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Off the Beaten Renin–Angiotensin–Aldosterone System Pathway: New Perspectives on Antiproteinuric Therapy

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Abstract

CKD is a major public health problem in the developed and the developing world. The degree of proteinuria associated with renal failure is a generally well accepted marker of disease severity. Agents with direct antiproteinuric effects are highly desirable therapeutic strategies for slowing, or even halting, progressive loss of kidney function. We review progress on therapies acting further downstream of the renin–angiotensin–aldosterone system pathway (e.g., transforming growth factor-beta antagonism, endothelin antagonism) and on those acting independent of the renin–angiotensin–aldosterone system pathway. In all, we discuss 26 therapeutic targets or compounds and 2 lifestyle changes (dietary modification and weight loss) that have been used clinically for diabetic or nondiabetic kidney disease. These therapies include endogenous molecules (estrogens, isotretinoin), biologic antagonists (monoclonal antibodies, soluble receptors), and small molecules. Where mechanistic data are available, these therapies have been shown to exert favorable effects on glomerular cell phenotype. In some cases, recent work has indicated surprising new molecular pathways for some therapies, such as direct effects on the podocyte by glucocorticoids, rituximab, and erythropoietin. It is hoped that recent advances in the basic science of kidney injury will prompt development of more effective pharmaceutical and biologic therapies for proteinuria.

Keywords

Proteinuria; Albuminuria; Podocyte; Glomerulus; Diabetes; Novel therapies

Overview of Non-Renin–Angiotensin–Aldosterone System Agents

Looking beyond renin–angiotensin–aldosterone pathway inhibitors, which are addressed elsewhere in this issue, there are diverse antiproteinuric therapies. Several recent reviews have recently been written on novel agents used for slowing the progression of CKD.^{1,2} We will highlight novel mechanisms for established antiproteinuric therapies, as well as progress on the development of new antiproteinuric agents. Some of these agents may work by stabilizing or normalizing podocyte phenotype.^{2–6} Most molecular entities have pleiotropic effects, and, collectively these therapies also have anti-inflammatory, immunosuppressive, antifibrotic, and cytoprotective properties. Given the complexity of kidney injury and repair, it is seldom possible to disentangle these effects in vivo. We will focus in this brief overview on antiproteinuric effects, particularly on publications in the past 5 years. A summary of these agents and their proposed cell targets and mechanisms of action is presented in Table 1.

Glucocorticoids

Glucocorticoids have efficacy in both inflammatory and noninflammatory glomerular disease, suggesting complex modes of action. In human podocytes and rodent adriamycin nephrosis, dexamethasone was shown to restore suppressed nephrin and reduced vascular endothelial growth factor (VEGF) proteins.^{7,8} Nephrin is an integral component of the slit diaphragm structure; when reduced in quantity, as in nephrotic syndrome, it causes disruption of the glomerular barrier. VEGF is involved in angiogenesis regulation. Fuji and colleagues found in cells expressing nephrin, that the stress of glucose deprivation induces endoplasmic reticulum (ER) stress and subsequent underglycosylation of nephrin and retention in the ER.⁹ Dexamethasone was shown to restore nephrin release, apparently by stimulating mitochondrial ATP generation, but it could not improve nephrin processing in another form of ER stress (calcium imbalance), raising questions about the generalizability of the findings. In cultured mouse podocytes, it was shown that dexamethasone administered either before or after the toxin puromycin aminonucleoside prevented or reversed actin depolymerization and increased RhoA activity, which promotes stress fiber assembly.¹⁰ Although it is unclear how these activities relate to in vivo conditions, it is conceivable that dexamethasone might have favorable effects on disordered podocyte structure.

Adrenocorticotrophic Hormone

Adrenocorticotrophic hormone (ACTH), also known as corticotropin, is derived from the pituitary peptide proopiomelanocortin. ACTH is further processed into alpha-melanocyte-stimulating hormone (MSH) and corticotropin-like intermediate lobe peptide. A synthetic, active form of ACTH known as cosyntropin (synacthen) consists of the first 24 amino-acid residues of the hormone. Beginning in the 1950s, ACTH was widely used for nephrotic syndrome, but was later supplanted by synthetic glucocorticoids. More recently, an open-label trial in 23 subjects with diverse glomerular disease showed benefit,¹¹ and a randomized controlled trial by Ponticelli and colleagues showed similar efficacy for cytotoxic therapy versus ACTH in membranous nephropathy.¹² Beyond stimulating adrenal production of cortisol, ACTH may be acting by other mechanisms. ACTH specifically binds one of the MSH receptors, which is expressed on all 3 glomerular cell types, with highest expression by podocytes. In Heymann nephritis, a rat model of membranous nephropathy, MSH and an MSH agonist peptide both reduced proteinuria.¹³

Calcineurin Inhibitors

The observation that cyclosporine reduces proteinuria in nonimmune-mediated renal disease has prompted a search for additional mechanisms of action. Two recent observations may explain the role of cyclosporine in proteinuric disease. First, cyclosporine blocks the calcineurin-mediated dephosphorylation of synaptopodin, and this action protects synaptopodin from cathepsin L-mediated degradation; because synaptopodin is an actin-binding protein, it therefore helps maintain the integrity of the actin cytoskeleton.¹⁴ Second, cyclosporine downregulates expression of transient receptor potential channel, subfamily 6 (TRPC6) in adriamycin nephropathy, the animal model of focal glomerulosclerosis. TRPC6 gain-of-function mutations cause focal segmental glomerulosclerosis (FSGS) and TRPC6 is upregulated by angiotensin II, thus downregulation of TRPC-6 may contribute to the antiproteinuric effect of cyclosporine.¹⁵

Mycophenolate

Mycophenolate has been widely used for proteinuric diseases, but their antiproteinuric mechanisms have remained poorly defined. Apart from possible effects on systemic immunity, there is evidence of reduced interstitial mononuclear cell accumulation (but not reduced proteinuria) in the rat 5/6 renal ablation model,¹⁶ and reduced proteinuria and

glomerular and tubular injury in the obese Zucker rat model.¹⁷ Few randomized controlled trials have addressed the use of mycophenolate in primary glomerular disease¹⁸; the recently completed, but unpublished, FSGS controlled trial suggested similar efficacy between the cyclosporine arm and the mycophenolate plus intermittent oral dexamethasone pulse.

Rituximab

A serendipitous observation that rituximab anti-CD20 therapy used for post-transplant lymphoproliferative disease was associated with remission of recurrent FSGS has led to widespread use of this agent for recurrent and native kidney FSGS. In general, response rates have been variable, with the most beneficial effects in case series of glucocorticoid-sensitive children with nephrotic syndrome as a result of native kidney FSGS and minimal change disease.^{19,20} In 10 children on long-term cyclosporine therapy for steroid-dependent minimal change disease, a single infusion of rituximab was associated with decreased relapse rates and steroid use at a mean follow-up of 17 months.²⁰ The salutary effect in podocytopathies has been puzzling because B cells have not been implicated in pathogenesis. Recently, it has been shown that in cultured podocytes, recurrent FSGS serum disrupts the actin–myosin cytoskeleton and reduces activity of acid sphingomyelinase. Rituximab binds sphingomyelinase-like-phosphodiesterase-3b–precursor and restores acid sphingomyelinase activity, and this cytoprotective effect may explain the remittive potential.²¹

Omega-3 Fatty Acids

Omega-3 polyunsaturated fatty acids (PUFAs) have anti-inflammatory effects and may decrease proteinuria. Garman and colleagues showed that in rat diabetic nephropathy, canola oil (rich in omega-3 PUFAs) attenuated albuminuria, glomerulosclerosis, tubulointerstitial fibrosis, hypertension, and inflammation.²² The degree of reduction in nephrin and nestin normally associated with diabetic nephropathy was reduced when treated with canola. Transforming growth factor-beta (TGF- β) immunostaining in mesangial cells improved after canola supplementation. Omega-3 PUFAs may affect previously noted TGF- β -related injury of podocytes in glomerular diseases. In addition, markers of inflammation, such as MCP-1, and CD68-positive cells were also attenuated by canola oil, which would point to an additional anti-inflammatory mechanism. In cultured mesangial cells, docosahexaenoic acid, an omega-3 PUFA, suppressed proliferation and growth of cultured mesangial cells. In rodent acute mesangial proliferative glomerulonephritis, omega-3 PUFAs significantly reduced proteinuria and mesangial cell proliferation and matrix expansion.²³

A recent meta-analysis of 17 trials (10 of them randomized controlled trials) examined omega-3 PUFA effects on proteinuria. It involved 626 patients with glomerular diseases that included immunoglobulin A (IgA) nephropathy, diabetic nephropathy, lupus nephritis, and mixed etiologies.²⁴ The doses used ranged considerably (by 8-fold) and the median follow-up was 9 months. The estimated pooled effect size was -19% (95% CI: -34%, -4%; $P = .01$). Although the effect size was modest, this therapy is well-tolerated and merits continued consideration.

Tumor Necrosis Factor Antagonism

Chronic inflammation and cytokines such as tumor necrosis factor (TNF; the cytokine formerly known as TNF α) have been implicated in diabetic nephropathy and may contribute to other glomerulopathies. Several approaches to block TNF activity are available, including anti-TNF monoclonal antibodies (infliximab, adalimumab) and a soluble TNF receptor (etanercept). TNF antagonism may have direct effects on glomerular cells. Thus, TNF suppresses nephrin expression in cultured podocytes through the cyclic adenosine monophosphate–protein kinase A pathway²⁵ and reorganizes the actin cytoskeleton.²⁶

Human studies of TNF antagonism for primary kidney disease continue to remain at an early stage. In patients with membranous nephropathy, etanercept showed no improvement.²⁷ Adalimumab, a human monoclonal antibody directed against TNF, was tested in a single administration, dose escalation design and safety was demonstrated in patients with FSGS.²⁸ A case report described membranous nephropathy after the use of infliximab; although causation was not established, this does sound like a note of caution.²⁹

TGF- β Antagonism

TGF- β is mostly accepted as a profibrotic molecule, a major factor in diabetic nephropathy, and is found to be overexpressed in hyperplastic podocytes in glomerular diseases.³⁰ TGF- β inhibition has been shown to inhibit podocyte apoptosis by affecting the expression of p21 and Smad-7 and reversing increases in proapoptotic protein Bax and classical effector caspase-3.^{31,32}

In streptozotocin-induced diabetic nephropathy, both lisinopril and 11D11 (an anti-TGF- β antibody) decreased proteinuria, and when used in a combined form almost normalized proteinuria.³³ Smad-3 knockout mice with diabetic nephropathy had improved renal function and less severe renal hypertrophy and glomerular basement membrane (GBM) thickening, but without effects on albuminuria.³⁴ Thus, the antiproteinuric effect of inhibition of TGF- β seems to be at best indirect by influencing podocyte differentiation and apoptosis.

Retinoids

Retinoids are essential for embryogenesis, in particular for nephron development, and have an established therapeutic role in promoting cell differentiation in cancer. In vitro studies indicate that all-trans retinoic acid (ATRA), a potent ligand for the retinoic acid receptor, has differentiating effects on cultured podocytes. In murine podocytes, ATRA stimulates nephrin RNA and protein expression, acting through a retinoic acid receptor element in the nephrin promoter.^{35,36} HIV-expressing podocytes exhibit dedifferentiation and podocyte proliferation; subsequent ATRA treatment was shown to be associated with G1 cell cycle arrest and differentiation, with increased expression of synaptopodin, nephrin, podocin, and Wilms tumor-1.³⁷

In vivo studies in animals and humans support a role for ATRA to promote podocyte differentiation in various models, including HIV-transgenic mice and puromycin aminonucleoside nephrosis (PAN) in rats.^{37,38} In streptozotocin-diabetic rats, ATRA reduced proteinuria and monocytic infiltrates.³⁹ In autoimmune nephritis characterized by anti-GBM antibodies, ATRA ameliorated multiple features, including antibody deposition, cytokine production, and lymphocyte infiltration.⁴⁰ To date, no clinical studies using retinoid for medical renal disease have been reported.

Statins

HMG-CoA inhibitors (statins) manifest anti-inflammatory effects and podocyte-specific cytoprotective effects.⁴¹ In immortalized mouse podocytes, rosuvastatin protects against podocyte apoptosis, but only in cells with p21 expression, which suggests a p21-dependent antiapoptotic mechanism.⁴² In obese diabetic db/db mice, pitavastatin reduces albuminuria, mesangial expansion, and oxidative stress markers (possibly because of downregulation of NAD(P)H oxidase 4).⁴³ In the rat model of minimal change disease, such as in PAN, fluvastatin administered before development of nephrosis markedly improved proteinuria and foot process effacement and prevented decline in nephrin and podocin expression. Fluvastatin decreased excessive Rho-kinase activation, and a specific inhibitor of RhoA

resulted in amelioration of podocyte injury, concordant with the known role of Rho kinase in cytoskeleton rearrangement.⁴⁴

In a meta-analysis of clinical studies, statins reduced proteinuria, with a greater proportional effect in subjects with more proteinuria.⁴⁵ The favorable effects of statins have been attributed to lipid lowering, reduction in inflammation and fibrosis, reversal of mesangial proliferation, and effects on podocytes.

Calcium Channel Blockers

Afferent glomerular arterioles express T- and L-type calcium channels, whereas efferent arterioles express only T-type calcium channels. T- and L-type calcium channel blockers (nondihydropyridines and certain newer dihydropyridines, including efonidipine, which has been approved in Japan but not in the United States) dilate afferent and efferent arterioles and reduce proteinuria more effectively, compared with L-type calcium channel blockers (dihydropyridines such as amlodipine and nifedipine), which only dilate the efferent arteriole. Inhibition of both T- and L-type leads to a greater reduction in glomerular capillary pressure and reduced filtration fraction.⁴⁶ Furthermore, dihydropyridine calcium channel blockers also antagonize aldosterone-mediated activation of the mineralocorticoid receptor, and efonidipine reduces plasma aldosterone concentrations and glomerular injury in Dahl salt-sensitive rats.^{47,48}

Despite the possible benefits of nondihydropyridines, in a trial involving 304 hypertensive diabetic patients with macroalbuminuria, there was no difference in albuminuria between the verapamil and nifedipine groups.⁴⁹ Among 21 patients with chronic glomerulonephritis in a randomized crossover trial, efonidipine reduced proteinuria more effectively than nifedipine; the effect was statistically significant but quite modest.⁵⁰ In 40 diabetic patients with impaired glomerular filtration rate (GFR), proteinuria rose and GFR fell to a greater extent with amlodipine, compared with efonidipine.⁴⁷

Thiazolidinediones

Thiazolidinediones (TZD) are antiglycemic, insulin-sensitizing agonists of the peroxisome proliferator-activated receptor gamma, which is widely expressed in fat, muscle, liver, and kidney.⁵¹ The TZD pioglitazone stimulates nephrin promoter activity in vitro, as well as reduces proteinuria and increases nephrin RNA and protein expression in the passive Heymann nephritis model.⁵² TZDs reduce proteinuria in several rat models of diabetic nephropathy, including streptozotocin rats and obese SHR-ND-mcr-cp rats.^{53,54}

A recent meta-analysis identified 15 randomized controlled trials, including 5 with rosiglitazone and 10 with pioglitazone, involving 2860 diabetic patients with albuminuria. In the 3 studies that involved a total of 140 subjects with overt proteinuria, TZDs (pioglitazone in all 3 studies) reduced proteinuria by 37% (95% CI: -58%, -7%).⁵⁵ The effect size in studies involving subjects with microalbuminuria or normoalbuminuria at baseline was similar. Rosiglitazone is currently available in the United States, but under restricted access, because of reports of increased cardiovascular event rates. By comparison, the present data on pioglitazone suggest no increased cardiovascular events, with the exception of congestive heart failure because of the stimulatory effect of peroxisome proliferator-activated receptor gamma agonists on renal tubular sodium reabsorption.

Vitamin D and Analogs

Vitamin D and vitamin D analog therapy have shown efficacy in experimental animal models of glomerular disease in studies extending back over the past 25 years. Vitamin D was initially proposed to act as an immunosuppressive agent, and indeed, it functions in part

to suppress NF- κ B-mediated inflammatory pathways. Vitamin D receptor null mice manifest increase in renin levels and develop more severe diabetic nephropathy, and fibroblasts isolated from these mice showed a proinflammatory phenotype.⁵⁶ In animal models of podocyte injury, calcitriol pretreatment stabilizes podocyte phenotype, with preserved expression of nephrin and podocin.⁵⁷ Mechanisms demonstrated in animal models have included reduced renin–angiotensin–aldosterone system activity,⁵⁸ blockade of Wnt/beta-catenin signaling,⁵⁹ and activation of PI-3-kinase/AKT pathway.⁶⁰ In the recently published VITAL study, a randomized controlled trial of 281 diabetics with type 2 diabetes mellitus and microalbuminuria or macroalbuminuria, a high dose of paricalcitol reduced albuminuria by 20%.⁶¹ With its favorable toxicity profile, this is a promising agent to combine with other antiproteinuric therapies.

Pentoxifylline

Pentoxifylline, a methylxanthine derivative and nonselective phosphodiesterase, has been approved by the Food and Drug Administration for treating claudication associated with peripheral artery disease. Preclinical renal studies of pentoxifylline, which may modulate diverse pathways, including TNF production and signaling, are too numerous to review here.

Pentoxifylline has been used in clinical studies of kidney diseases for nearly 30 years. A meta-analysis of randomized controlled trials in diabetic nephropathy published through 2006 focused on 10 trials.⁶² Higher baseline proteinuria correlated with greater treatment response. Among 6 trials involving subjects with macro-proteinuria, the pooled estimate of the pentoxifylline effect was -502 mg/d proteinuria (95% CI: -805 , -198 ; $P < .01$). The authors concluded that quality of the existing studies was low.

In a pilot study of 10 patients with membranous nephropathy, pentoxifylline reduced mean proteinuria from 11 g/d to 2 g/d, with nearly 90% drop in serum and urine TNF levels.⁶³ In a randomized trial (lacking a placebo and thus lacking blinding) that involved 85 subjects with moderate-to-severe CKD, pentoxifylline group had a median 28% reduction in proteinuria, whereas the control group had a median 14% increase.⁶⁴

Adequately powered clinical trials are needed; however, because pentoxifylline is off patent, this may require noncommercial sponsorship or the development of novel compounds that target the same pathway.

Pirfenidone

Pirfenidone is an antifibrotic agent with anti-TGF- β and anti-TNF properties, and at high concentrations, it is a scavenger of reactive oxygen species (ROS). In rats with anti-GBM glomerulonephritis, both candesartan and pirfenidone decreased proteinuria and glomerulosclerosis, and the combination of both drugs showed additive effects.⁶⁵ Pirfenidone reduces interstitial fibrosis in the remnant nephron rat model but does not reduce proteinuria.⁶⁶ Similarly, it reduces mesangial matrix expansion in db/db mice, but without affecting proteinuria.⁶⁷ In a one-arm trial involving 21 patients with idiopathic and postadaptive FSGS, pirfenidone treatment slowed the GFR decline rate, but did not affect proteinuria.⁶⁸ Thus, pirfenidone holds promise as an antifibrotic drug, but seems to lack consistent antiproteinuric effect. This example of dissociation between fibrosis and proteinuria sounds a cautionary note on relying exclusively on anti-proteinuric properties to select agents for progressive kidney disease.

Tranilast

Tranilast, *N*-[3,4-dimethoxycinnamoyl]-anthranilic acid, is an antifibrotic agent approved in Japan for treatment of keloids. Tranilast inhibits signaling and/or production of TGF- β ,

platelet-derived growth factor (PDGF), interleukin-1, and MCP-1. In vitro, tranilast reduced TGF- β -induced fibronectin synthesis and hydroxyproline accumulation in human cortical fibroblasts.⁶⁹ In diabetic rats, tranilast reduced tubulointerstitial fibrosis and tubular atrophy, while almost normalizing albuminuria. In subnephrectomized rats, tranilast attenuated albuminuria, TGF- β activation, downstream Smad 2 phosphorylation, macrophage accumulation, glomerulosclerosis, and tubulointerstitial fibrosis.^{70,71} In a model of IgA nephropathy, tranilast reduced mesangial cell proliferation, macrophage infiltration, glomerular type IV collagen deposition, and subsequently proteinuria.⁷²

Tranilast was tested as an approach to retard coronary artery restenosis after stenting (the PRESTO trial); although the drug lacked efficacy, there was a generally favorable safety profile.⁷³ There have been 2 small studies of tranilast for diabetic kidney disease. In an uncontrolled study of 9 patients with diabetic nephropathy, tranilast slowed progressive loss of renal function (assessed as 1/serum creatinine).⁷⁴ In a randomized controlled trial involving 20 diabetic patients with albuminuria and receiving ACE inhibitor or ARB therapy, tranilast treatment for 1 year reduced albuminuria and urinary collagen IV.⁷⁵ Further clinical development seems to be warranted.

Triptolide

Triptolide (PG-490) is an active component of the Chinese herb *Tripterygium wilfordii*, used widely for immunologic diseases in traditional Chinese medicine. Its actions in glomerular disease have been explored, using both an immunologic model (passive Heymann nephritis) and a non-immunologic model (PAN).^{76,77} Triptolide treatment decreased proteinuria in PAN and reduced immune deposits in membranous nephropathy, consistent with its immunosuppressive properties (possibly by stimulating T-rags). Triptolide may act on cultured podocytes through suppression of various injury pathways, including p38 MAP kinase activation and ROS generation, and by stimulating Rho activity. Crude herbal preparations have moderate toxicity, including gastrointestinal effect and cytopenias. Recently, a phase I trial of a semisynthetic triptolide derivative (F6008) in advanced solid tumors resulted in 2 deaths and associated monocyte apoptosis.⁷⁸ Further development with better understanding of the molecular pathways and less toxic derivatives are needed.

Antiplatelet Drugs

Antiplatelet drugs have been previously used for glomerular diseases,⁷⁹ but have generally fallen out of favor, and have been replaced by more effective therapy. One exception is childhood IgA nephropathy,^{80,81} particularly in Japan; a randomized trial involving 80 children with newly diagnosed IgA nephropathy found combination therapy (prednisolone, azathioprine, heparin/warfarin, and dipyridamole) to be superior to prednisolone alone.⁸² Proposed mechanisms of benefit have included suppression of platelet adhesion and glomerular mesangial cell proliferation, improved glomerular hemodynamics, altered GBM anionic charge, and scavenging of ROS.⁸³ To our knowledge, there have been no adequately powered randomized trials that isolated the effect of anti-platelet agents.

Erythropoietin

Darbepoetin has been shown to reduce proteinuria and facilitates glomerular repair in Thy-1 rat glomerulonephritis model,⁸⁴ proteinuria in rat PAN,⁸⁵ and proteinuria and podocyte apoptosis in antiglomerular serum-induced mouse glomerulonephritis.⁸⁶ In the latter study, the effects were attributed to activation by darbepoetin of the AKT cell survival pathway.

Retrospective analysis of patients with CKD treated with erythropoietin showed a slower rate of progression of renal function decline, although the studies were not randomized and involved a total of 140 subjects.^{87,88} In contrast, no renoprotective effects were seen in 2

large randomized controlled trials.^{89,90} Till recently, no study has addressed an antiproteinuric role for erythropoietin in human proteinuric diseases, and issues of dosing and safety would have to be addressed before initiating such studies.

Novel Agents in Diabetic Nephropathy

Novel therapies specific to diabetic nephropathy have been reviewed elsewhere in detail.^{91–94} We will address 9 agents and approaches that may help reduce proteinuria.

VEGF Antagonism

VEGF antagonism, either in the setting of cancer therapy or in experimental mouse models, is associated with thrombotic microangiopathy and glomerular injury. Conversely, it may have a role in the treatment of diabetic nephropathy.⁹⁵ VEGF and its receptor are upregulated in podocytes and tubular cells of streptozotocin-diabetic rats. Antibodies to VEGF improved renal function in this model.⁹⁶ Similarly, nephropathy in db/db mice is ameliorated by a small molecular inhibitor of angiogenesis⁹⁷ and by VEGF tyrosine kinase inhibitor.⁹⁸ Nevertheless, caution is warranted. As noted previously, tilting the balance against VEGF may cause harm. Also, progressive human kidney disease is associated with loss of interstitial capillaries, and this correlates with increased VEGF RNA expression, presumably to promote angiogenesis.⁹⁹ Chronic VEGF suppression may worsen interstitial fibrosis, which the rodent models may fail to model adequately. Much work is still needed to truly assess for beneficial mechanisms and safety of VEGF antagonism for proteinuria.

AGE Antagonism

Advanced glycation end products (AGEs) are produced following nonenzymatic glycosylation of proteins and lipids, and this process is accelerated in diabetes.¹⁰⁰ In human diabetic nephropathy, AGEs accumulate in the mesangium and the GBM and there is increased expression of the AGE receptor (RAGE) in podocytes.¹⁰¹

RAGE activation promotes podocyte production of ROS and profibrotic growth factors in PAN.¹⁰² In diabetic db/db mice, RAGE expression in podocytes is associated with increase in VEGF production and inflammatory molecule accumulation, both reversed by the administration of a soluble RAGE.¹⁰³ The upregulation of VEGF may promote altered glomerular permeability and proteinuria. When diabetic rats were treated with perindopril or the AGE-formation inhibitor aminoguanidine, both agents restored nephrin depletion and reduced albuminuria and tubulointerstitial injury.¹⁰⁴ Mice lacking RAGE and subjected to streptozotocin-induced diabetes did not develop albuminuria.¹⁰⁵

Therapeutic targets for inhibiting AGE accumulation include the following: AGE-formation inhibitors (e.g., aminoguanidine), AGE crosslink breakers, RAGE antagonists (e.g., soluble RAGE, RAGE antibody), and RAGE signaling pathway molecules (protein kinase C inhibitors).¹⁰⁶ As reviewed in the study by Turgut and Bolton, aminoguanidine reduced proteinuria in the ACTION trial but did not affect the primary outcome, the time to creatinine doubling. Several other agents are being tested in phase I or II trials. Continued research is needed before AGE antagonism may eventually prove to be beneficial in controlling proteinuria.

Endothelin Antagonists

The vasoactive peptide endothelin-1 has been implicated in diabetic nephropathy. In apolipoprotein E knockout and diabetic mice, an endothelin receptor A antagonist, avosentan, ameliorated albuminuria, extracellular matrix accumulation, and oxidative stress.¹⁰⁷ In addition, it reduced expression of angiotensin I, endothelin receptor gene,

proinflammatory cytokines (VEGF, NF- κ B), and fibrosis-related molecules (TGF- β , fibronectin). In a randomized controlled trial of diabetic patients, avosentan added to an ACE inhibitor regimen further reduced albuminuria, but with the side effect of significant volume overload and congestive heart failure, causing termination of one study.^{108,109} The antiproteinuric mechanism may be related to the changes in renal blood flow, normalized endothelial function, and/or reduced inflammation and fibrosis.

Antioxidants

ROS are important mediators of progressive kidney disease, but finding an effective antioxidant therapy has proven to be challenging, despite studies using a broad array of agents with in vitro antioxidant activity.¹¹⁰ One promising new approach involves nuclear factor erythroid-related factor 2 (Nrf2), a redox-sensitive transcription factor, which plays a central role in the cellular response to oxidative stress.¹¹¹ Nrf2 null mice were shown to have more ROS production and oxidative DNA and renal injury, and Nrf2 overexpression was shown to inhibit TGF- β 1 in these mice. Nrf2 may be protective in diabetic nephropathy through reduction in oxidative damage, and perhaps, a mechanism related to pathways involving TGF- β . In addition, glomeruli of patients with diabetic nephropathy were found to have upregulated oxidative stress and Nrf2 levels. Bardoxolone, an inducer of Nrf2, currently is in phase IIb clinical trials of diabetic nephropathy.¹¹² Antioxidants may prove to be renoprotective, but specific antiproteinuric pathways have not been found.

Ruboxistaurin

Ruboxistaurin, a selective inhibitor of protein kinase C-beta (PKC- β), has been studied in diabetic nephropathy, as protein kinase C-regulated activation has been accepted to play a role in vascular complications of diabetes. In diabetic animal models, PKC- β inhibition attenuates albuminuria and diabetic morphological changes.¹¹³ PKC- β inhibitor treatment of diabetic mice improved albuminuria and mesangial expansion in the setting of normalizing PKC- β activity and TGF- β levels.¹¹⁴ In a randomized controlled trial of 123 patients with type 2 diabetes, already on ACE and/or ARB therapy, the addition of ruboxistaurin reduced albuminuria and stabilized creatinine clearance.¹¹⁵ In contrast, after a 3-year follow-up of 1157 patients with diabetic retinopathy, there was no significant difference seen in progression of kidney disease between control subjects and those treated with ruboxistaurin.¹¹⁶ Ruboxistaurin is thought to decrease the overexpression of TGF- β and extracellular matrix proteins, but without direct evidence of antiproteinuric effects.

PDGF Antagonism

PDGF has also been implicated in the pathogenesis of diabetic nephropathy. In streptozotocin-diabetic apolipoprotein E knockout mice, a tyrosine kinase inhibitor of PDGF (imatinib) attenuated albuminuria, although it did not normalize impaired GFR.¹¹⁷ In addition, imatinib decreased mesangial matrix expansion, macrophage infiltration, and expression of profibrotic cytokines TGF- β and CTGF. We did not find evidence for specific antiproteinuric role, but the antagonism of PDGF deserves further studies in diabetic nephropathy.

Sulodexide

Sulodexide, a glycosaminoglycan with anticoagulant and antithrombotic properties, has shown evidence of anti-proteinuric effects. In rats with radiation nephropathy, sulodexide did not change renal function, but lowered proteinuria; and in diabetic mice, sulodexide had no effect on proteinuria.¹¹⁸ In rats with adriamycin nephropathy, sulodexide markedly decreased albuminuria and reverted from diffuse to segmental foot process involvement. It also reduced levels of a molecule known to degrade the proteoglycan layer of endothelium

(heparanase messenger RNA), a cytoskeleton stabilizer (desmin), and a slit diaphragm component (CD2AP).¹¹⁹

In diabetic patients, sulodexide treatment after 3 and 6 months significantly reduced albuminuria.¹²⁰ In a small randomized trial of diabetic patients, Broekhuizen and colleagues intricately demonstrated the presence of endothelial glycocalyx perturbation, increased permeability in retinal and sublingual vessels in type 2 diabetic patients, and showed that sulodexide diminished these effects.¹²¹ Sulodexide seems to exert its antiproteinuric property by stabilizing podocyte cytoskeleton and the glomerular endothelial glycocalyx.

Diet

A recent meta-analysis examined the effect of low protein diet in diabetic renal disease.¹²² There was weak evidence of decreased proteinuria with low protein diet, but no effect on renal function was observed. The short duration of interventions and the heterogeneity of the studies prevent a strong conclusion.

The role of plant proteins, rich in phytoestrogens (isoflavones and lignans), has been investigated in the progression of renal diseases.¹²³ In vitro studies show evidence of inhibition of mesangial cells by the isoflavone genistein. Lipid-lowering effect of phytoestrogens may also be contributing to the renoprotective effect. In various animal models of kidney disease, diets high in isoflavones were shown to have a protective effect on renal disease progression, even improving proteinuria in some cases. In small trials of patients with a range of renal diseases, vegetarian diets high in soybean protein and flaxseed, both major sources of phytoestrogens, have shown a decrease in proteinuria. Phytoestrogens may modify gene transcription by disrupting the binding of 17 β -estradiol to the intranuclear estrogen receptor protein. Cell proliferation and signal transduction pathways through protein kinases and their downstream growth factors, such as PDGF, TGF- β , or nuclear transcriptor factors (such as NF- κ B), are thought to be involved. In a more recent study of murine lupus, phytoestrogens attenuated the overall disease process, including glomerulonephritis, likely through anti-inflammatory effects.¹²⁴ The use of phytoestrogens seems hopeful for their anti-proliferative and antioxidant properties in renal diseases, and perhaps, these effects indirectly contribute to their antiproteinuric effects.

Weight Loss

In addition to improving metabolic parameters and decreasing glomerular hyperfiltration, weight reduction reduces proteinuria. In a diabetic mouse model, moderate exercise, independent of glycemic control, reduced weight, albuminuria, glomerular expansion, proapoptotic caspase and TNF expression, and renal oxidative damage.¹²⁵ Ibrahim and Weber reviewed the effect of weight loss in human CKD. Surgical and nonsurgical forms of weight loss seemed to improve blood pressure, proteinuria, and inconsistent GFR.¹²⁶ Shen and colleagues carried out a single-arm trial involving 63 patients with biopsy-proven obesity-related glomerulopathy.¹²⁷ Of 48 patients who completed the study at month 24, 27 subjects had reduced body mass index (BMI) (by a mean of 3 kg), and these individuals had reduced proteinuria from a mean of 1.7 g/d to 0.9 g/d, compared with smaller proteinuria reduction in those with stable BMI and increased proteinuria in those with increased BMI. Although these data are interesting, the lack of a control group is an important limitation.

Conclusion

Recent work has improved our understanding of how antiproteinuric agents work at the cellular and molecular level, and this has the potential to speed the development of new therapies. Nonetheless, given the limitation of current therapies, both those in clinical use

and those with efficacy in animal models, new pathways and new molecular entities are needed. Examples of promising new pathways would be agents that enhance the process by which podocyte progenitor cells self-renew, differentiate, and replace lost podocytes, and agents that reprogram the mesangial cell away from exuberant matrix production. Some of the agents described may fit into these categories, but have limited use because of reported adverse effects, most of which are described elsewhere, and thus, this will require more specific targeting or use of alternative options.

Molecular libraries can be most effectively exploited by using high-throughput assays that are designed to report on effective stimulation or inhibition of specific molecular pathways. Drug development involves a path extending from identification of a promising molecular entity targeting a particular pathway through preclinical work in relevant animal models to clinical trials, with considerable attrition at each step. Given the magnitude of the CKD epidemic, it is important that appropriate societal resources are devoted to this process.

Finally, proteinuria is the major therapeutic target in progressive CKD, and to date, all clinically approved agents that suppress proteinuria also slow CKD progression. We need to keep in mind that agents may act as cytoprotective agents, or perhaps in other ways, and may slow progressive loss of kidney function without altering proteinuria.

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Table 1**Non-RAAS Antiproteinuric Therapies: Major Recognized and Proposed Mechanisms**

Agent	Primary Activity	Proposed Antiproteinuric Target	Major Proposed Mechanism
Glucocorticoids	Immunosuppressant	Podocyte	Transcriptional or translational regulator of nephrin and other podocyte proteins
ACTH	Immunosuppressant	Systemic	Increase in endogenous steroids
		Podocyte	Melanocortin receptor agonist
Calcineurin inhibitor	Immunosuppressant	Podocyte	Arterial vasoconstriction leading to decreased albumin delivery; transcriptional or translational regulator of TRPC6; blockade of synaptopodin dephosphorylation
Mycophenolate	Immunosuppressant	Unknown	No specific mechanism identified
Rituximab	Immunosuppressant	Podocyte	Restoration of sphingomyelinase signaling
Omega-3 fatty acids	Anti-inflammatory, lipid-lowering	Podocyte	Transcriptional or translational regulator of nephrin and other podocyte proteins; reduction of profibrotic and proinflammatory molecules
		Mesangial cell	Reduces mesangial cell proliferation
TNF antagonism	Anti-inflammatory	Podocyte	Transcriptional regulation of nephrin, paxillin, focal adhesion kinase; preservation of cytoskeleton rearrangement with additional unclear mechanism; regulation of MAP kinase
		Mesangial cell	Anti-inflammatory
TGF- β antagonism	Antifibrotic, cytoprotective	Podocyte	Antiapoptotic
Retinoids	Cell differentiation Apoptosis Anti-inflammatory Antiproliferative	Podocyte	Transcriptional regulator of nephrin, synaptopodin, podocin, WT1; inhibition of cell differentiation and proliferation, anti-inflammatory
Statins	Lipid metabolism	Podocyte	Antiapoptotic, anti-inflammatory, antioxidant
		Mesangial cell	Antiproliferative
Nondihydropyridine calcium channel blockers	Hemodynamic	Glomerular capillaries	Glomerular capillary pressure reduction
Thiazolidinediones	Antiglycemic/metabolic	Podocyte	Transcriptional regulator of nephrin; anti-inflammatory, antifibrotic
		Glomerular cell	Preservation of permeability regulation
Vitamin D	Anti-inflammatory	Podocyte	Transcriptional regulator of nephrin, podocin; blockade of Wnt/beta-catenin signaling; activation of PI-3-kinase/AKT
Pentoxifylline	Cytokine antagonism	Unknown	Unknown
Pirfenidone	Antifibrotic	Mesangial cell	Antifibrotic
		Unknown	RNA processing; antioxidant
Tranilast	Antifibrotic	Fibroblasts	Antifibrotic
		Mesangial cell	Antiproliferative
Triptolides	Immunosuppressant	Podocyte	Antioxidant; regulation of MAP kinase activation
Antiplatelet drugs	Anticoagulant	Glomerular arterioles	Regulation of renal blood

Agent	Primary Activity	Proposed Antiproteinuric Target	Major Proposed Mechanism
Erythropoietin	Erythropoiesis	Glomerular capillaries	Suppression of platelet coagulation, adhesion; preservation of permeability, unclear mechanism
		Mesangial cell	Antiproliferative
		Unknown	Antioxidant
		Podocyte	Antiapoptotic; transcriptional regulator of nephrin and other podocyte proteins
VEGF	Angiogenesis	Podocyte	Transcriptional or translational regulator of nephrin
AGE antagonism	Product of nonenzymatic glycoxidation from diabetes	Endothelial cell	Regulator of vasodilation and permeability
		Podocyte	Transcriptional or translational regulator of nephrin; anti-inflammatory, antioxidant, antifibrotic, VEGF suppression
Endothelin antagonist	Vasoconstriction	Unknown	Regulation of renal blood flow; anti-inflammatory, antifibrotic
Antioxidants (Nrf2, bardoxolone)	Antioxidant	Unknown	Antioxidant, anti-inflammatory
Ruboxistaurin	Protein kinase-C inhibition	Unknown	Antifibrotic unknown
PDGF antagonism	Angiogenesis	Unknown	Antifibrotic
Sulodexide	Anticoagulant, antithrombotic	Endothelial cell	Preservation of endothelial layer
Diet	Low protein	Unknown	Unknown and no clear evidence
	Plant proteins	Mesangial cell	Antiproliferative
		Unknown	Antiproliferative, antioxidant
Weight loss	NA	Glomerular cell	Decreasing glomerular hyperfiltration, antioxidant, antiapoptotic, anti-inflammatory

Abbreviations: RAAS, renin–angiotensin–aldosterone system; ACTH, adrenocorticotrophic hormone; TRPC6, transient receptor potential C6; TNF, tumor necrosis factor; MAP, mean arterial pressure; TGF- β , transforming growth factor-beta; WT1, Wilms tumor-1; PI-3, phosphatidylinositol-3-kinase; VEGF, vascular endothelial growth factor protein; AGE, advanced glycation end product; Nrf2, nuclear factor erythroid-related factor 2; NA, not applicable.