2005). Hypertension-induced CKD can be also the consequence of non-mechanical damage (e.g. increased angiotensin II (ANG-II) or decreased NO). Epidemiological correlations between hypertension and renal damage can be interpreted in both ways. In fact, hypertension may arise from subtle renal lesions (atherosclerosis and endothelial dysfunction) resulting from acquired (genetic) traits and environmental insults (Johnson et al., 2005a,b). Accordingly, nephropathy can also be viewed as a primary renal lesion that progresses in parallel to and initiates the rise in blood pressure. The multidirectional and complex relation among cardiovascular and renal disease, atherosclerosis, fibrosis and tissue hypoperfusion and ischemia, is reinforced by the fact that (i) all these conditions share common risk factors, and (ii) the same gene polymorphisms (e.g. of renin–angiotensin aldosterone system—RAAS-components) seem to be related to many (if not all) of them (Rosario & Wesson, 2006). A vicious circle is closed by renal damage, renal vascular disease and hypertension. Therefore, it is likely that entering the circle through any of these avenues ultimately generates and results in an indistinguishable pattern of disease.

A growing body of evidence suggests that hypertension arises from a renal vasculopathy initially affecting renal arteries, or from renal microangiopathy involving preglomerular arteries that eventually causes preglomerular vascular dysfunction or maladaptive remodeling and intrarenal focal ischemic damage (Johnson et al., 2005a; López-Hernández & López-Novoa, 2006). Vasculopathies and microangiopathies may be (i) caused by genetic determinants; (ii) provoked by environmental insults; (iii) secondary to systemic conditions, like atherosclerosis or the metabolic syndrome; or (iv) derived from transient and intermittent fluctuations in blood pressure, which overtime slowly inflict progressive damage to intrarenal vessels (even in the presence of a good renal autoregulation; see below in this section). Such fluctuations may be derived from hyperactive sympathetic activity arising from diverse factors that include stress, exacerbated cytokine responses, type A personality, etc. (Johnson et al., 2005).

Clinical, epidemiological and experimental correlations between hypertension and renal microvascular disease (with or without relevant renal dysfunction) are strong (Goldblatt, 1947; Sommers et al., 1958; Tracy et al., 1986; Rodriguez-Iturbe et al., 2004; Johnson et al., 2005a). Ultimately, abnormal preglomerular resistance for any given level of blood pressure and skewed autoregulation alter the precise medullary blood flow that signals for the appropriate level of natriuresis and blood pressure, leading to hypertension (López-Hernández & López-Novoa, 2006), as Goldblatt envisioned about 60 years ago (Goldblatt, 1947). It is easy to postulate how alterations in a few glomerular vessels would be able to reset the whole renal function. Affected nephrons have modified endocrine profiles (e.g. renin secretion) that alter the function of healthy or mildly injured nephrons (Sealey et al., 1988). Then, hypertension-associated renal damage would paradoxically originate from renal damage itself or, more precisely, from subtle, focal renovascular damage, where hypertension would be another mere consequence acting as a magnifying amplifier in the vicious circle of malignancy.

2.2. Hypertensive nephropathy

In general terms, both genetic [e.g. spontaneously hypertensive rats (SHR; Camp et al., 2003); stroke-prone SHR (SHR-SP; Nakamura et al., 1996)] and induced [e.g. uninephrectomy (UNX) + DOCA salt (Kretzler et al., 1994), or L-NAME administration (Van Dokkum et al., 1998)] animal models of hypertension replicate most of the essential events associated with human glomerulopathies. Hypertensive animals usually develop a clear nephropathy with renal dysfunction biomarkers and histological alterations, starting with glomerulosclerosis and progressing to nephron degeneration and tubulointerstitial fibrosis, similar to the findings in human hypertensives (Kriz et al., 1998). An exception is observed with Milan hypertensive rats, whose age-dependent nephrosclerosis is less evident than in the Milan normotensive rat (Brandis et al., 1986; Menini et al., 2004). However, in general, animal models of hypertension and hypertensive renal
damage recapitulate specific elements of the corresponding human situation to a yet undetermined degree. For example, it is unknown to what extent genetic models of hypertension mimic the genetic background that determines not only pressure rise, but also pressure-independent neuroendocrine influences that modulate the extent and mechanisms of pressure elevation and end organ damage (Stoll & Jacob, 2001).

In this sense, the human corresponding idiopathic (also termed primary or genetic) hypertension is difficult to represent using a single animal model. First, because animal models usually have homogeneous genetic backgrounds derived from in-bred selection, which is at odds with the inter-racial and inter-individual heterogeneity of the human genome. Second, because the term idiopathic hypertension probably embraces etiologically different diseases, phenotypically indistinguishable with present-day knowledge, further variability is introduced. For example, atherosclerosis is present in a high proportion of cases of human idiopathic hypertension, whereas it is not observed in genetically hypertensive rat strains such as SHR and SHR-SP, except when specifically induced by a high lipid diet (Yamori et al., 1984). Similarly, the activity of systemic RAAS is variable in human idiopathic hypertensives whereas it is homogeneously lower in SHR (Watanabe et al., 1983; Zhang et al., 1996) and LHR, compared to their respective normotensives, the Wistar rat and the Sprague–Dawley rat (Sassard et al., 2003), especially as blood pressure increases.

2.3. Renal autoregulation: a key to hypertensive nephropathy

Hypertension-associated CKD progression is highly dependent on (i) renal blood flow autoregulation and renal hemodynamics, (ii) artificial maneuvers or genetically-determined factors that modify renal function or renal tissue homeostasis, independently of their action on blood pressure or renal hemodynamics, and (iii) genetic susceptibility factors. Renal autoregulation endows the kidneys with the capacity to maintain constant glomerular flow and pressure upon changes in systemic and renal perfusion pressure. Autoregulation is attained through vasoconstriction and vasodilatation of preglomerular (afferent) arteries and arterioles. In addition to the insulation of renal function from the influence of fluctuations in systemic blood pressure, one of the most important physiological functions of autoregulation is believed to be the protection of renal tissues from mechanical overload derived from high blood pressure (Louzenhiser et al., 2006). Accordingly, animal strains and experimental models that keep autoregulation physiologically functional are significantly less prone to developing hypertensive renal damage than those that have lost autoregulatory capacity. For example, fawn hooded rats are believed to bear a genetic susceptibility to renal damage. Fawn hooded normotensive (FHN) rats are more susceptible than other normotensive strains to aging-induced renal damage or to artificially induced hypertensive nephropathy, as by L-NAME administration (Van Dokkum et al., 1997, 2000).

Similarly, FHH rats are more susceptible to renal damage than better autoregulating strains, such as the SHR. Both FHN and FHH have a defective autoregulatory capacity (Van Rodijnen et al., 2002). Genetic susceptibility to renal damage in FH rats has been ascribed to five quantitative trait loci (QTL, named RF-1 thru RF-5) mapping to chromosome 1 (Van Dijk et al., 2005; Lopez et al., 2006; Van Dijk et al., 2006). Interestingly, the RF-1 region is related to renal autoregulation impairment (Van Dijk et al., 2005, 2006). Cross-breeding experiments between FH rats and renal damage resistant August Copenhagen Irish (ACI) rats have shown that heterozygosity provides some (but not complete) protection against L-NAME induced hypertensive nephropathy (Van Dokkum et al., 2000). Similarly, the transfer of chromosome 1 from Brown Norway (BN) rats to FHH reduces L-NAME hypertensive renal damage in the latter (Mattson et al., 2005). Furthermore, susceptibility to renal damage appears to be independent from susceptibility to hypertension in FH rats (Brown et al., 1996).

Other models with impaired autoregulation and, thus, accelerated hypertension-related renal damage include rats with extensive renal mass reduction (RMR; Bidani et al., 2003), rats rendered hypertensive by constant administration of angiotensin II with subcutaneously implanted minipumps over a period of at least 15 days (Inscho et al., 1999; Wang et al., 2000), the unclipped kidney in the two-kidney, one-clip (2K1C) model (Turkstra et al., 2000) and experimentally induced hypertension in the BN rat (Wang et al., 2000). Recent experiments performed on RMR rats further support the important role of autoregulation in the preservation of renal integrity. RMR rats treated with polysulphate pentosan or mycophenolate mofetil did not develop proteinuria, glomerular hypertension or hyperfiltration, despite persisting hypertension. The protective effect correlates with a higher afferent resistance (Sanchez-Lozada et al., 2003). The angiotensin II model mimics the situation of the non-clipped kidney in the 2K1C Golblatt model. The role of renin release from the clipped kidney in 2K1C, is replaced by angiotensin II administration. The hypertensive and renal damaging actions of angiotensin-II require long term action, coinciding with the time frame necessary to detect an augmented intrarenal amount of angiotensin II (Zou et al., 1996). Indeed, angiotensin II-mediated effects on pressure-natriuresis, blood pressure and GFR need long term blockade by angiotensin receptor blockers (ARA; Kline & Liu, 1994), demonstrating mechanistically different effects of angiotensin II on the short and long term pressure regulation. Like the FH rat, the Brown Norway (BN) rat bears a genetic region on chromosome 1 responsible for renal damage susceptibility. When this 22 cM chromosomal region (identified by D1Mit3 and Igf2r markers) is transferred to the SHR background, the resulting crossed animals experiment a greater renal damage than the parental SHR after accelerated hypertension with DOCA salt (St Lezin et al., 1999).

In contrast, in well autoregulating strains, such as the SHR or MHR, hypertensive renal damage develops significantly later and more slowly, in parallel with an aging-dependent loss or a slow hypertension-induced upward shift of autoregulatory capacity, towards higher levels of pressure (Palmer, 2004). In these strains, uninephrectomy (UNX) forces the remaining kidney to vasodilate, which accelerates renal damage (Lopez-Hernandez et al., 1998; Reverte et al., 1998; Kinuno et al., 2005). Very interestingly, a genomic region for renal damage susceptibility has been identified in African Americans (who are more susceptible than Caucasians) that appears located at chromosome 10 within the orthologous region of Rf-1 in the FH rat (Hunt et al., 2002). Further genetic analysis is necessary to determine if Caucasians who develop such renal damage bear comparable genetic polymorphisms as African Americans.

2.4. Consequences of glomerular stretch

Even in well autoregulating models, autoregulation is very, but not absolutely, effective. The absence of complete protection is derived from the fact that (i) autoregulation mechanisms (i.e. myogenic response and tubuloglomerular feedback) take time to respond, and (ii) skewed neuroendocrine influences may modulate the extent of the response (Persson, 2002) by confounding afferent arterioles to properly set the precise resistance through wall tone, or by progressively inducing an arteriopathy which results in maladaptive function (Sanchez-Lozada et al., 2003). As a consequence, upon systemic pressure oscillations, undetermined renal blood flow and glomerular capillary pressure fluctuations occur (Persson, 2002) which, in the long term, have been associated with the development of hypertension and target organ (including renal) damage (Persson, 2002). This is how systemic hypertension translates into intrarenal hypertension and progressive damage to the glomerular tuft and filtration barrier (even reaching very proximal tubular areas). The more slowly the process the better the autoregulatory capacity. Upon
mechanical overload, glomerular cells adopt a secretory phenotype and produce cytokines and growth factors, which finally are involved in the aberrant replacement of functional tissue by fibrotic connective tissue (Ljutic & Kes, 2003). Mechanical stretch derived from an elevated intraglomerular pressure exerts direct physical actions on glomerular structures, as well as cell signaling regulatory influences.

Stretching of glomerular structures directly increases the permeability of the filtration barrier. In addition, stretch probably mediates responses of the glomerular structures that attempt to compensate for the increased stress and filtration barrier disruption; however, these responses eventually become pathological and malignant. As for all initial responses to damage or evolving circumstances affecting the kidneys (and probably any organ, tissue and cell in the organism), these initial responses could be considered merely adaptive. Why and what turns them pathological are, as commented through the manuscript, key but unfortunately ignored issues. They include rapid (contraction, transcriptional activity, etc.) and medium term responses (proliferation, remodeling, fibrosis, etc.) that embrace glomerular endothelial cells, mesangial cells, podocytes, basement membranes and extracellular matrix (ECM). Paradoxically, extracellular and cellular signaling mediators of these responses also seem to play pathological roles under pathological circumstances. As a consequence of stretch (and of variations in shear stress) or as a response to it: (i) endothelial cells proliferate, synthesize extracellular matrix, reorient their cytoskeleton, remodel their shape and change their secretory and signaling patterns (Lacolley, 2004; Wang & Thampatty, 2006; Chien, 2007); (ii) mesangial cells proliferate (Ingram et al., 1999), and augment the production of factors known to participate in the inception and progression of the disease. These include vascular permeability factor (VPF, Gruden et al., 1997), transforming growth factor beta (TGF-β) and fibroactin (Gruden et al., 2000), a cellular renin–angiotensin system (RAS, Becker et al., 1998), and ECM (Ingram et al., 1999); (iii) podocytes reduce their proliferation (Perrtman et al., 2002) and undergo hypertrophy (Petermann et al., 2005), activate a local RAS (Durvasula et al., 2004) and also modify their signaling pattern with undetermined consequences on glomerular filtration (Morton et al., 2004).

3. Diabetic nephropathy

3.1. Pathophysiology of diabetic renal damage

Diabetic nephropathy is the most common glomerulopathy, and the leading cause of ESRD in the USA and Europe (Molitch et al., 2004). In fact, about 50% of ESRD patients (in the USA) are diabetic (U.S. Renal Data System, 2009). It is important to consider that hyperglycemia is a primary initiator of diabetic nephropathy. In the absence of elevated glycemia, nephropathy does not develop. However, diabetic nephropathy holds a genetic component at two levels: first, the elevation of glycemia; and second, at establishing a genetic background where nephropathy can occur (in the presence of hyperglycemia). Only 30% of patients with type 1, and 35–40% of patients with type 2 diabetes develop diabetic nephropathy irrespective of glycemic control (Diabetes Control & Complications, 1995). The clinical history of a typical patient starts with symptoms of hyperfiltration (elevated values of GFR) and occasional microalbuminuria, which may last approximately 5 years. During the next ~20 years, microalbuminuria turns into progressively higher proteinuria, whereas GFR declines. Finally, the patient undergoes renal insufficiency with severe proteinuria, which eventually evolves towards ESRD (Schena & Gesualdo, 2005).

Very early, hyperglycemia increases endothelial NO synthase (eNOS) expression in afferent arteries and glomerular capillaries, which leads to vasodilation and increased GFR (Sugimoto et al., 1998). Progressively, glomerular distension causes endothelial dysfunction and hemodynamic alterations, loss of the glomerular basement membrane (GBM) electric charge and GBM thickening, decreased number of podocytes, foot process effacement and mesangial expansion have been shown to underlie the initial glomerular injury (Lehmann & Schleicher, 2000; Wolf & Ziyadeh, 2007; Munusamy & MacMillan-Crow, 2009), which eventually leads to glomerulosclerosis. Damage to podocytes is emerging as a critical event in glomerulosclerosis (Kretzler, 2005; Reddy et al., 2008), which has lead to propose diabetic nephropathy as a disease of podocyte loss. Besides direct effects of hyperglycemia on tubular cells (Munusamy & MacMillan-Crow, 2009), glomerular damage causes tubular injury resulting in tubular cell death, epithelial to mesenchymal transition (EMT), cell infiltration, tubule degeneration and interstitial fibrosis, by different mechanisms (Kriz et al., 1998; Remuzzi et al., 2006; Wolf & Ziyadeh, 2007; Ziyadeh & Wolf, 2008): (i) the proteinuria derived from GBM alterations activates tubular cells to produce mediators (TGF-β, angiotensin-Ii, etc.) and proinflammatory cytokines; (ii) growth factors derived from glomerular cells (TGF-β, insulin-like growth factor—IGF-1, angiotensin II, etc.) stimulates the uptake of proteins, which amplifies the effect of proteinuria, and activates cell death and the profibrotic program in tubule cells; (iii) microangiopathy results in reduced postglomerular blood flow to peritubular capillaries. Renal structures become gradually impaired through changes that start diffusely and spread through the glomerulus, or focally localized as FSGS (Kimmelsteil–Wilson disease). Initially localized sites of focal sclerosis may extend and coalesce through the glomeruli, giving rise to a greater diffuse sclerosis. Alternatively, initial focal sclerosis may progress more rapidly towards tubule degeneration as opposed to glomerular collapse (as described below).

Alternatively, diffuse and focal glomerulosclerosis can be contemplated as etiologically or mechanistically different events. Typically, an early histological finding is the adhesion of a glomerular capillary to Bowman’s capsule at a podocyte deprived basement membrane point. These adhesions create gaps in the parietal epithelium that allow ectopic filtration out of Bowman’s capsule into the paraglomerular, interstitial space.

Two primary pathological pathways have been identified in total nephron degeneration. Through pathway I, glomerular collapse occurs before or parallels tubular degeneration, whereas through pathway II, tubular atrophy precedes glomerular degeneration (Kriz et al., 1998). Pathway I involves the propagation of glomerular adhesions at the vascular pole, and the formation of a progressively larger paraglomerular space (PGS) which contains ectopic filtrate and the remains of capillary tufts, which eventually reaches the urinary pole and the tubular structure. The PGS is formed initially within the GBM, which progressively disappears as the paraglomerular space enlarges and becomes surrounded by a sheath of fibroblasts (at the interstitial side). When the PGS reaches the urinary pole, it penetrates within the tubular basement membrane and separates it from the tubular epithelium, which correlates with tubule degeneration. Focal adhesions become areas of increasing sclerosis, capillary and perfusion collapse, all of which eventually embraces the whole glomerulus. In pathway II, the PGS initiated by the original focal adhesion reaches the urinary pole before further damage occurs at the vascular pole. As such, tubular fibrosis and degeneration proceeds extensive glomerular damage. As tubule flow decreases, pressure within Bowman’s capsule increases and it dilates. Finally, total tubule collapse occurs and the urinary orifice in Bowman’s capsule disappears, giving rise to the physical separation of the glomerulus and the tubule, and the formation of a glomerular cyst with substantial remnant perfusion. The PGS content is proposed to play a significant role in the initiation of damage and in the connection of glomerular and tubule disease. PGS contains renal filtrate (or exudate), cell debris from podocytes, ECM and basement membrane material, which may prospectively trigger proinflammatory, profibrotic and cell death-inducing responses. Interestingly, these histopathological patterns have been observed both in diabetic rats and humans (Kriz et al., 1998).
Traditionally, diabetic nephropathy has been considered a glomerular disease, in which tubular damage is a consequence of primary glomerular events. However, some results challenge this concept (Lapsley et al., 1993; Hong et al., 2003; Thomas et al., 2005; Thomson et al., 2006; Singh et al., 2008). Signs of impaired renal function have been detected before the evidence of glomerular malfunction in animals and humans, including increased excretion of small proteins (Lapsley et al., 1993; Hong et al., 2003). Accordingly, diabetic nephropathy is now presented in a holistic view as a disease affecting the whole nephron simultaneously. More investigation is necessary to unravel the mechanisms through which hyperglycemia injures the tubules in an early and glomerulus-independent manner.

### 3.2. Cellular effects of hyperglycemia

An unsolved question is through which mechanisms hyperglycemia triggers the observed histopathological alterations. Hyperglycemia acts on renal endothelial and mesangial cells, podocytes and also tubular cells. The cellular consequences derived from hyperglycemia resulting from types 1 and 2 diabetes are largely similar (Kanwar et al., 2005). Also, the cellular consequences of exposure to high glucose are similar in all renal cells (Orasanu & Plutzky, 2009). Hyperglycemia initiates cell signaling pathways in renal cells (Orasanu & Plutzky, 2009), including hyperactivation of protein kinase C (Ishii et al., 1996; Koya et al., 1997), oxidative stress through an excessive production of reactive oxygen species (ROS) (Palm et al., 2003; Tesch & Nikolic-Paterson, 2006) and others. This activation results in overexpression of (i) growth factors like TGF-β (Di Paolo et al., 1996; Heilig et al., 1997; Weigert et al., 2000), PDGF (Di Paolo et al., 1996) and connective tissue growth factor (CTGF; Connolly et al., 2003; ii) inflammatory cytokines (Connolly et al., 2003; Lorz et al., 2009), ECM elements (fibronectin, collagens I and IV) (Connolly et al., 2003) and matrix deposition (Bolick et al., 2003), cytoskeleton reorganization (Dai et al., 2006), cell cycle arrest (Masson et al., 2006), and hypertrophy (Fan & Weiss, 2004; Masson et al., 2006).

ROS attenuation in vivo (Brezniceanu et al., 2008) and in vitro (Munusamy & MacMillan-Crow, 2009) significantly reduces hyperglycemic cell damage.

Besides glucose, other mediators of hyperglycemia-associated damage include the advanced glycosylation end-products (AGEs), which result from the non-enzymatic glycation of proteins and lipids. Accumulation of AGEs in the kidney is associated with the development of nephropathy (Singh et al., 2001; Tesch & Nikolic-Paterson, 2006), both in type 1 (Cohen et al., 2000) and type 2 diabetic mice (Lassila et al., 2004). Interaction of AGEs with their membrane receptor (RAGE) is necessary for their action (Flyvbjerg et al., 2004; Wendt et al., 2004). On the other hand, hyperglycemia also increases the ubiquitous glucose transporter GLUT-1 mRNA and protein, and glucose transport in mesangial cells (Schena & Gesualdo, 2005). This has been proposed as a positive feedback mechanism in the appearance of glucose-induced damage.

Hyperglycemia induces hyaluronan (an anionic, non-sulfated glycosaminoglycan) overproduction in the mesangial matrix. Hyaluronans are potential binding sites for monocytes and macrophages in vitro and in vivo (Wang & Hascall, 2004). Hyperglycemia also induces ROS-mediated apoptosis in podocytes (Susztak et al., 2006), tubule cells (Verzola et al., 2004) and endothelial cells (Tawfik et al., 2005).

In the vasculature, hyperglycemia induces an oxidative stress (Giardino et al., 1996; Bellin et al., 2006) that results in endothelial dysfunction (Price et al., 2001; Yu & Lyons, 2005; Zuvrova-Nedelcheva et al., 2006) and impaired relaxations (Sercome et al., 2004). High blood glucose is also involved in the development of dyslipidemia (Veiraiah, 2005) and atherosclerosis (Price et al., 2001). In all renal cells, high glucose and AGEs induce the production of angiotensin II and growth factors (TGF-β, CTGF, vascular endothelial growth factor—VEGF, etc.) that act in an auto or paracrine manner to activate a fibrotic and inflammatory pathway in glomeruli and tubules (Lehmann & Schleicher, 2000; Wolf & Ziyadeh, 2007).

It is expected that, at least initially, all endothelial cells in the glomerular capillaries are subject to a similar glycemic stress, whereas only a few initially undergo a damage that likely translates to neighboring areas. It thus can be speculated that (i) for undetermined reasons every cell processes stimuli differently; or (ii) cell–cell and cell–matrix interactions, in conjunction with neuroendocrine influences modulate (and even null) the effect of hyperglycemia on most cells. It is only when these and certain other localized conditions (e.g. hypoxia, rupture or disorganization of basement membranes or ECM) merge in a particular manner and localization that hyperglycemia-associated damage begins. On the contrary, a homogeneous glycemic stress on all mesangial cells, inducing a similar effect in all or most cells would better correlate with the appearance of a diffuse glomerular sclerosis. The question is why a particular glomerulus shows diffused sclerosis, whereas a neighboring one exhibits focal adhesions.

### 4. Renal mass reduction

Regardless of etiology, the number of nephrons decreases during the progression of CKD. The space formerly occupied by glomeruli and tubuli becomes replaced with an extracellular matrix through a fibrotic process largely resembling scarring (Garber et al., 2003; Prieto et al., 2005). The remaining nephrons increase their filtration rate in order to maintain the excretory need of the organism. Renal dysfunction appears when the remaining nephrons cannot cope with the sustained extra load. However, over time the adaptive mechanisms contribute to the deterioration of the remnant nephrons. This situation has been modeled in experimental animals by surgically dissecting a large part of the renal mass in order to accelerate the progression of nephron loss towards the verge of renal dysfunction.

#### 4.1. Relevance

Experimentally, renal mass reduction (RMR) is almost exclusively practiced in rats. It is achieved by either surgical ablation, ligation of the renal artery branches, or a combination of both procedures, so that 1/2 to 5/6 of the total renal mass becomes functionally nullified. The extent of RMR depends on the desired degree of damage to be inflicted, the evolution time course and the concomitant presence of other renal insults such as pre-existent hypertension. Mainly, two RMR models have been used for the study of renal disease: (i) unilateral nephrectomy plus polycystome of the remnant kidney resulting in approximately 5/6 RMR (Rodriguez-Peña et al., 2001); and (ii) unilateral nephrectomy plus complete ligation of 2 branches of the contralateral renal artery, resulting in infarction of approximately 2/3 of the remnant kidney, which produces an overall 5/6 renal mass nullification (Flores et al., 1998). Uninephrectomy (UNX) models are not true models of RMR, because one kidney is capable of assuming the whole renal function without evident signs of damage, under otherwise normal conditions. UNX in SHR is a model of accelerated hypertensive nephropathy (as commented in Section 2). RMR is a very good model for the study of the mechanisms involved in compensatory adaptations to nephron loss (Kim et al., 2003). As a consequence of RMR, the number of functional nephrons is drastically and suddenly reduced. Remnant nephrons set on functional and structural compensations, and renal damage eventually appears in a manner and time course dependent on the experimental maneuver, extent of RMR, genetic background, sex and other determinants. A limitation posed by this model is the fact that this abrupt and extensive loss of renal mass occurs very rarely in human diseases, where a more gradual loss of nephrons is observed.
4.2. Structural and functional adaptations

Early in the aftermath of RMR, the remaining renal mass undergoes an adaptive and compensatory response in order to maintain renal function. This response comprises (i) hypertrophy of remnant nephrons mediated by cellular hyperplasia and hypertrophy; and (ii) hemodynamic alterations including an increase in blood flow due to efferent and, especially, afferent vasodilation, as a mechanism to increase glomerular filtration (Neuringer & Brenner, 1993; Griffin et al., 2000). Consequently, intraglomerular pressure increases (Hostetter et al., 1981; Brenner, 1985) which causes distension of glomerular structures. Initially, pressure and stretch induce a number of adaptive responses, which eventually corrupt and become malignant (as commented throughout this manuscript). These responses include (i) mesangial cell proliferation and fibrogenesis (Mertens et al., 1998; Riser et al., 1998; Cortes et al., 1999); (ii) mesangial increased expression of TGF-β receptors (Riser et al., 1999) and activation of the Raf/mitogen-activated protein kinases (MAPKs) pathway (Krepinsky et al., 2005); (iii) local activation of RAS (Ichikawi & Harris, 1991) with potent effects on glomerulosclerosis partially mediated by TGF-β (Kagami et al., 1994) and CTGF (Gupta et al., 2000); (iv) podocyte alterations, such as cytoskeleton reorganization (Endlich et al., 2001), RAS activation (Durvasula et al., 2004), increased expression of fibrosis-related molecules like osteopontin (Endlich et al., 2002), and TGF-β-mediated podocyte proliferation and apoptosis (Schiffer et al., 2001; Petermann et al., 2002). All these events eventually progress to a generalized fibrosis and loss of glomerular and tubular cells (Makino et al., 1996), finally leading to glomerulosclerosis and tubular atrophy (Fig. 3).

4.3. Pathophysiology of RMR-induced renal damage

Initially, the RMR-induced increment of glomerular volume reflects the compensatory response of the remnant renal mass. RMR develops different degrees of systemic and glomerular hypertension and proteinuria, initially focal in nature but developing to global glomerulosclerosis accompanied by a gradual reduction of GFR and progressive tubulointerstitial damage. This culminates in renal failure and death (Griffin et al., 2000; Kim et al., 2003). Ultrastructural changes in the GBM after 5/6 RMR, which resembles the characteristic phenotype of focal glomerulosclerosis, includes alterations in the filtration membrane which contributes to glomerular dysfunction, proteinuria and renal disease progression (Aunapuu et al., 2003). In addition, podocytes show hypertrophy, proliferation and foot process effacement. It has been proposed that proteinuria occurs speciﬁcally as a proliferative defect of epithelial cells after glomerular and filtration surface expansion, and TGF-β1-induced podocyte apoptosis (Schiffer et al., 2001), favoring the separation of the podocyte layer, and thus creating a more permeable barrier (Noel, 1999; Petermann et al., 2002). Numerous studies have revealed a correlation between proteinuria and interstitial and tubular damage (Kim et al., 2003). Immunohistochemical studies reveal an expansion of the glomerular tuft compartment and focal sclerosis two weeks after 5/6 RMR (Floge et al., 1992a). Macrophage infiltration and a progressive deposition of collagens I and IV, fibronectin, laminin, heparans and entactin are detected in the next weeks, correlating with the degree of glomerulosclerosis that develops (Floge et al., 1992a). Infiltrated lymphocytes, monocytes and platelets in the interstitium also contribute to renal scar progression (El Nahas, 1992).

From early phases of glomerular damage, mesangial cell proliferation and ECM overproduction occur (Cortes et al., 1996). In vitro experiments have detected and proposed as potential mitogens for mesangial cells: angiotensin II (Ichikawi & Harris, 1991), PDGF, basic fibroblast growth factor (bFGF), TGF-β (Floge et al., 1992b,c) and endothelin-1 (Bruzzi et al., 1997). These factors also cause vasoconstriction, reduction of renal blood flow (RBF) and, consequently, a decrease of GFR. Reduced RBF, and a progressive reduction in renal inducible NO synthase (iNOS) and NO production all have important roles in mesangial and vascular contraction (Aiello et al., 1998). Angiotensin II seems to be important for fibroblast and macrophage proliferation in the remnant renal mass, as demonstrated by RAAS inhibition with angiotensin converting enzyme inhibitors (ACEIs) and ARA (Wu et al., 1997). Indeed, the observed tubular expression of RAAS components might contribute to tubulointerstitial damage in this model (Gilbert et al., 1999). Simultaneously, along with proliferation, apoptosis takes place as well in glomeruli and tubules of 5/6 RMR rats, and both processes contribute to the damage, in which TGF-β appears to be involved (Thomas et al., 1998). In support of this hypothesis, simultaneous glomerular and tubular cell proliferation and apoptosis has been already described in the uninephrectomized spontaneously hypertensive rat (SHR) (Rodriguez-Lopez et al., 1998, 2002). Tubular EMT is detected 3 weeks after 5/6 RMR, and tubular cells acquire de novo expression of alpha smooth muscle actin (α-SMA), lose apical-basal polarity, transform into myofibroblasts, and migrate to the interstitium where they undertake a pivotal task in fibrogenesis (Ng et al., 1998).

In RMR animals, hypertension develops very soon after renal mass ablation (Benchetrit et al., 1999; Pires et al., 2007). In these models, hypertension contributes to glomerular damage, especially because of the compensatory afferent vasodilation occurring after RMR (Figs. 1 and 3).

5. Obstructive nephropathy

Obstruction of the urinary pathway (chiefly resulting from blockade of one or, exceptionally, both ureters) causes progressive deterioration of renal structures leading to chronic dysfunction. Ureteral obstruction gives rise to the hydronephrotic syndrome, characterized by kidney enlargement due to urine collection in the renal pelvis or calyces (Mendelsohn, 2004). It appears clinically as decreased renal function due to anatomical or functional abnormalities compromising urine flow through the urethra, bladder, ureters or renal pelvis (Klahr, 1998). Congenital obstructive nephropathy constitutes the principal cause of renal failure in children of the United States, accounting for 16% of pediatric kidney transplants, in contrast to the 0.3% in adults (Benfield et al., 2003). Though incidence of obstructive nephropathy decreases after childhood, it has been detected an increase after the age of 60–65, especially in men as a consequence of prostate hyperplasia and cancer (Klahr, 1998). Unilateral ureteral obstruction (UUO) in animals is also used as an experimental model for the investigation of the pathological mechanisms implicated in tubulointerstitial fibrotic diseases (Chevalier et al., 2009).

5.1. Relevance

A clear etiological parallelism exists between experimental UUO and human obstructive nephropathies. Experimental UUO is a maneuver that causes a complete obstruction of the manipulated ureter. This is an extreme situation that is rarely observed in humans, which however accurately reproduces the fibrotic sequence of events seen in patients, through an accelerated time course (Klahr & Morrissey, 2002). Extensive work has demonstrated the usefulness of this model for the study of histopathological, cellular and molecular alterations, for the identification of extracellular and intracellular mediators, and also for its predictive capacity for drug discovery processes (Chevalier, 1998; Klahr & Morrissey, 1998, 2002; Bascands & Schanstra, 2005). Contralateral kidneys undergo compensatory physiological alterations such as increased blood flow and hypertrophy. They also suffer structural damage posed by glomerular edema, congested blood vessels, dilated tubuli, epithelial necrosis and apoptosis (Ekinci et al., 2003).
5.2. Pathogenesis of the obstructed kidney

Following complete UUO, there is a progressive fall in renal blood flow and GFR, and an increased intratubular pressure ensues which stretches the tubule walls. As a result, the plasma and renal RAAS becomes activated, which determines subsequent pathological alterations through the activation of TGF-β, immune cell infiltration, fibrosis and renal hypoperfusion (Klahr & Morrissey, 2002). Immediately after total UUO, the increase in ureteral pressure translates into an incremental increase in tubular pressure, which decreases the hydraulic pressure gradient across the glomerular filtration barrier. This increase in intratubular pressure is thought to be the result (at least partially) of cyclic refractory peristaltic-like contractions of the ureter from the point of occlusion upward. However, as early as five hours after UUO, intratubular pressure declines. Notwithstanding, glomerular filtration rate continues to fall because of the reduction in renal blood flow (secondary to renal vasoconstriction) and in the ultrafiltration coefficient (Kf). It has also been postulated that upon UUO cortical interstitial pressure also increases. The transient hydrodynamic perturbations that develop shortly after UUO in the obstructed kidney produce a mechanical disturbance to the proximal tubular epithelium (e.g. membrane stretch), which causes the increased production of components of the renin–angiotensin II axis within the proximal tubular epithelium, and consequent auto and paracrine chemokine generation (Diamond et al., 1998).

The pathology of obstructed kidneys faithfully recreates the common alterations observed in most tubulointerstitial diseases, including tubular atrophy, proliferation and apoptosis of epithelial tubular cells, EMT, interstitial cell infiltration, ECM deposition, and accumulation of fibroblasts and myofibroblasts (Klahr & Morrissey, 2002). According to Young et al. (Young et al., 1998) and Klahr (Klahr, 1998), within the first few hours following a complete UUO, intratubular pressure, increased tubular volume, and appearance of glomerular pressure and renal blood flow alterations have been
described. During the first phase (0–90 min), renal blood flow and glomerular pressure augment because of rapid, prostaglandin-mediated vasodilation of the afferent arterioles (Klahr, 1998). In the second phase (90 min–5 h), renal blood flow decreases but glomerular pressure rises due to vasoconstriction of efferent arterioles, as a consequence of intrarenal release of vasoconstrictors such as angiotensin II (Frokiaer et al., 1996), thromboxane A2 (TXA2) (Klahr, 1998) and endothelin-1 (Kahn et al., 1997). From 5 to 18 h post obstruction, afferent arterioles also contract, and glomerular pressure and blood flow decrease (Young et al., 1998).

5.3. Involvement of cell infiltration

In normal kidneys, macrophages are scarce and restricted to the renal cortex (Schreiner & Unanue, 1984). As a response to the mechanical stress, ligated kidneys synthesize chemoattractants that evoke interstitial monocyte and T lymphocyte infiltration during the first 4 h (Schreiner et al., 1988). The infiltrating cells contribute to development of the lesion by synthesizing profibrogenic TGF-β1 (Diamond, 1995) and by reducing renal blood flow and GFR (Klahr, 1998) through the release of vasoactive compounds such as interleukin 1 (IL-1), thromboxane A2 and leukotriene D4 (Schreiner & Kohan, 1990). T lymphocytes and macrophages also contribute to the progression of renal disease by inducing dysfunction of tubular transport, tubular atrophy and interstitial fibrosis. In the obstructed kidney, increased and sustained expression of the adhesion molecules ICAM-1 and VCAM-1 in the tubuli and interstitium, and their decreased glomerular expression suggest an important role for these molecules in the infiltration of inflammatory cells into the tubulointerstitium during chronic hydronephrosis (Shappell et al., 2000). Interestingly, the pattern of tubular ICAM-1 expression (increased between days 6 and 25, and normalized afterward), coincides with tubular apoptosis and proliferation, suggesting a role for activated inflammatory cells in these processes (see below in this section for further details). Experiments carried out by inducing UUO in KO mice have demonstrated that other cell-to-cell and ECM-to-cell interaction-related molecules, such as selectins (Lange-Sperandio et al., 2002), osteopontin and its receptor, CD44 (Ophascharoensuk et al., 1999; Rouschop et al., 2004), intervene to a significant degree in cell infiltration, tubular atrophy, apoptosis and interstitial fibrosis. In addition, a gradual increased expression of the chemoattractant and profibrotic cytokine MCP-1 is observed in tubular cells after 12, 48 and 96 h of ligation (Diamond et al., 1994). Three days post-occlusion, expansion of the cortical interstitial volume, deposition of collagens I,
III and IV in the cortical interstitium, and collagen IV overexpression in tubular basement membranes, are detected (Kaneto et al., 1994). Maintenance of ligation originates a generalized interstitial fibrosis, proliferation of activated fibroblasts and myofibroblasts during the first week, and parenchymal scars afterwards (Gonzalez-Avila et al., 1988; Klahr, 1998). Fifteen days after obstruction, a pronounced interstitial fibrosis is observed with accumulation of fibronectin, laminin and collagens I and IV (Rodriguez-Peña et al., 2002). An increased expression of TGF-β1 and the TGF-β type III receptor endoglin mRNA levels are also observed (Rodriguez-Peña et al., 2002).

5.4. Participation and fate of renal cells

Ureteral obstruction induces complex changes in renal cells including phenotypic alterations and loss of renal mass that reflects a skewed balance between cell death and proliferation. From the first week after ligation, loss of tissue mass is detected (most rapidly occurring between 2 and 4 weeks) which correlates with the extent of tubular cell apoptosis and tubular atrophy observed (Gobe & Axelsen, 1987). These data suggest that tubular apoptosis may play an important role in tubular atrophy and renal mass loss. It has also been demonstrated that tubular cells undergo proliferation just before detecting an increase in apoptosis rates, which then return to those levels associated with healthy kidneys (Truong et al., 1996). In contrast, proliferation and apoptosis of interstitial cells progressively increase in the obstructed kidney from day 1, and both proliferation and apoptosis seem also to participate in the renal damage (Truong et al., 1996; Rodríguez-Peña et al., 2008; Grande & López-Novoa, 2009). Ex vivo studies demonstrate that cortical interstitial fibroblasts obtained from ligated kidneys show a higher proliferation rate than those obtained from contralateral and healthy kidneys (Davis et al., 1983). Proliferation and apoptosis do not become altered in glomerular cells from UUO kidneys (Truong et al., 1996).

Studies carried out in cyclin kinase inhibitor p21^{WAF/CIP1} or p27^{KIP1} KO mice subject to UUO reveal that p21 and p27 are, respectively, important controllers of myofibroblast (Hughes et al., 1999) and...
tubular cell proliferation (Ophascharoensuk et al., 1998), with no effect detectable on interstitial fibrosis. Moreover, increased p53 expression has been detected in kidneys after UUO (Morrissey et al., 1996), and a role in obstructive pathogenesis has been demonstrated for this proapoptotic transcription factor since UUO p53 KO mice present 50–70% lower tubular and interstitial apoptosis, lesser tubular atrophy, and reduced interstitial volume, compared with UUO wild type mice (Choi et al., 2001).

Tubular EMT is also a characteristic hallmark of obstructed kidneys. Tubular cells dedifferentiate to fibroblasts and migrate to the interstitium. Cells undergoing EMT acquire mesenchymal markers such as α-SMA and vimentin, lose epithelial markers like E-cadherin, while they maintain tubular markers (e.g. lectin) and synthesize ECM products such as fibronectin and collagen I (Yang & Liu, 2001; Iwano et al., 2002; Yang & Liu, 2002). The biphasic expression time course of the myofibroblast marker α-SMA suggests that during days 1–3 after obstruction myofibroblasts originate by activation of resident fibroblasts, whereas the second peak (at the 7th day) respond to myofibroblasts derived from tubular EMT (Yang et al., 2002a; Liu, 2004). Tissue plasminogen activator (tPA) is a central mediator of EMT as demonstrated some studies performed with tPA null mice, which show reduced tubulointerstitial fibrosis after UUO (Yang et al., 2002b). This inhibition, which seems specific for the EMT process, appears to be based on a marked decrease in MMP-9 (Yang et al., 2002b).

Activation of the small GTPase Ras plays a major role in UUO-induced EMT. It has been demonstrated that both Ras and its downstream pathways ERK1/2 and PI3K/Akt are activated in the induced EMT. It has been demonstrated that both Ras and its downstream pathways ERK1/2 and PI3K/Akt are activated in the induced EMT. (Diamond et al., 1994); (ii) ECM degradation of MMPs (Klahr, 1998); (iii) induction of adhesion molecules, interstitial accumulation of immune cells, fibroblast proliferation; and (iv) tubular EMT (Klahr & Morrissey, 1998; Yang et al., 2002a).

5.5. Role of the renin–angiotensin aldosterone system

IECA-mediated RAAS inhibition (Kaneto et al., 1994; Ishidoya et al., 1995; Klahr & Morrissey, 1997) and mice KO of RAAS-elements (Ma et al., 1998; Satoh et al., 2001) have provided demonstration for an important role of angiotensin II in UUO renal damage. After UUO, there is a considerable increase in all the components of renal RAAS. Renal parenchymal cells synthesize angiotensin II, which decisively participates in renal damage through direct hemodynamic and profibrogenic actions (Kellner et al., 2006), and the induction of profibrotic mediators like nuclear factor kappa B (NFκB) and TGF-β1 (Pimentel et al., 1995; Klahr & Morrissey, 1998). In addition, angiotensin II synergizes with TGF-β1 to induce tubular EMT in UUO (Yang et al., 2002a). TGF-β1 is a pivotal executor of fibrogenesis in UUO (Klahr, 1998). Ligation induces an increment in TGF-β1 expression in epithelial tubular cells (Kaneto et al., 1993) and peritubular interstitial cells (Diamond et al., 1994). TGF-β1 overexpression induces (i) macrophage infiltration, and synthesis and interstitial accumulation of fibronectin and collagens I, III and IV in the obstructed kidney (Diamond et al., 1994); (ii) ECM degradation inhibition through the stimulation of TIMP expression and inhibition of MMPs (Klahr, 1998); (iii) induction of adhesion molecules, chemotaxis of fibroblast and immune cells, fibroblast proliferation; and (iv) tubular EMT (Klahr & Morrissey, 1998; Yang et al., 2002a).

6. Common mechanisms of progression

Animal models have provided valuable insights into the pathogenesis of chronic kidney diseases. They have proved to be extremely useful for studying and understanding the pathogenetic mechanisms involved in their onset and progression, and for testing new treatments. Knowledge gathered from animal models increases our understanding of human pathology, which in turn helps us to develop new and more specific animal models in which to evaluate novel therapeutic interventions at the preclinical level. In this intertwined relation, animal models play a pivotal role between pathological information and clinical development.

Whereas the pathophysiological events underlying the inception of every nephropathy are different, those involved in the progression of the disease are, to a certain extent, common to many nephropathies (see Figs. 1–4). This may explain why, as different diseases progress, an increasingly common renal phenotype of tissue destruction, inflammation and scarring ensues, regardless of etiology. Persistent insults to renal cells by chemical or physical action eventually activate inflammatory and fibrotic responses that not only interfere with the repair processes, but also redirect the renal tissue status through similar mechanisms of irreversible degeneration. Regardless of whether the disease starts mostly as a glomerular (i.e. hypertensive nephropathy) or tubular (RMR and UUO) insult, the initial cellular damage eventually activates responses that finally damage other nephron structures. This leads to a vicious circle of malignancy where nephrons progressively disappear and are substituted by scar tissue. As depicted in Figs. 1–4, cell damage and activation leads in all cases to inflammation and cytokine imbalance, which activates other cell types and contributes to unleashing fibrosis, mesangial and vascular contraction contributing to the reduced GFR, tubule degeneration and scarring (Fig. 5). These are logical targets for pharmacological intervention aimed at slowing down disease progression (Remuzzi et al., 2006). Yet, a challenge for the future is to unravel the key molecular events that specifically drive each disease beyond the no return point, and to identify early and etiologically specific markers, that allow us to detect the inception of malignant damage and intervene appropriately.

7. Current treatments

7.1. Renin–angiotensin aldosterone system inhibition

RAAS inhibition has proved to be the most effective therapy at reducing proteinuria and slowing CKD progression in animals and humans (Perico et al., 2008). Furthermore, RAAS inhibition has proved to cause regression and prevent certain aspects of CKD, such as glomerular fibrosis, in experimental models (Fogo, 2006a,b; Remuzzi et al., 2006).

Angiotensinogen is cleaved by the enzyme renin into the decapeptide angiotensin I, which in turn is cleaved by the zinc metallopeptidase angiotensin converting enzyme (ACE) and other carboxypeptidases into the highly active octapeptide angiotensin II, with potent functional and structural effects on the vascular wall, heart, renal tissues, nervous system and many others (Mire et al., 2005). Two pharmacologically distinct, G-protein coupled, seven-transmembrane spanning, cell surface receptors for angiotensin II have been identified (i.e. AT-1, AT-2). AT-1 and AT-2 have been cloned and characterized. Two additional high affinity binding sites for angiotensin II have been proposed, designated AT-3 and AT-4. These have not been molecularly characterized to date (Bernstein et al., 2001; Berry et al., 2001). Most pivotal effects of angiotensin-II in relation with CKD and hypertension are mediated by activation of the AT-1 receptor. Activation of the AT-2 receptor exerts actions rather antagonistic to those mediated by AT-1 (Ardailou et al., 1999; Kim & Iwao, 2000; Touyz & Schiffrin, 2000), although its physiological and pathological roles are not clearly ascertained. AT-3 and AT-4 receptors are functionally still poorly characterized (Touyz & Schiffrin, 2000).

RAAS inhibition can be achieved with two families of compounds angiotensin–converting enzyme inhibitors [i.e. ACEIs (Sica, 2005)] and angiotensin receptor antagonists [ARA (Sica, 2006)]. Both drug types are clinically approved and commonly used for the treatment of
hypertension as well as other cardiovascular disorders (Fig. 6). As a class, both families of compounds have good pharmacological profiles with high efficacy and low toxicity. ACEIs (e.g. enalapril, trandolapril, ramipril, lisinopril, benazepril and fosinopril) prevent angiotensin II from being produced by ACE, whereas ARA (e.g. losartan, valsartan, irbesartan, candesartan, eprosartan, olmesartan and telmisartan) prevent angiotensin II from binding and activating its cell receptors. ARA are selective for the AT-1 receptor. Other RAAS blockers include renin inhibitors (e.g. aliskiren) (Azizi et al., 2006), which might have a potential usage for the therapeutic management of CKD (Perico et al., 2008) although to date this has not been investigated. Recently, aliskiren has been approved by the Food and Drug Administration (FDA) of the United States of America for use in the treatment of hypertension. Aliskiren (Tekturna) has also recently been approved in combination with valsartan (Valturna) for hypertension.

The accumulated clinical experience during the last several decades and knowledge acquired from landmark clinical trials with RAAS blockers, plus information derived from clinical meta-analyses (MacKinnon et al., 2006) have demonstrated that RAAS inhibition using ACEIs and ARA is the most effective single therapy to attenuate progression of CKD, despite being almost equally effective to other antihypertensive drugs at reducing blood pressure (Epstein, 2002a,b). RAAS inhibitors are considered as first line therapy for patients with diabetic and non-diabetic kidney disease (Brewster & Perazella, 2004). Their distinctive effect is more evident the more advanced and more proteinuric the kidney disease (Nigbor & Lewis, 2003; Rahn, 2005).

Clinical studies involving RAAS inhibitors include the Irbesartan Type 2 Diabetic Nephropathy Trial (IDNT) (Pohl et al., 2005), the Reduction of Endpoints in NIDDM with Angiotensin II Antagonist Losartan (RENAAL) (Shahinfar et al., 2006), the Valsartan Antihypertensive Long-term Use Evaluation (VALUE) trial (Julius et al., 2006), the International Verapamil–Trandolapril Study (INVEST) (Pepine et al., 2003), the combination treatment of an ARA and an ACEI in non-diabetic renal disease (COOPERATE) study (Nakao et al., 2003) study, and others.

RAS inhibitors are superior to other antihypertensive agents at reducing progression of CKD, proteinuria and mortality end-points
ARA decrease PAI-1 levels, but the effect is more lasting with the ACEI (Brown et al., 2002). Taken as a whole, these studies highlight a potentially important difference between ACEIs and ARA in renal disease whose clinical consequences need to be evaluated.

(ii) Bradykinin, a vasodilatory peptide that also provides cardio-protection in hypertensive rats (Tanaka et al., 2004), is inactivated by ACE (Yang et al., 1970). Accordingly, in contrast to ARA, ACEIs increase bradykinin levels. Bradykinin affords considerable renoprotection in diabetic nephropathy (Doggrell, 2005). Indeed, a part of the renoprotective effect of IECAs is mediated by bradykinin because bradykinin antagonists partially block IECA-induced benefits. This has been shown in several experimental models including diabetic (Tschope et al., 2003) and hypertensive nephropathy (Yokota et al., 2003; Seccia et al., 2006). These results are, however, disputed by others who found no effect of bradykinin antagonists on IECA-induced protection in rats rendered hypertensive by subtotal (5/6) nephrectomy (Nabokov et al., 1998), and in 5/6 nephrectomized SHR (Kohzuki et al., 1995). Other peptidases such as neutral endopeptidase, basic carboxypeptidase and aminopeptidase P also degrade bradykinin, although their relative contribution to overall bradykinin degradation (as compared with ACE) appears to be negligible (Bagate et al., 2000).

(iii) Peptidases other than ACE (chiefly the chymotrypsin-like serine protease, chymase) have been shown to be responsible for an 80% of angiotensin II generation in the heart, and a 60% in blood vessels (Richard et al., 2001; Miyazaki & Takai, 2006). Furthermore, angiotensin II levels are not significantly altered in ACE−/− mice, which have been attributed to the compensatory enhanced chymase activity observed in these mice (Wei et al., 2002). Interestingly, ACEIs do not inhibit chymase (Richard et al., 2001). Chymase levels are low in normal kidneys and seem to contribute little to RAAS-dependent renal function (Ugata et al., 1994; Hollenberg et al., 1998; Hollenberg, 2000). However, chymase is overexpressed in the diseased kidneys of different experimental models of CKD and patients with different chronic nephropathies, not only in resident mast cells but also in renal glomerular, tubular and interstitial cells (Huang et al., 2003; Ritz, 2003; McPherson et al., 2004; Miyake-Ogawa et al., 2005; Morikawa et al., 2005; Sadjadi et al., 2005).

Unfortunately, the effect of chymase inhibitors on experimental models of nephropathy and in patients has not been characterized, such studies are needed.

(iv) Pleiotropic actions of ACE beyond angiotensin II generation may also respond to differences in the action of ACEIs and ARA. For instance, ACE is involved in signaling cascades independent of angiotensin II production and bradykinin degradation. In fact, ACE mediates the activation of cyclooxygenase-2 (COX-2) and prostacyclin expression, in cultured endothelial cells, in the presence of ACEIs (Kohlstedt et al., 2005). Moreover, (i) ACEIs increase casein kinase (CK)-2-mediated serine1270 phosphorylation of ACE; and (ii) mitogen-activated protein kinase (MAPK) kinase (MAPKK) 7 and JNK coprecipitates with ACE, and ACEIs increase ACE-associated JNK activity (Kohlstedt et al., 2004).

Because of the distinct differences that exist between the mechanisms of action of ACEIs and ARA it could be anticipated that differences in their clinical effects exist; however such effects are not readily observed. ACEIs and ARA utilized at the highest possible dose (considering their particular therapeutic and toxic ranges) appear equally effective in the management of CKD. Head to head clinical comparisons are scarce, although a few can be found in combination studies, where single therapy groups are included as controls.
Interestingly, combination of ACEIs with ARA exerts additive nephroprotection (reviewed in Song et al., 2006 and Tsouli et al., 2006). As depicted in Fig. 6, both drug families interfere with direct actions of angiotensin-II, and with the production of other key factors such as transforming growth factor beta. RAAS blockade not only reduces hemodynamic and pro fibrictic effects (Brewster & Perazella, 2004), but also cardiovascular events derived from uremia (Kim & Iwao, 2000), and controls comorbid factors such as hypertension (López-Hernández & López-Novoa, 2006) and renal atherosclerosis (Suganuma et al., 2006).

8. Therapeutic perspectives

The horizon for the clinical handling of CKD may be analyzed from different perspectives. First, diagnosis must be improved for an earlier detection of progression and for identification of prognostic markers that anticipate which patients will progress more rapidly than others. Second, new forms of therapy are needed to improve our capacity to slow and even halt progression, and to reverse the damage and regenerate renal tissues.

8.1. Pharmacological inhibition of common mechanisms of progression

According to the discussion in Section 6, the key, common pathological mechanisms of CKD progression pose attractive targets for pharmacological intervention. Inhibition of inflammation, fibrosis, specific mechanisms involved in progressive tubular degeneration and nephron loss (García-Sánchez et al., 2010), cell activation and aberrant production of cytokines and their effects leading to a reduced RBF, mesangial contraction and increasingly lower GFR, are specific drugable candidates. Indeed, several drugs are under different degrees of experimental and clinical development (reviewed in Perico et al., 2008; also see Grande & López-Novoa, 2008 and Gonçalves et al., 2010). Prominent, prospective antifibrotic and anti-inflammatory drugs and strategies under different levels of development are outlined in Table 1. Besides these inhibitory drugs, other strategies exist that are based on the exogenous administration of endogenous molecules known to counteract the action of profibrotic mediators. Chiefly, it is the case of bone morphogenetic protein 7 (BMP-7) and hepatocyte growth factor (HGF), which outweigh the profibrotic effect of TGF-β (Chatziantoniou & Dussaule, 2005) and are known to reduce and even reverse fibrosis, inflammation and renal damage in experimental CKD (García-Sánchez et al., 2010).

As indicated in Fig. 5, an imbalance in cytokines and inflammatory mediators can reduce RBF and GFR independently from progressive nephron loss, which then limits filtration in remnant nephrons and further contributes to developing renal injury. Thus, inhibition of the effects of these molecules may contribute to decelerating disease progression by improving the outcome of functional nephrons, increasing their lifespan. In the pathological scenario, the equilibrium between molecules that cause vascular and mesangial contraction (ANG-II, endothelin-1—ET-1, platelet activating factor—PAF, TXA2, etc.) and those causing relaxation (NO, PGE2, etc.) is displaced towards the former. The effects of ANG-II can be blocked with renin inhibitors, ACEIs and ARA (reviewed in Perico et al., 2008). The potential efficacy of ET-1 antagonists (such as avosentan and tezosentan) is also under development for the treatment of CKD (reviewed in Longaretti & Benigni, 2009).

A potential candidate to which little attention has been addressed is PAF. PAF is an acetylated alkyl phosphoglyceride synthesized by many cell types, including different renal cells (López-Novoa, 1999). PAF contracts mesangial cells and blood vessels and exerts a pro-inflammatory action by contributing to leukocyte infiltration (Dui et al., 2006). Increased production of PAF has a key role in maintaining a reduced GFR in different models of acute renal injury (AKI; López-Novoa, 1999), and has pro-fibrotic effects as a result of stimulating the synthesis of ECM components on mesangial and tubular cells (Torras et al., 1999). Inhibition of PAF effects with PAF receptor antagonists reduces AKI in vivo (López-Farré et al., 1990; Dos Santos et al., 1991; Rodríguez-Barbero et al., 1992; Bagini et al., 1996; Rodríguez-Barbero et al., 1997), and direct cellular effect in vitro, such as mesangial cell contraction (Martínez-Salgado et al., 2008). Interestingly, inhibition of PAF with the PAF receptor (PAF-R) antagonist UR-12670, not only retards but also significantly reduces the intensity of the progressive and chronic renal damage induced by ischemia in uninephrectomized rats during 52 weeks of treatment (Torras et al., 1999). PAF-R antagonists are classified into five groups (Chen et al., 2008): nitrogen...
**Table 1**

Drugs under development for the treatment of progressive chronic kidney disease (CKD).

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<th>Drugs under development</th>
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<tr>
<td><strong>Statins</strong></td>
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<td>Fluvastatin, atorvastatin</td>
<td>Perico et al., 2008</td>
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<td><strong>Inhibitors of cytokines and growth factors</strong></td>
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<td>Anti TGF-β antibodies</td>
<td>X</td>
<td>X</td>
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<td></td>
<td>mAbs</td>
<td>Perico et al., 2008; García-Sánchez et al., 2010</td>
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<td>TGF-β1 production inhibitors</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>Pefedinide</td>
<td>Perico et al., 2008</td>
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<tr>
<td>TGF-β1 receptor inhibitors</td>
<td>X</td>
<td>X</td>
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<td>Imatinib, AG490</td>
<td>Gonçalves et al., 2010; Perico et al., 2008</td>
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<td>ALK inhibitors</td>
<td>X</td>
<td>X</td>
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<td>IN-1130, SB431542, GW788388</td>
<td>Moon et al., 2006</td>
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<td>CTGF inhibitors</td>
<td>X</td>
<td>X</td>
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<td>anti-CTGF</td>
<td>Perico et al., 2008</td>
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<td>PDGF, EGF, FGF, VEGF inhibitors</td>
<td>X</td>
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<td>Mozesanib, afibriccept, cediranib</td>
<td>Grande &amp; López-Novo, 2008; Perico et al., 2008</td>
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<td>MCP1 production</td>
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<td>CCR1 antagonists</td>
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<td>RX471</td>
<td>Perico et al., 2008</td>
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AF: anti-inflammatory; Al: antiinflammatory; ALK: Activin receptor-like kinase; BMP-7: bone morphogenetic protein-7; CCRs: CC chemokine receptors; CDK: cyclin-dependent kinase; CTGF: connective tissue growth factor; EGF: epidermal growth factor; ET: endothelin; FGF: fibroblast growth factor; FTIs: farnesyltransferase inhibitors; HGF: hepatocyte growth factor; mAbs: monoclonal antibodies; MCP1: monocyte chemotactic protein-1; PAF: platelet activating factor; PDGF: platelet derived growth factor; PPAR: peroxisome proliferator-activated receptor; RN: renal; RAS: renin-angiotensin system; RBF: renal blood flow; TI: tubular injury; VEGF: vascular endothelial growth factor.

AF: anti-inflammatory; Al: antiinflammatory; ALK: Activin receptor-like kinase; BMP-7: bone morphogenetic protein-7; CCRs: CC chemokine receptors; CDK: cyclin-dependent kinase; CTGF: connective tissue growth factor; EGF: epidermal growth factor; ET: endothelin; FGF: fibroblast growth factor; FTIs: farnesyltransferase inhibitors; HGF: hepatocyte growth factor; mAbs: monoclonal antibodies; MCP1: monocyte chemotactic protein-1; PAF: platelet activating factor; PDGF: platelet derived growth factor; PPAR: peroxisome proliferator-activated receptor; RN: renal; RAS: renin-angiotensin system; RBF: renal blood flow; TI: tubular injury; VEGF: vascular endothelial growth factor.

heterocyclic compounds (such as WEB2086 and WEB2170), PAF analogues (e.g. CV3988 and SDZ63072), dihydropyridines (e.g. PCV4233 and PVA4248), plant extracts (e.g. BNS50201—Ginkolide B—and kadsurenone), and others (e.g. 52 770 RP and TCV309). PAF antagonists should be further developed in CKD therapeutics.

Many of these drugs and therapeutic approaches exert effects at different levels. The case of PAF is not the only one. For example, ET-1 and RAS besides modulating renal vascular resistance, also participate in fibroblast and infiltrated cell stimulation and secretion of profibrotic mediators. Their inhibition improves RBF and reduces fibrosis (Perico et al., 2008). EGF has been shown to be involved in fibrosis, EMT and vascular homeostasis. However, its inhibition not only has antifibrotic effects but also improves renal hemodynamics (Helle et al., 2009). Because many mediators and signaling pathways seem to be redundant at inducing specific pathological effects, very probably, an effective prevention of progression must count on the identification of crucial targets and should contain a cocktail of different molecules targeting all these key pathophysiological mechanisms at different levels (Perico et al., 2008). Moreover, specific actions require strict control of other comorbid factors. For example, increasing GFR through elevating RBF must be accompanied by a strict control of blood pressure and of tubular damage, in order to prevent glomerular hypertensive injury (especially if hypertension is present), and excessive water and electrolytic loss, respectively. RAS inhibitors are probably effective (to a certain extent) because they combine multiple effects improving at the same time RBF and GFR, but also limiting tubulointerstitial damage.

### 8.2. Gene therapy

Gene therapy is another therapeutic strategy under preclinical development. The renal delivery of gene-carrying vectors can be attempted through the renal artery, the renal vein, and direct injection into the renal parenchyma (Imai & Isaka, 2004). Tissue barriers posed by capillary endothelium, basal membranes and renal epithelia limit the access of vector and cells to target sites from the circulation (Imai & Isaka, 2004). Alternatively, gene therapy may be addressed to the skeletal muscle so it continuously produces the transgenic protein product and releases it to the circulation for a sustained bioavailability (Imai & Isaka, 1999). Gene therapies are still in a developmental state, but they hold promising potential for the treatment of CKD.

For gene therapy, an appropriate vector containing a gene of interest or genetic materials (antisense oligonucleotides, decoy DNA or interference RNAs), either alone or as a part of a genetic construct (plasmid, etc.) is introduced into the target (and other) cells. Vectors are viral and non-viral. Exceptionally, small genetic materials (oligonucleotides) can be directly endocytosed by cells without the help of a vector. Viral vectors have the intrinsic capacity for infection since the genetic material is incorporated into engineered retro, lenti or adenoviruses.
Non-viral vectors must be forced into the cells though different biological complexes, including in vivo transfections with liposome-based complexes, electroporation, and ultrasound-microbubbles (Imai & Isaka, 2004; Lien & Lai, 2004). Gene therapy has been first attempted towards monogenetic diseases, like Alport syndrome and polycystic kidney disease (reviewed by Imai & Isaka, 2004). Monogenetic diseases with identified mutated genes are clear candidates for genetic therapy. Skeletal muscle erythropoietin-based gene therapy has also been attempted to treat CKD-associated anemia (Oh et al., 2006). In the last decade, intense research has been conducted at the preclinical level towards the application of gene therapy to the treatment of CKD. A number of strategies have been attempted with variable results on specific pathological events and disease progression in different experimental models and human nephropathies. These strategies are aimed at (i) introducing functional genes substituting for mutated ones [type-IV collagen α5 chain—COL4A5 (Heikkila et al., 2001; Imai et al., 2004), carbonic anhydrase II—Call (Lai et al., 1998)]; (ii) overexpression of transgenes involved in the regulation of renal function and structure [like lipoxygenase (Munger et al., 1999)]; (iii) interrupting signaling cascades involved in the development of pathological events [e.g. c-myc (Ricker et al., 2002), egr-1 (Carl et al., 2003)]; (iv) inhibiting the expression of pro disease mediators [e.g. TGFβ (Akagi et al., 1996)]; (v) producing factors that prevent or soften disease [HGF [Herrero-Fresneda et al., 2006]]; (vi) producing molecules addressed to improvement of CKD-derived systemic effects [e.g. anemia, with erythropoietin [Lippin et al., 2005]]. Future perspectives contemplate DNA repair (through homologous recombination of small DNA fragments for mutation reversal) as a more definitive therapeutic strategy, envisioned initially for monogenic diseases (Goncz et al., 2002; Yoon et al., 2002). Gene therapy can be combined with drugs (e.g. ACEIs) in the search for synergistic effects (Yang et al., 2002a,b,c); it can also be applied on cell therapy (see below) by priming and genetically engineering stem cells with specific and effective tissue regenerating properties, which opens new frontiers in therapeutics (Hanss & Bruggeman, 2003).

8.3. Cell therapy

Cell-based regeneration of degenerated tissues by redirecting renal or extrarenal cells to proliferate or differentiate, or both, into specific renal cell lineages is also an area of active preclinical development. Cellular therapy is currently also under development, both for the regeneration of damaged tissues and for the design of bioartificial kidneys (Humes, 2005; Schachinger & Zeiher, 2005). The rapidly evolving field of cell biology has changed many old concepts regarding cell differentiation and plasticity. Mature, terminally differentiated cells have now been demonstrated to hold the capacity to de-differentiate and cis- or trans-re-differentiate into distinct phenotypes, with different degrees of pluriotypicity (Odelberg, 2002; Bonventre, 2003). Moreover, the discovery of pluripotent and multi-potent stem cells has revolutionized the field of tissue engineering and tissue repair through cell therapy (Bianco & Gehron Robey, 2000; Korbling & Estrov, 2003; Passier & Mummery, 2003). An advantage of cell-based therapy is to supercede the difficulty of correcting the skewed homeostasis of diseased cells, where a myriad of interconnected and altered pathways seems to be difficult to reverse through the pharmacological or genetic manipulation of a single or a few molecular targets (Humes & Szczypka, 2004). The kidney possesses an extraordinary capacity for spontaneous regeneration and self repair (Anglani et al., 2004). Restitution of cells lost to damage or pathological events seems to be closing up through a variety of possibilities. Epithelial (e.g. tubular) cells destroyed during renal damage (especially during acute injury) have been shown to be replaced and the epithelium reconstituted. Sources for renal (tubule) epithelial cells are hypothesized to include (Humphreys et al., 2006): (i) surviving renal epithelial (or even interstitial) cells, which might directly proliferate or de-differentiate into a mesenchymal phenotype, proliferate to restore the integrity of the basement membrane and then re-differentiate into a normal epithelium (Rookmaaker et al., 2004); (ii) surviving renal stem cells or resident tubule progenitors which selectively proliferate after the damage. Recent evidence strongly suggests that adult renal stem cells exist, although rigorous corroboration and precise intrarenal location (i.e. the papilla) await to be established (Oliver et al., 2004); (iii) extrarenal, bone marrow-derived or circulating cells. Several cell types derived from the bone marrow (also found in the circulation) bear transdifferentiating capability, including mesenchymal stem cells and hematopoietic stem cells. Circulating monocytoid cells (derived from the neural crest) and fibrocytes are postulated as candidates (Anglani et al., 2004).

Consequently, cell therapy can theoretically be approached (i) by appropriately driving resident renal cells (stem, progenitor or differentiated) to restore the damaged tissues, or alternatively (ii) by injecting renal cells or bone marrow-derived cells with transdifferentiating and repair capabilities. So far, only the second option has been experimentally tested, with variable outcomes. Primarily, bone marrow-derived mesenchymal cells (with distinct phenotypes) have been used (reviewed in Brodie & Humes, 2005) for their ability to transdifferentiate into renal epithelial tubular (Kale et al., 2003; Herrera et al., 2004) and mesangial (Masuya et al., 2003) cells, and endothelial cells for re- and neo-vascularization (Takahashi et al., 1999; Patschan et al., 2006). Bone marrow-derived mesenchymal cells have been reported to contribute to the repair of drug- or ischemia/reperfusion-injured kidneys (Herrera et al., 2004), although the efficacy of these cells has also been challenged (reviewed in Brodie & Humes, 2005).

Adult tubule epithelial cells have been used to construct a bioartificial kidney. This biosynthesis device consists of a synthetic hemofilter coupled in series with a bioartificial tubule which contains approximately one billion tubule cells (Humes et al., 2004). A bioartificial kidney has been used to treat a CKD patient with multi-organ failure in the intensive care unit, resulting in some improvement of cardiovascular and renal parameters (Humes et al., 2004). Other cell based therapies conducted at the experimental level include encapsulated cell implants to remove uremic toxins and deliver therapeutic agents (see Brodie & Humes, 2005), and foetal kidney transplantation (Hammerman, 2003).

9. Final remarks

A greater specific knowledge regarding the pathophysiological mechanisms underlying the inception, transformation and progression of pathologies that lead to the development of chronic kidney disease is expected to enable, in the near future, the development of better and earlier diagnostics. This, in turn, should provide a better clinical management of disease progression in order to prevent the need for renal replacement therapy (RRT) or secondary health complications during the patient’s lifespan. This improvement will provide an enormous human benefit based on a better quality of life, and will reduce the disproportionate socioeconomic cost associated to CKD. Therapeutic improvement will be based (i) on the discovery of better molecular targets on which to act with existing and new drugs; and (ii) on therapy optimization through drug combinations, through the development of novel molecules bearing different biological activities, and through drug targeting to specific action sites.

However, a greater level of understanding of CKD must be achieved in order to facilitate effective control of this disease. On the one hand, it is not well known why and how renal repair mechanisms, which function reasonably well under such circumstances as acute renal injury, eventually become non-functional and precipitate disease (Garcia-Sánchez et al., 2010). Understanding the components involved in this transformation is of grave importance in order to prevent rather than only slow the progression of CKD. The second aspect for future further consideration, which is dependent...
upon the previous goal, is the capability of tissue regeneration and restoration of renal function.

References


