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The pathogenesis of diabetic nephropathy (DN) and current anti-DN agents are summarized, providing potential guidance for the future design and discovery of novel anti-DN agents.

Therapeutic strategies of diabetic nephropathy: recent progress and future perspectives

Meng Lv, Zhuo Chen, Gaoyun Hu and Qianbin Li

Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Central South University, 410013 Changsha, Hunan, China

Diabetic nephropathy (DN) is one of the most common complications of diabetes with high mortality rates worldwide. The treatment of DN has posed a formidable challenge to the scientific community. Simple control of risk factors has been insufficient to cope with the progression of DN. During the process of anti-DN drug discovery, multiple pathogeneses such as oxidative stress, inflammation and fibrosis should all be considered. In this review, the pathogenesis of DN is summarized. The major context focuses on a few small molecules toward the pathogenesis available in animal models and clinical trials for the treatment of DN. The perspectives of novel anti-DN agents and the future directions for the prevention of DN are discussed.

Introduction

With the worldwide increase in the prevalence of diabetes in recent years, diabetic nephropathy (DN), a common complication of diabetes, has become a threat to human health and mortality [1]. In 2013, there were 347 million people with diabetes all over the world, and WHO projects that diabetes will be the seventh-leading cause of death by 2030 [2]. The incidence increases particularly with the improvement of living standards and the change of lifestyle in developed countries such as the USA [3]. Approximately one-third of all diabetic individuals (with either type 1 or type 2 diabetes) are affected by DN [4], which produces significant social and economic burdens [5].

The pathogenic essence of DN is kidney fibrosis, which is clinically characterized by an initial increase in glomerular filtration rate (GFR) and microalbuminuria [6]. Mounting evidence suggests that genetic predisposition, hyperglycemia, hypertension and dyslipidemia mainly contributed to the induction and progression of DN. Although DN is becoming increasingly recognized as a leading cause of morbidity and mortality in most kidney diseases, there are no treatment strategies available that specifically target the pathogenesis of DN, instead they control, for example, high blood pressure and glucose levels. Nevertheless, during the past five years of global investigation efforts, it is deemed that oxidative stress [7], inflammation [8] and fibrosis are the key links in the progress of DN, which has provided scientific researchers with an enormous number of potential targets. Given the drawbacks of angiotensin-converting enzyme inhibitors

Corresponding authors: Hu, G. (hugaoyun@csu.edu.cn), Li, Q. (qbli@csu.edu.cn)

Meng Lv is currently a postgraduate researcher in medicinal chemistry at the Laboratory of Small Molecule Drug R&D for Major Diseases from Central South University. He received his BS degree in pharmaceutical sciences from Zhengzhou University in 2008. He has been concentrating on the design and synthesis of priding derivatives as antidiabetic nephropathy agents.

Zhuo Chen is a lecturer at the Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Central South University. He received his PhD degree of chemical biology at Peking University in 2012. His current research interest is focused on chemical biology and drug discovery of traditional herbal extract.

Gaoyun Hu is a professor at the Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Central South University. He received his BS degree in medicine at the School of Medicine, Peking University in 1983. He visited Rutgers University, New Brunswick, USA, during 2009 and 2010. His current research interest is focused on drug discovery in the field of diabetic nephropathy and fibrotic disorders.

Qianbin Li is an associate professor at the Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Central South University. He received a BS degree in pharmacy in 2003 and a PhD in medicinal chemistry in 2008 from Shandong University. He joined the School of Pharmacy, Virginia Commonwealth University, as a postdoctoral fellow in February 2008. Li was named associate professor at Central South University in April 2010. His current research is focused on medicinal chemistry, namely in structure-based drug design and discovery in the field of diabetic nephropathy, cancer and inflammatory disorders.

1359-6446/06/$ - see front matter http://dx.doi.org/10.1016/j.drudis.2014.10.007

Please cite this article in press as: Lv, M. et al. Therapeutic strategies of diabetic nephropathy: recent progress and future perspectives, Drug Discov Today (2014), http://dx.doi.org/10.1016/j.drudis.2014.10.007

BOX 1

Microalbuminuria
Microalbuminuria (MAU), also known as urine albumin, occurs when the kidney leaks small quantities of albumin into the urine – in other words there is an abnormally high permeability for albumin in the renal glomerulus. MAU is usually defined when urine albumin is between 30 and 299 mg over a 24-hour collection period. MAU is frequently used as an important prognostic marker to assess the stage of certain diseases such as diabetic nephropathy (DN) and cardiovascular disease.

Diabetic nephropathy
DN is a progressive kidney disease caused by long-standing diabetes mellitus, and is a big problem in many western countries. Clinically, DN is characterized by a progressive increase in proteinuria and decline in glomerular filtration rate (GFR). Generally, DN is divided into five stages: (i) hyperfunction with raised GFR and increased kidney volume; (ii) normalbuminuria with the thickening of the glomerular basement membrane (GBM) and the expansion of the mesangial matrix; (iii) incipient DN with MAU (30–299 mg per day); (iv) overt DN with macroalbuminuria (>300 mg per day); (v) uremia with end-stage renal disease.

(ACEIs) and angiotensin receptor blockers (ARBs) [9], such as for the risk of kidney dysfunction and the low efficiency to terminal DN [9,10], the trend of anti-DN agent studies has been oriented to target the multiple mechanisms of DN; for instance targeting interrelated enzymes, interrupting cell signal transduction pathways and reducing extracellular matrix (ECM) accumulation. The drugs designed are expected to have high affinity to multifactorial pathogenesis, which can inhibit the production of ECM and speed up decomposition. This article will review the pathogenesis of DN, novel anti-DN targets and corresponding inhibitors or manipulators discovered in recent years (Box 1).

Key links for the development of DN

Other than genetics, age and obesity [11], at least two theories regarding the development and progression of DN have been proposed: that is metabolic and hemodynamic, including blood glucose and lipid metabolism disorders. Additionally, increasing studies suggest that oxidative stress, inflammation and fibrosis appear to be the key links in the progression of DN.

Oxidative stress is a normal defense mechanism in which the body responds to stimuli outside [12]. During the progress of diabetes, stimuli such as high blood glucose and high blood pressure cause the excessive production of reactive oxygen species (ROS), breaking the oxidation–reduction balance and destroying the mechanism of autocontrol oxidation–reduction regulation, which consequently initiates oxidative stress. Moreover, numerous research studies indicated ROS cause direct and indirect damage on renal interstitium induced by oxidative stress under the long-term ongoing hyperglycemia condition: (i) renal vascular sclerosis; (ii) increased vascular permeability; (iii) structure and function damage; (iv) activating downstream mediators such as extracellular regulated protein kinases (ERK), p38 mitogen-activated protein kinases (p38 MAPK), nuclear factor κB (NF-κB) and activator protein-1 (AP-1), triggering a series of cellular responses, which are thought to contribute to the development of DN. Activation of nicotinamide adenine dinucleotide phosphate oxidases (NOX) [13] and protein kinase C (PKC) [14], increased formation of advanced glycation endproducts (AGEs) [15] and the polyol pathway [16] are the major resources of ROS (Fig. 1).

As discussed above, oxidative stress is the initial part of DN and activates a variety of pathological pathways. However, fibrosis is the most fundamental and prominent feature of DN and inflammation appears to be the central role [17] in the onset and progression of kidney fibrosis if uncontrolled. In animal models and live specimens with DN, it turns out that the accumulation of lymphocytes, macrophages, dendritic cells, mast cells and other inflammatory cells in the kidney tissue is closely associated with DN [18]. In the local microenvironment, these inflammatory cells synthesized and secreted various fibrogenic cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and ROS as well, which directly damage kidney architecture or subsequently trigger the epithelial-to-mesenchymal transition (EMT) process [19], resulting in ECM accumulation. In addition, other factors such as chemokine C–C motif ligand 2 (CCL2) also known as monocyte chemotactic protein 1 (MCP-1), are also involved in the mechanisms of DN.

Regardless of which type the injury belongs to, oxidative stress or inflammation, kidney fibrosis is commonly the final outcome of kidney diseases, which results in significant destruction of the normal structure and function of the kidney. DN has the following characteristics: the thickening of glomerular basement membrane, glomerular hypertrophy and increase of mesangial matrix, all of which are important pathogenic factors of fibrosis of the kidney. Excessive ECM is produced by the prolonged stimuli of a series of cytokines and the decomposition decreases. Under normal circumstances, moderate ECM is synthesized to maintain normal cellular structure and function. However, once there is persistent existence of injury as a result of oxidative stress or inflammation, profibrotic cytokines are over-released to activate fibroblasts to express α-smooth-muscle actin (α-SMA), contributing to ECM overproduction and deposition in renal tissues. During the fibroblast activation process, transforming growth factor β (TGF-β) exhibits great effect on the modulation of subsequent downstream signaling, especially the Smad pathway [20]. Upon TGF-β binding to the receptors present in mesangial cells, the pathogenic Smad signaling pathway initiates, which upregulates the transcription of target genes [21,22] such as *Mix2*, produces excessive ECM, accelerates mesangial expansion and promotes kidney tissue fibrosis.

Nevertheless, each of the described pathways is not independent but closely cross-talked and mutually reinforcing. Inflammation and fibrosis pathways can be induced by oxidative stress, which in turn enhances the oxidative stress; inflammation prompts kidney fibrosis, which exacerbates inflammatory response. For example, ROS acts as a second messenger, activating transcription factors NF-κB and AP-1, increasing expression of CCL2 and resulting in tubular and glomerular fibrosis; infiltration into tubular and glomerular cells by inflammatory cells is enhanced when high blood glucose prolongs, thus producing ROS. Meanwhile, the inflammatory pathways can produce profibrotic cytokines to regulate oxidative stress positively. As an example, TGF-β, connective tissue growth factor (CTGF) and platelet-derived growth factor (PDGF) can not only stimulate ECM production and inhibit matrix degradation by suppressing the activity of matrix metalloproteinases [23] but also bind to cell surface receptors, activating PKC and NOX to generate ROS.

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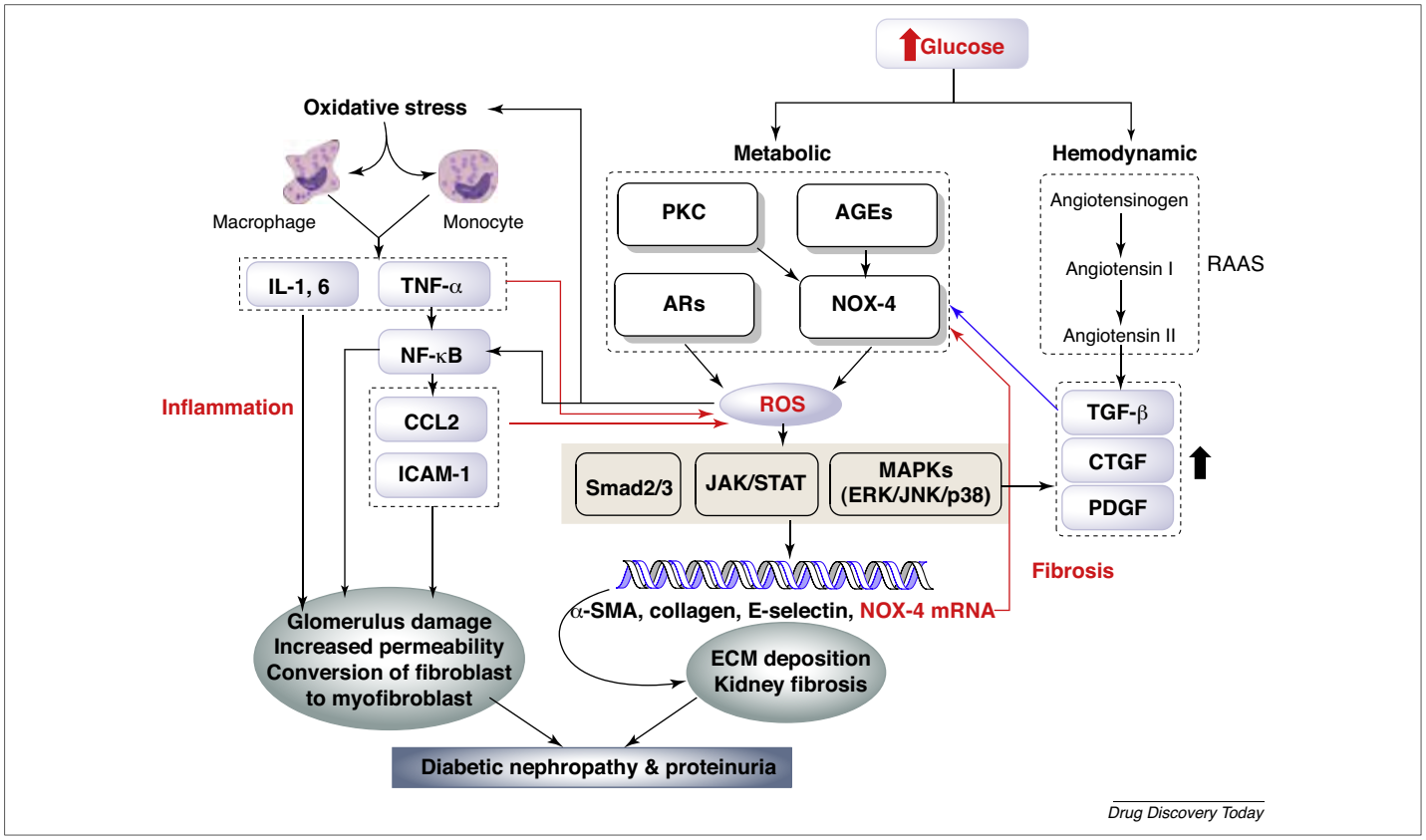

FIGURE 1

The pathogenesis of diabetic nephropathy (DN). Oxidative stress, inflammation and fibrosis contributed to the occurrence and development of DN and each of the pathways is closely cross-talked and mutually reinforcing. Oxidative stress is the initial part of DN and activates inflammation and fibrosis, which in turn enhances oxidative stress; inflammation prompts kidney fibrosis, which exacerbates inflammatory response. *Abbreviations*: IL-1,6, interleukin-1,6; TNF-α, tumor necrosis factor α; NF-κB, nuclear factor κB; CCL2, chemokine (C–C motif) ligand 2, also called monocyte chemotactic protein 1 (MCP-1); ICAM-1, intercellular cell adhesion molecule 1; PKC, protein kinase C; AGEs, advanced glycation end-products; NOX-4, nicotinamide adenine dinucleotide phosphate oxidase-4; Smad2/3, drosophila mothers against decapentaplegic protein 2/3; JAK/STAT, janus kinase-signal transducer and activator of transcription; MAPKs, mitogen-activated protein kinases; ERK, extracellular regulated protein kinases; JNK, c-Jun N-terminal kinase; α-SMA, α-smooth muscle actin; ECM, extracellular matrix; RAAS, renin-angiotensin-aldosterone system; TGF-β, transforming growth factor β; CTGF, connective tissue growth factor; PDGF, platelet-derived growth factor.

Current strategies for the treatment of DN

As discussed above, DN is a multifactorial progressive disease involving many different cells, molecules and factors. The exact causes of DN are still not fully elucidated, but various postulated mechanisms are: hyperglycemia (causing hyperfiltration and renal injury), oxidative stress, inflammation and fibrosis. Therefore, to control blood sugar level to alleviate the problem or to block the pathological process will be of significant value in DN.

Current strategies to control the risk factors

Renin–angiotensin system (RAS) blockades

In 2005, a randomized, double-blind and placebo-controlled irbesartan DN trial was conducted in 209 clinics worldwide among 1590 hypertensive patients with type 2 diabetes and the data indicated that a target systolic blood pressure (BP) of 120–130 mmHg provided tremendous benefit to renal outcomes [24]. Another study investigated by the Action to Control Cardiovascular Risk in Diabetes (ACCORD) showed the consistent outcomes [25]. In STZ-induced diabetic rats, ACEIs and ARBs reduced ROS, mesangial expansion and associated proteinuria [26]. Hence, RAS blockades should be recommended in type 2 DN patients.

Aliskiren, an orally active direct renin inhibitor, has been intensively researched in drug combinations with ACEIs and ARBs. It was co-developed by the Swiss pharmaceutical companies Novartis and Speedel [27,28], and was approved by the FDA in 2007 for the treatment of primary hypertension. Further study showed that the accumulation of aliskiren in kidney can block RAS and significantly ameliorate albuminuria and glomerulosclerosis in db/db mice [29]. Dong *et al.* [30] demonstrated that a combination of aliskiren with an ARB such as valsartan enhanced renal protective effect compared with valsartan alone in mice with type 2 DN. In DN patients, addition of aliskiren to losartan reduced urinary protein excretion by an additional 20% compared with losartan monotherapy [28]. Another study was aimed to evaluate the efficiency of aliskiren to interfere with kidney dysfunction in patients with type 2 diabetes [31]. After discovering increased incidence of nonfatal stroke, renal complications and other safety concerns [32], Novartis had to halt the clinical trial and warned to avoid the use of aliskiren with ARBs or ACEIs for patients with moderate-to-severe renal impairment.

Dipeptidyl peptidase-4 (DPP-4) inhibitors

Glucose-lowering medications with insulin and/or oral hypoglycemic agents (Fig. 2) have been demonstrated to improve albuminuria and other measures of DN in type 1 and 2 diabetes, which aroused great interest from scientific researchers [33,34]. A small

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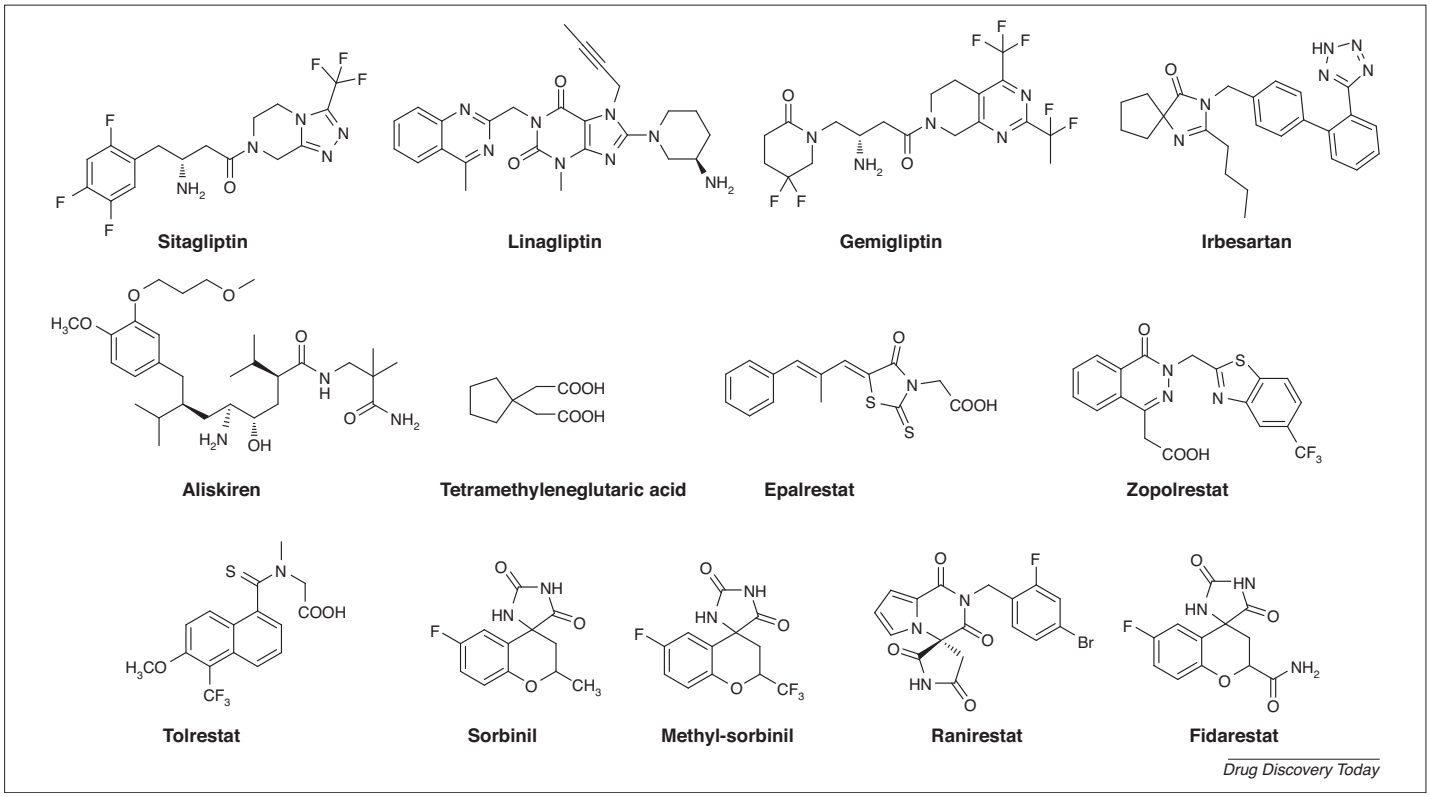

FIGURE 2

Chemical structures of sitagliptin, linagliptin, gemigliptin, irbesartan, aliskiren, tetramethyleneglutaric acid, epalrestat, zopolrestat, tolrestat, sorbinil, methyl-sorbinil, ranirestat and fidarestat.

clinical trial among 36 type 2 diabetic patients showed that sitagliptin reduced albuminuria by 20% [35]. Linagliptin further reduced albuminuria by 28% beside that from conventional therapy with a RAS blockade [36]. The Phase III clinical trial is ongoing (http://www.clinicaltrials.gov/show/NCT01792518). Moreover, a Phase IV clinical trial is underway to analyze the effect of gemigliptin on albuminuria over a 40-week period as a secondary outcome in patients with moderate-to-severe DN (http://www.clinicaltrials.gov/show/NCT01968044).

Mineralocorticoid receptor (MR) antagonists

Currently, with the identification of the MR [37], eplerenone, an aldosterone antagonist, has been investigated for the treatment of DN. *In vivo*, eplerenone attenuated albuminuria and suppressed glomerulosclerosis in a dose-dependent manner in the Dahl salt-sensitive hypertensive model [38]. Pfizer has designed and synthesized a novel scaffold of nonsteroidal pyrazoline MR antagonists, among which the compound PF-3882845 showed the most potency with an IC_{50} of 9 nM [39]. As a consequence of good efficiency and tolerability in preclinical trials, PF-3882845 has entered Phase I clinical trials in patients with DN [40].

However, monotherapy with ACEIs or ARBs prevents DN with a low efficiency and drug combinations enhance protective effects on kidney dysfunction with the drawback of increasing risk of severe cardiovascular disease [9,10]. Therefore, there is a pressing need for a new chemical entity and a novel strategy for DN treatment.

Drug discovery targeting oxidative stress

Various scaffolds of drugs for DN therapy have been developed, which mainly fall into the following categories: antioxidants, anti-inflammatories and antifibrosis agents. Given the important roles of ROS in DN, direct scavenging of ROS and blockades of production are potential strategies for the drug discovery of anti-DN agents and will be of great value for the relief of DN.

Direct scavengers of ROS

Enhanced stimulation by high levels of sugar and blood pressure and weakened antioxidant capacity on account of the excessive consumption of NADPH and the decreased production of glutathione (GSH) have been shown to contribute to the overproduction of ROS. A randomized, double-blind and placebo-controlled Phase II clinical trial sponsored by Shahid Beheshti Medical University has been completed to evaluate the impact of vitamin and mineral supplementation on neuropathy and nephropathy complications in 69 type 2 diabetic patients [41,42]. The administration of vitamins C and E (Table 1) decreased levels of urinary albumin excretion after 3 months. Moreover, the addition of minerals to vitamins further decreased fasting serum glucose ($P = 0.035$) and levels of systolic, diastolic and mean blood pressure ($P = 0.008$, $P = 0.017$ and $P = 0.009$, respectively), which are the risk factors of DN.

In addition, alpha-lipoid acid (Table 1) and its reduced form, dihydrolipoate, is currently in a randomized, single-blind Phase IV clinical trial at Wuhan General Hospital of Guangzhou Military

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TABLE 1

The anti-DN pipeline in clinical research

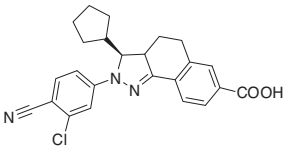
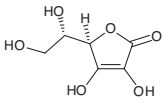
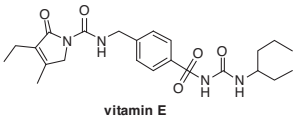
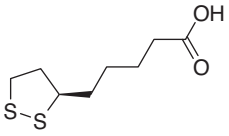
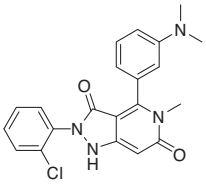
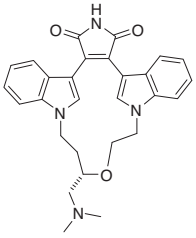
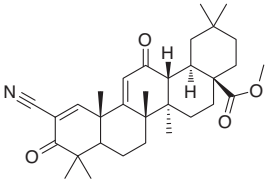
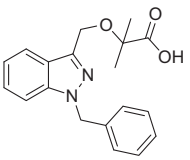
Compounds	Structures	Target	Clinical details			Status	Refs
			Treatment dosage	Treatment duration	Primary endpoints		
Mineralocorticoid receptor (MR) antagonists							
PF-3882845		MR	NA	NA	NA	Phase I (ongoing)	[40]
Drug discovery targeting oxidative stress							
Vitamins C and E	 vitamin C  vitamin E	ROS	Vitamins C (100 mg) plus E (100 IU)	3 months	Change in urine microalbumin concentration	Phase II (completed)	[41,42]
Alpha-lipoic acid		ROS	600 mg	6 months	Acute kidney injury; changes of endothelium dependent arterial dilation before and after contrast administrated	Phase IV (recruiting)	http://clinicaltrials.gov/ct2/show/NCT01978405
GKT137831		NOX-1,4	100 mg twice daily	12 weeks	Change in albuminuria and UACR	Phase II (recruiting)	http://clinicaltrials.gov/ct2/show/NCT02010242
Drug discovery targeting protein kinase C							
Ruboxistaurin		PKC	32 mg/day	1 year	A reduction in the ACR	Phase II (completed)	[94]
Drug discovery targeting inflammation							
Bardoxolone Methyl		Anti-inflammation	20 mg/day	16 months	ESRD (need for chronic dialysis or renal transplantation) cardiovascular death	Phase III (terminated)	http://clinicaltrials.gov/show/NCT01351675
CCX140-B	NA	CCL2	5 and 10 mg/day	1 year	Incidence of adverse events	Phase II (active, not recruiting)	http://clinicaltrials.gov/show/NCT01447147
Bindarit		CCL2	300 mg twice daily	12 months	Change in albuminuria	Phase II (completed)	http://clinicaltrials.gov/ct2/show/NCT01109212

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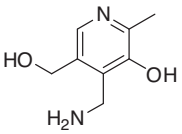
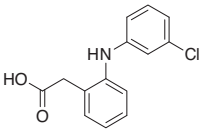
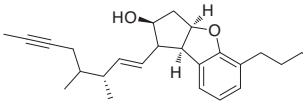
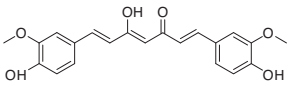
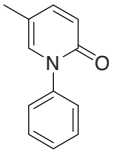
TABLE 1 (*Continued*)

Compounds	Structures	Target	Clinical details			Status	Refs
			Treatment dosage	Treatment duration	Primary endpoints		
Pyridoxamine		AGEs	150 and 300 mg twice daily	52 weeks	Change in serum creatinine and other biomarkers of kidney disease	Phase III (completed)	[105]
GLY-230		AGEs	125, 250 and 375 mg twice daily	2 weeks	Change in glycated albuminuria concentration	Phase Ib/IIa (completed)	[106]
Beraprost		AGEs	0.02 mg twice daily	12 weeks	Change of brachial ankle PWV	Phase III (recruiting)	http://clinicaltrials.gov/ct2/show/NCT01796418
Drug discovery targeting fibrosis							
FG-3019(antibody)	NA	CTGF	3 and 10 mg/kg every 2 weeks	22 weeks	Change in 24-hour urinary ACR	Phase II (terminated)	http://clinicaltrials.gov/ct2/show/record/NCT00913393
Drug discovery targeting multifactorial pathogenesis							
Curcumin		ROS, SOD, TGF-β and PDGF	NA	8 weeks	Change in proteinuria	Phase III (completed)	http://clinicaltrials.gov/ct2/show/NCT01831193
Pirfenidone		ROS, TGF-β, CTGF and PDGF	1200 and 2400 mg/day	54 weeks	Change in eGFR	Phase II (completed)	[122]

Abbreviations: ROS, reactive oxygen species; UACR, urine albumin:creatinine ratio; ACR, albumin:creatinine ratio; ESRD, end-stage renal disease; PWV, pulse wave velocity; SOD, superoxide dismutase; TGF-β, transforming growth factor β; CTGF, connective tissue growth factor; PDGF, platelet-derived growth factor; eGFR, estimated glomerular filtration rate; NA, not available.

Command targeting the contrast-induced acute kidney injury and endothelial dysfunction in type 2 diabetes (http://clinicaltrials.gov/ct2/show/NCT01978405). Moreover, superoxide dismutase (SOD) mimetic tempol (Table 2), which enhanced the catalytic process of the dismutation of ROS into hydrogen peroxide, further to hydrogen oxide, reduced the peroxide production in glomeruli and mesangial matrix expansion, but failed to reduce proteinuria as a result of the damage to the glomerular basement membrane (GBM) owing to the increased myeloperoxidase [43].

However, application of antioxidants for the treatment of DN is still limited to human clinical trials [44]. In view of the potential harm [45] such as increased morbidity [46] and increased cardiovascular complications [47], routine vitamin or mineral supplementation is not generally recommended in diabetic individuals despite a number of apparent benefits in experimental studies and some clinical trials. Nevertheless, using these drugs as adjunctive therapies in diabetic patients might be a better choice. To sum up, with increased understanding of the mechanisms of production and release of free radicals, it becomes clear that a more fundamental and comprehensive approach aimed at preventing the generation of these reactive species rather than merely scavenging reactive radicals might prove more beneficial.

Aldose reductase (AR) as a target

AR is the rate-limiting enzyme of the polyol pathway, which comprises two consecutive steps [48]. The first step is to reduce glucose to sorbitol with the help of AR, requiring NADPH as the cofactor. Secondly, sorbitol is further converted to fructose by sorbitol dehydrogenase with its cofactor NAD^+ (Fig. 3). During the process, excessive consumption of NADPH and decreased levels of GSH break redox balance and abundant ROS are produced. Moreover, accumulated sorbitol causes osmotic stress, which can lead to cell swelling and eventually cell death and maybe another underlying promoter of DN [49]. Currently, there is also evidence that genetic deficiency of AR in all tissues but the renal medulla results in significant amelioration of DN in STZ-induced diabetic C57BL/6 mice [50]. Therefore, AR has gained increasing attention in the past two decades as a promising therapeutic target and the inhibition of AR is an effective approach to prevent DN.

Currently, many AR inhibitors (ARIs) (Fig. 2) have been shown to be effective in the prevention or reversal of DN in animal models, warranting more-focused and dedicated future clinical investigations [51]. At present, numerous ARIs are in various development stages for the treatment of DN. Although natural products such as flavonoids have been researched as ARIs,

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TABLE 2

Compounds in preclinical trials

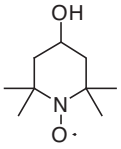
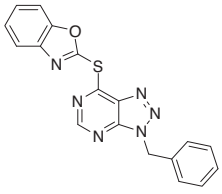
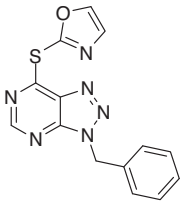
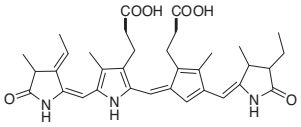
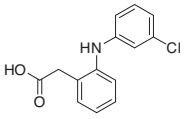
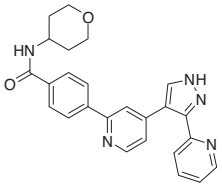
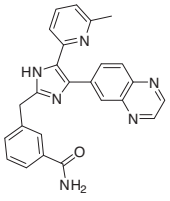
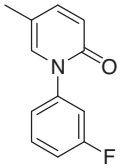
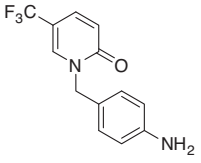
Compounds	Structures	Possible mechanisms	Status	Refs
Drug discovery targeting oxidative stress				
Tempol		Nonspecific NOX inhibitors with IC_{50} of 0.9 μM by forming a covalent adduct with FAD, withdrawing an electron transfer	*In vitro* and animal experiment	[43]
VAS2870		Inhibiting NOX activity in NOX-2-containing HL-60 cell line and in vascular endothelial cells containing NOX-2 and NOX-4	*In vitro*	[77,78]
VAS3947		Reducing NOX-derived ROS production in several cell lines with low micromolar potency, irrespective of the specific isoforms expressed	*In vitro*	[79]
Phycocyanobilin		Downregulating the expression of NOX-4	Animal experiments	[84]
Drug discovery targeting inflammation				
TLK-19705		CCR2 antagonist	Animal experiments	[99]
Drug discovery targeting fibrosis				
GW788388		Inhibition of TGF-β-induced Smad2 phosphorylation and Smad2/3 nuclear translocation	Animal experiments	[109,110]
IN-1130		Inhibition of Smad2 phosphorylation	Animal experiments	[110]
Drug discovery targeting multifactorial pathogenesis				
Fluorofenidone		Inhibition of TGF-β, CTGF and PDGF	Animal experiments	[125]
ZHC-104		NA	Cell analysis (IC_{50} = 0.3 mM against NIH3T3 cell line)	[127]

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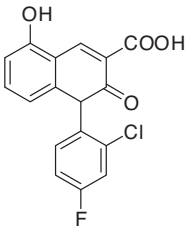
TABLE 2 (*Continued*)

Compounds	Structures	Possible mechanisms	Status	Refs
ZHW-001		NA	Cell analysis (IC_{50} = 0.09 mM against NIH3T3 cell line)	[128]

Abbreviations: NOX, nicotinamide adenine dinucleotide phosphate oxidase; FAD, flavin adenine dinucletide; CCR2, C–C chemokine receptor 2; ROS, reactive oxygen species; TGF-β, transforming growth factor β; CTGF, connective tissue growth factor; PDGF, platelet-derived growth factor; eGFR, estimated glomerular filtration rate; MA, not available. NIH3T3: mouse embryonic fibroblast cell line.

synthetic ARIs attract more attention for treatment of DN, which are mainly classified into two different kinds according to their structure scaffold: carboxylic acids and hydantoins.

Long-chain fatty acids, such as tetramethyleneglutaric acid, could inhibit AR in lens homogenates [52–54]. Given the solubility problems, some water-soluble carboxylic acid ARIs are synthesized [55] such as epalrestat, ponalrestat, tolrestat, alrestatin and zopolrestat. Of note, epalrestat, also known as ONO2235, is the only ARI on the market in Japan and has been recently been approved in China and India [56]. In STZ-induced DN rats, oral administration of epalrestat prevented renal hypofunction and morphologic changes such as the reduction of mesangial expansion [57]. Administration of zopolrestat of 100 mg/kg/day for 4 months decreased proteinuria to 15.07 ± 2.17 mg/day compared with 49.97 ± 7.94 mg/day in the untreated group of STZ-induced diabetic rats [58]. Furthermore, in recent *in vitro* studies, zopolrestat was shown to reduce the high glucose-induced expression of fibronectin and prevent ECM deposition in human mesangial cells [59]. Increased glomerular permeability to albumin, raised glomerular filtration and renal hypertrophy were impeded by treatment with tolrestat in STZ-induced diabetic rats. And fractional mesangial expansion was unchanged in tolrestat-treated diabetic rats compared with untreated animals [60].

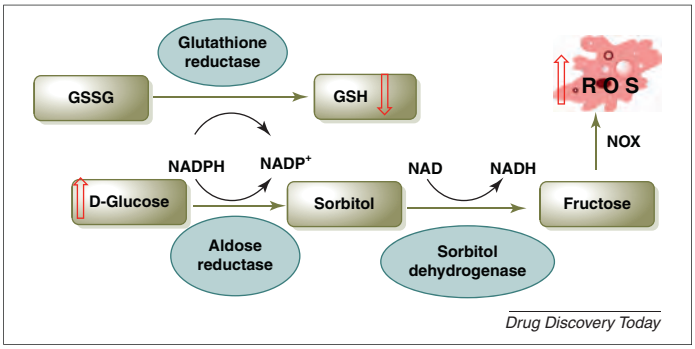


FIGURE 3

The polyol pathway and the pivotal role of aldose reductase (AR). The polyol pathway comprises two consecutive steps. The first step is the reduction of glucose to sorbitol with the help of AR and subsequently sorbitol is converted to fructose by sorbitol dehydrogenase with its cofactor NAD^+, resulting in excessive consumption of NADPH, decreased level of GSH and the generation of abundant ROS. *Abbreviations*: GSSG, glutathione (oxidized form); GSH, glutathione; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form); $NADP^+$, nicotinamide adenine dinucleotide phosphate (oxidized form); NAD, nicotinamide adenine dinucleotide (oxidized form); NADH, nicotinamide adenine dinucleotide (reduced form); NOX, nicotinamide adenine dinucleotide phosphate oxidase; ROS, reactive oxygen species.

By contrast, since the first *in vivo* effective oral hydantoin ARI sorbinil with better inhibitory activity but bad selectivity was subsequently discontinued in the early 1990s [51,61], medicinal chemists have been making efforts to design ARIs with a hydantoin scaffold. A 2-methyl substituent compound processed the apparently similar pharmacokinetics with a compromise of liver function. Ranirestat, optimized by KY-ORIN and Dainippon Sumitomo and with better potency and pharmacokinetics [62], has completed a Phase III clinical trial in the USA to evaluate the efficacy and safety (40 and 80 mg) in mild-to-moderate diabetic sensorimotor polyneuropathy (http://www.clinicaltrials.gov/ct2/show/NCT00927914).

Increased AR activity and oxidative/nitrosative stress have contributed to the occurrence and development of DN. *In vitro* studies revealed that D-glucose rather than L-glucose induced accumulation of nitrosylated and poly(ADP-ribosyl)ated proteins in cultured human mesangial cells, which can be stopped by D-glucose plus fidarestat. *In vivo*, excessive accumulation of sorbitol and fructose in the renal cortex of STZ-induced diabetic rats was completely prevented by fidarestat treatment. In conclusion, fidarestat treatment, at least in part, counteracted nitrosative stress and polymerase activation by reducing nitrotyrosine and poly(-ADP-ribose) concentrations in the diabetic renal cortex and prevented diabetes-induced increase in kidney weight, justifying new beneficial properties of fidarestat in experimental and, potentially, human DN [63].

Although a great deal of evidence indicates that AR and the polyol pathway represent a potential and promising therapeutic target for DN [64], the low tissue permeation of ARIs with carboxylic acid scaffold attributed to their low pK_a values is the common problem that limits the bioavailability *in vivo*. Meanwhile, liver toxicity and hypersensitivity reactions are common in the hydantoin-based structures of ARIs. Worse still, negative results from preclinical and clinical trials showed poor amelioration or some unacceptable toxicity, which could account for high cross-reactivity with other AR-similar enzymes, thus damaging the protective effect. As we all know, AR is a protective enzyme that has a crucial role in the detoxification of metabolite products such as the detoxification of methylglyoxal, a toxic 2-oxo-aldehyde [65]. Hence, the alteration of the aldehyde-detoxifying role of AR could be one of the reasons for withdrawal of current AR inhibitors, which indicates that the strategy to design specific ARIs is a

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remarkable success. Regardless of the positive effects of ARIs on disrupting the progression of DN [56], further studies regarding the structural features of AR and identification of more-specific ARIs toward the treatment of DN are still formidable challenges to the scientific community.

NOX as a target

As mentioned above, members of the NOX family generate ROS that modulate redox-sensitive cellular responses and are essential mediators of normal cell physiology. However, excessive ROS production by an overactive NADPH oxidase system probably mediates constitutive activation of signaling pathways involved in the initiation and progression of DN. This occurs through the selective oxidation of specific signaling enzymes and/or proteins that are linked to processes such as activation of transcription factors, secretion of cytokines or altering signaling proteins such as protein kinases and phosphatases [66].

Currently, the NOX family consists of seven members: the dual oxidases DUOX-1 and -2 as well as NOX-1–5. NOX-2, earlier called gp91phox, was first found in neutrophils and phagocytic cells, together with p22phox, a small regulatory subunit, modulating respiratory burst (Fig. 4) [67]. Further studies showed that p67phox, p47phox, p40phox and the small GTPase Rac also have a vital role in activating the NOX family. Apparently, inhibiting the activity of the NOX family can significantly prevent oxidase stress, which is a more direct means to decrease the generation of ROS and one of the efficient therapeutic strategies of DN.

Historically, when it comes to NOX inhibitors, the two nonspecific NOX inhibitors diphenylene iodonium (DPI) and apocynin (Fig. 5) should be mentioned. DPI, with IC_{50} value of 0.9 μM [68], is commonly used as a potential inhibitor of NADPH oxidases by forming a covalent adduct with FAD and withdrawing electron transfer [69]. Nevertheless, flavin-dependent enzymes are involved in many signal pathways such as nitric oxide synthase regulating vascular tone, insulin secretion and xanthine oxidase playing an important part in the catabolism of purines in humans. As a result, the off-target effects impede its potential as a therapeutic candidate in *in vivo* trials. Apocynin (4-hydroxy-3-methoxy-acetophenone) (Fig. 5), isolated from *Picroria kurroa*, prevents the binding of p47phox to membrane protein gp91phox as a nonspecific NOX-2 inhibitor [70]. Moreover, recent studies have shown that apocynin substantively possesses a direct and strong antioxidative ability (>100 μm) such as scavenging HOCl and H_2O_2 and other nonradical oxidant species [71]. In STZ-induced DN rats, administration of apocynin with 16 mg/kg/day significantly reduced H_2O_2 excretion and the expression of p47phox and gp91phox, reducing the mesangial matrix expansion and proteinuria [72]. In conjunction with the outcome, these data obtained by Nam *et al.* demonstrated the reduction of urinary protein and albumin excretions after apocynin treatment in OLETF rats with DN [73]. However, there is a great deal of support that suggests myeloperoxidase (MPO) is required to activate apocynin inhibitory effect [74], which results in a dramatic change in inhibitory activity across cells and tissues. Thus, apocynin failed to inhibit overexpressing of NOX-1, 2 and 4 in MPO-free HEK293 cells even at high concentrations [75]. Notably, NOX-4, predominantly expressed in kidney [76], maintains continuous activation even without the presence of cytosolic subunits. Without question, whether apocynin is also able to block the constitutive activity of these proteins and prevent occurrence and progression of DN is currently unknown. The use of apocynin should be cautious and further study should be continued.

It is clear that nonspecific NOX inhibitors actually decreased NOX-dependent ROS generation and suppressed oxidative stress. Although there are many subtypes of NOX, the present studies have demonstrated that it is mainly NOX-4 that is the most abundantly expressed in the kidney that regulated myofibroblast

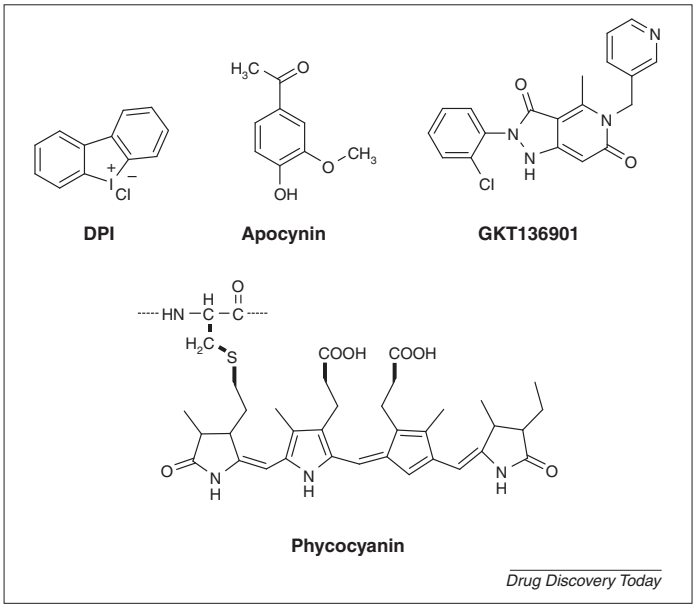

FIGURE 5

Chemical structures of DPI, apocynin, GKT136901 and phycocyanin. *Abbreviations*: DPI, diphenylene iodonium.

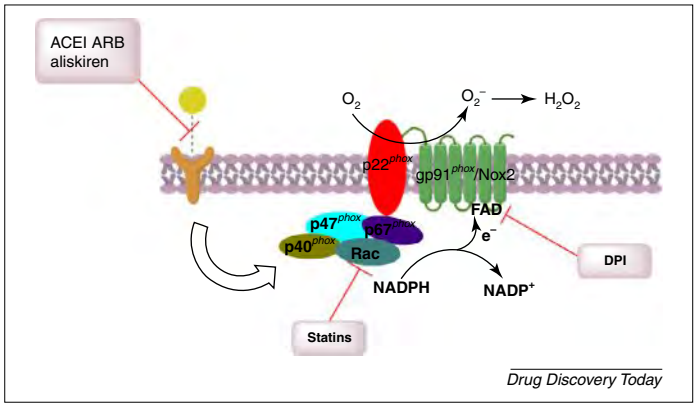

FIGURE 4

The activation mode of NOX and some blockades of the key link. NOX is activated by numerous factors including cytokines, angiotensin II and AGEs. Upon activation, NOX-1 and -2 with regulatory cytosolic subunits such as p47phox, p40phox, p67phox and Rac formed active enzyme complexes in the similar mechanism. ACEIs and ARBs also block ligand from binding to membrane receptor thus blocking the upstream signaling required for NOX activation; DPI inhibits NOX activity by withdrawing an electron transfer; apocynin prevents the binding of p47phox to membrane protein gp91phox as a nonspecific NOX inhibitor. In addition, statins prevent the binding of GTP-binding protein Rac to the membrane components of NOX. *Abbreviations*: NOX, nicotinamide adenine dinucleotide phosphate-oxidase; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; DPI, diphenylene iodonium; FAD, flavin adenine dinucleotide.

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activation and tissue fibrosis in glomeruli and tubules from db/db mice [76]. Therefore, NOX-4 might be an ideal therapeutic target for attenuating the chronic production of toxic ROS underlying progressive kidney damage and preventing the progress of DN. And it is likely that NOX-4 special inhibitors will be of improved therapeutic efficiency.

In vitro, VAS2870 (Table 2) inhibits ROS generation with an IC_{50} value of 10.6 mM in cell-free systems and could possess a NOX-specific inhibitory effect [77]. But it still needs further study. In vascular smooth muscle cells and endothelial cells, VAS2870 is characterized by an inhibitory effect on PDGF-derived ROS production without affecting basal ROS production [78]. However, it is frustrating that animal experiments have not yet been conducted to evaluate the pharmaceutical profile of VAS2870. VAS3947 (Table 2), a triazolo pyrimidine derivative of VAS2870, shows an improved solubility without difference in its inhibition profile. However, this compound presents different inhibitory activity against different NOX isoforms independent on the cell model tested, with IC_{50} values of 2 μM for HL-60 cells (mainly NOX-2 and NOX-5), 12 μM for CaCo-2 cells (mainly expressing NOX-1 and NOX-2) and 13 μM for A7r5 cells (mainly NOX-4) [79]. In summary, VAS2870 and VAS3947 appear to hold significant promise to be excellent pan-NOX inhibitors and an important treatment strategy of DN.

What is more, Sedeek *et al.* [80] found that GKT136901 (Fig. 5) reduced proteinuria by inhibiting the activity of NOX and played the renal-protective role by reducing oxidative damage and decreasing extracellular regulated protein kinase 1/2 (ERK1/2) activation, which prevented type 2 diabetes db/db mice from progression to DN. Another compound GKT137831 (Table 1), which is developed by Genkyotex, the leading developer of NOX inhibitors, has completed a Phase I clinical trial and demonstrated excellent safety and tolerability following single and multiple oral doses [81]. Currently, Genkyotex have announced the initiation of a multinational Phase II clinical study of GKT137831 in patients with DN.

Additionally, previous reports indicated that Gilbert syndrome, an innate cause of hyperbilirubinemia, reduced the prevalence of vascular complications, nephropathy included, and had striking reductions in markers of oxidative stress [82]. Inspired by this phenomenon, a series of experiments were conducted by Zheng and colleagues [83], which demonstrated that the administration of biliverdin, a precursor of bilirubin with much better water solubility, inhibits albuminuria and renal histological abnormalities in db/db mice by inhibiting oxidative stress. However, a superabundant daily intake of biliverdin is required to achieve a meaningful impact on serum for clinical use and tissue bilirubin levels. Moreover, chemical synthesis of biliverdin might be too complex and costly for human use. Taken together, phycobilins, the structural analogs of bilirubin, will be a better choice. Further studies conducted by Zheng *et al.* [84] showed that oral administration of phycocyanin and phycocyanobilin (Table 2) downregulated the expression of NOX-4, which is the main expressed isoform of NOX in the kidney and the major source of oxidase stress in DN. Oral administration of phycocyanin (300 mg/kg daily) for 10 weeks and treatment with phycocyanobilin (15 mg/kg daily) for 2 weeks protected against albuminuria and renal mesangial expansion in db/db mice by reducing renal oxidative stress, which provided a feasible and novel therapeutic approach to prevent DN.

One thing to note is that ACEIs and ARBs can not only target lowing blood pressure but also prevent ligand from binding to membrane receptor thus blocking the upstream signaling required for NOX activation [85]. The combination of eplerenone and ACEIs further reduced production of ROS and expression of NOX [86]. Furthermore, statins can not only reduce the level of cholesterol but also prevent the binding of GTP-binding protein Rac to the membrane components of NOX [87]. Existing evidence supports the fact that monotherapy of statins or combination use could reduce mortality and improve biomarkers of DN [88,89]. However, the positive effect on DN seems to be caused by the lipid-lowering and anti-inflammatory effect [90], which is not explained in detail. Collectively, recent research illustrates that NOX inhibitors show significant advantages in the treatment of DN and the discovery of specific NOX-4 small-molecule inhibitors will become an attractive approach in the future.

Drug discovery targeting PKC-β

There is increased understanding that activation of the PKC pathway appears to be a crucial mechanism in the occurrence and progression of DN. PKC enzymes are activated by signals such as increasing concentration of diacylglycerol (DAG) or calcium ions (Ca^{2+}). Long-term hyperglycemia is likely to lead to overproduction of DAG, which then activates one or more PKC isoforms in different tissues. Although it remains an additional consideration, there is also evidence that the PKC-α and PKC-β isoforms are most closely involved in DN [91–93], which provides us with a new potential way to treat DN.

Ruboxistaurin (RBX) (Table 1), also called LY333531, was discovered and investigated by Eli Lilly as an inhibitor of PKC-β. Owing to insignificant or no side-effects in a large amount of preclinical trials to prevent retinal and renal diseases, RBX has been investigated in several clinical trials to evaluate its efficacy in diabetic complications such as diabetic retinopathy, diabetic neuropathy and DN. A randomized, double-blind, placebo-controlled, multicenter, pilot clinical study showed that urinary albumin-to-creatinine ratio (ACR) significantly decreased ($-24 \pm 9\%$, $P = 0.020$) compared with the baseline in diabetes patients after treatment with RBX for one year based on drug therapy with renin–angiotensin system inhibitors. In addition, estimated glomerular filtration rate (eGFR) did not decline significantly in the RBX group (-2.5 ± 1.9 ml/min/1.73 m^2, $P = 0.185$), whereas the placebo group lost significant eGFR over one year (-4.8 ± 1.8 ml/min/1.73 m^2, $P = 0.009$) compared with the baseline. In summary, the added benefits of RBX lowering albuminuria and maintaining renal function in diabetic patients are noteworthy. Nevertheless, further research into DN is imperative to confirm drug safety and effectiveness [94].

Drug discovery targeting inflammation

It is well known that blood glucose and pressure control do not work in preventing and reversing renal fibrosis. Recently, great efforts have been put into the identification of the central role of inflammation in the development and progression of DN, which activates various inflammatory cells and releases a series of biological molecules [17] such as NF-κB and CCL2. Accordingly, new

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drug discovery is now focused on limiting the inflammation pathway related to DN.

NF-κB as a target

In recent years, numerous studies have shown that NF-κB regulating the expression of numerous genes plays a significant part in the inflammatory response during human and experimental kidney injury [95]. As a result, the downregulation of NF-κB will be of great value for the inhibition of inflammation.

Bardoxolone methyl (Table 1) induced the activation of powerful Nrf2-dependent phase 2 inducers, which selectively reduced the DNA binding of NF-κB to inhibit the inflammatory process [96,97]. Recently, the US Texas State Renal Association carried out a randomized trial including 227 cases of chronic kidney disease (CKD) and type 2 diabetic patients, discovering that bardoxolone methyl clearly improved patient eGFR (eGFR increased by an average 10 ml/min/1.73 m^2) in the short-term (24 weeks), which was dose-related. In addition, continuous administration (52 weeks) could further improve renal function [98]. However, Phase III clinical trials have yielded disappointing results owing to the serious adverse events, probably caused by increased cardiac events such as heart failure in the drug-treated group (http://www.clinicaltrials.gov/show/NCT01351675).

CCL2 as a target

In addition, a common finding that the high level of CCL2, a downstream chemokine, in the urine of patients with DN has been detected. Increasingly, research is showing that CCL2 is closely associated with the process of glomerular and interstitial inflammatory cell recruitment, such as macrophage cells. Hence, the blockade of the CCL2/CCL2 receptor (CCR2) pathway could have considerable therapeutic potential for DN. A group of db/db mice were treated with TLK-19705 (30 mg/kg/day) (Table 2), a novel CCR2 antagonist, for 8 weeks and the amelioration of urinary ACR was observed with administration dosage of 30 mg/kg/day or 10 mg/kg/day [99].

CCX140-B (Table 1) was discovered as an orally delivered therapy for the treatment of DN by ChemoCentryx. Preclinical trials of CCX140-B have shown satisfactory pharmacological action with sufficient potency and selectivity toward CCL2 with an IC_{50} of 8 nM. Additionally, several Phase I clinical trials with CCX140-B have been completed globally to evaluate the safety and efficacy of CCX140-B in healthy volunteers, which demonstrated an excellent pharmacokinetic and safety profile [100]. In a randomized, double-blind, placebo- and active-controlled Phase II study in 159 patients with type 2 diabetes, a dose-dependent effect on lowering fasting plasma glucose (FPG) was shown after 28 days of treatment with CCX140-B (http://clinicaltrials.gov/ct2/show/NCT01028963). Phase II clinical development for the treatment of DN with CCX140-B has been on the agenda (http://clinicaltrials.gov/show/NCT01447147).

The molecular scaffold of indazole has been known in non-hormonal, nonsteroidal antispermatogenic agents. However, it was indeed observed that the pharmacological properties changed as minimal modifications were conducted on the chemical structure of indazole carboxylic acids. Studies have indicated that bendazac, benzydamine and bindarit (Table 1) possess different pharmacological profiles: bendazac selectively inhibits protein denaturation, whereas benzydamine does not have any antispermatogenic activity but is used as a strong anti-inflammatory agent; bindarit shows inhibitory activity of protein denaturation and anti-inflammatory activity [101]. A proof-of-concept, prospective, randomized and double-blind Phase II clinical trial conducted by Angelini Farmaceutici evaluated the antagonistic activity against CCR2 through bindarit therapy in patients with systemic lupus. It was in fact observed that urine albumin excretion and the level of chemokine CCL2 decreased respectively by 90% and 50% when bindarit was administrated (1200 mg/day) over 24 weeks [102]. Further study showed that bindarit acted selectively on the NF-κB pathway by inhibiting phosphorylation of IκBα and p65, thus blocking the inflammatory pathway [103]. Another pilot Phase II, double-blind, multicenter, randomized and placebo-controlled clinical trial has been completed by Angelini Farmaceutic to estimate the efficiency of bindarit in patients with DN according to screening urinary albumin excretion and urinary CCL2 levels in the overnight urine specimen by adding bindarit to background therapy with irbesartan (http://clinicaltrials.gov/ct2/show/NCT01109212). However, the results have not been made available.

Blockade of the AGE–RAGE system

During long-standing hyperglycemic state in diabetes mellitus, glucose forms covalent adducts with the plasma proteins through a non-enzymatic process known as glycation. Recent studies suggest that AGEs interact with plasma-membrane-localized AGE receptors (RAGE) to release proinflammatory molecules and alter intracellular signaling, for example the activation of the RAGE/NF-κB pathway, which plays a crucial part in the pathogenesis of DN.

Pyridoxamine (PM) (Table 1) blocks the formation of AGEs probably by forming an adduct with the Amadori intermediate and is more efficient than pyridoxal (PL) or pyridoxine (PN), the other forms of vitamin B6. Animal experimental studies indicated that PM treatment of DN was successfully accompanied by scavenging of toxic ROS and carbonyls and decreasing the level of AGEs [104]. In a double-blind and randomized trial, 317 patients with proteinuric type 2 DN were randomly assigned to receive pyridorin (pyridoxamine dihydrochloride) treatment (150 mg twice daily or 300 mg twice daily) for 52 weeks. In fact, pyridorin solely displayed encouraging efficiency on patients with less serious renal damage (serum creatinine level lower than 1.9 mg/dl) [105]. Considering the fact that all fibrotic diseases are chronic illness, it is expected that every reparation or prevention is going to be a lengthy process, warranting a long-term pharmacological intervention. Therefore, just like any long-term drug use, it is very desirable to have antifibrotic drugs with low toxicity, which is one of advantages of PM. A Phase III clinical trial to assess the safety and efficacy is undergoing for the treatment of DN (http://clinicaltrials.gov/ct2/show/NCT02156843).

GLY-230 (Table 1) is a lead compound developed by Glycadia, which has completed Phase Ia and Phase Ib/IIa clinical trials and shown encouraging results. A single-blind, randomized and dose-ranging study in healthy subjects and patients with diabetes presented that plasma levels of urinary albumin were significantly reduced from 29.8 ± 10.4 μg/mg creatinine to 61.4 ± 15.8 μg/mg creatinine ($P = 0.001$) in diabetes patients with preexisting microalbuminuria, which consequently caused the decrease of glycated albumin. And there were no serious adverse events or laboratory

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abnormalities, and all safety parameters remained within normal limits. Collectively, as the first-in-class selective glycation inhibitor and the first agent in this class to enter human clinical trials, a series of positive findings promote unified efforts to evaluate GLY-230 further in the prevention and treatment of DN. Glycadia is putting its effort into proceeding with Phase II clinical development of GLY-230 for the treatment of DN [106].

Beraprost (BPS) (Table 1), also known as 2,3,3a,8b-tetrahydro-2-hydroxy-1-(3-hydroxy-4-methyl-1-octen-6-ynyl)-1H-cyclopenta[b]benzofuran-5-butanoic acid, is a prostaglandin I2 analog. A random but not blind clinical trial including 27 type 2 diabetes patients with incipient DN who showed signs of microalbuminuria revealed that beraprost sodium decreased albuminuria ($P < 0.05$) starting from month 18 of the study period in the beraprost sodium group (137.8 ± 98.8 mg/day) compared with the control group (76.8 ± 49.9 mg/day) [107]. Animal studies conducted by Watanabe *et al.* indicated that Otsuka Long-Evans Tokushima fatty (OLETF) rats with DN treated with beraprost sodium (400 μg/kg bodyweight) showed lower values for urine protein excretion and serum blood urea nitrogen than untreated rats. Indexes of glomerulosclerosis and glomerular volume were also significantly reduced compared with untreated rats and the level of AGEs and inflammatory cell infiltration in the glomerulus of treated rats were suppressed in a dose-dependent manner [108]. In conclusion, beraprost sodium is a stable orally active prostacyclin analog and the evaluation of beraprost sodium on arterial stiffness by pulse wave velocity (PWV) in patients with DN has been on the agenda in a Phase III clinical trial (http://www.clinicaltrials.gov/ct2/show/NCT01796418). These compounds have not been successfully approved for market, but bright prospects for application of anti-inflammatory compounds in the treatment of DN cannot be denied.

Drug discovery targeting fibrosis

Given the prime role of TGF-β in the onset and progression of DN, together with the report of TGF-β receptors, numerous efforts have been made to design and synthesize small molecules to regulate the TGF-β/Smad signaling pathway. A limited number of studies associated with small-molecule TGF-β inhibitors have been completed to address the therapeutic potential effect on the treatment of DN.

GW788388 (Table 2) suppresses TGF-β-induced Smad2 phosphorylation and Smad2/3 nuclear translocation in a dose-dependent manner and attenuated TGF-β-induced EMT responses *in vitro*. *In vivo*, oral administration of 2 mg/kg/day of GW788388 for 5 weeks in 6-month-old db/db mice with advanced-stage DN significantly reduced mesangial matrix expansion, mesangial hypertrophy and other features of DN, which indicated therapeutic potential in DN [109]. In addition, Moon *et al*. have demonstrated that the novel small molecule IN-1130 (Table 2) inhibited Smad2 phosphorylation with an IC_{50} of 5.3 nM and proved the efficiency of intraperitoneal administration of IN-1130 (10 and 20 mg/kg/day) in suppressing the expression of α-SMA and fibronectin in UUO rat kidneys [110].

By contrast, monoclonal anti-TGF-β antibody has been used long-term to abrogate the TGF-β signaling pathway. FG-3019 (Table 1) is an anti-CTGF monoclonal antibody developed by Fibrogen and is believed to prevent the development of kidney fibrosis in diabetes by blocking the final common pathway factor CTGF, which represents a first-in-class therapy that could provide a crucial renoprotective benefit. In an open-label, dose-escalation Phase I clinical study, ACR decreased significantly from 48 mg/g to 20 mg/g ($P = 0.027$) without evidence of dose-independence through treatment with FG-3019 for 56 days in diabetic patients with early-stage kidney disease [111]. Of note, another randomized, double-blind, placebo-controlled Phase I study to evaluate the safety, pharmacokinetics and pharmacodynamics of FG-3019 among 36 patients with type 1 or type 2 diabetes mellitus and DN indicated that it is safe and well tolerated in patients with DN on background ACEI and/or ARB therapy (http://clinicaltrials.gov/ct2/show/study/NCT00754143). However, a Phase II clinical trial to study the effect of FG-3019 in subjects with type 2 diabetes and kidney disease on ACEI and/or ARB therapy has been terminated (http://clinicaltrials.gov/ct2/show/record/NCT00913393).

Drug discovery targeting multifactorial pathogenesis

By analyzing the pathogenesis of DN, it can be concluded that, apart from individual factors such as race, genetics and so on, oxidative stress, inflammatory response and fibrosis greatly contributed to the development and progression of DN. What is worse, oxidative stress, inflammation and fibrosis pathways promote each other, causing irreversible damage on cell structure and function of kidney. So multitargeted drugs aimed at blocking as many aspects of the pathogenesis as possible are imminent.

Curcumin (Table 1) was extracted from *Curcuma longa linn* and further characterized by the potent anti-inflammatory and antioxidant properties. Its ability to directly scavenge free radicals is stronger than vitamin E [112], and it can enhance the activity of antioxidant enzymes such as superoxide dismutase (SOD) to reduce oxidative stress [113]. Research of curcumin has focused on its potential therapeutic use in DN. Experiments carried out by Sharma *et al*. [114] showed that curcumin could improve oxidative stress and proteinuria in STZ-induced DN mice. Research by Soetikno and colleagues [115] indicated that curcumin could downregulate TGF-β, PDGF and fibronectin and suppress ECM expansion in STZ-induced DN mice. Recently, a randomized, double-blind and placebo-controlled clinical trial including 40 patients has shown that administration of curcumin (66.3 mg/day) for 2 months could significantly reduce proteinuria from 4328.7 ± 3038.2 mg/24 h to 2354.7 ± 1800.1 mg/24 h ($P < 0.001$), and other mediators such as TGF-β and IL-8 as well [116]. Moreover, a randomized and double-blind Phase III clinical trial has been carried out to evaluate the effect of oral supplementation with curcumin in patients with proteinuric CKD (http://www.clinicaltrials.gov/ct2/show/NCT01831193); however, the data are unavailable. In short, owing to the low cost and few side-effects of curcumin, it could become an adjuvant treatment of proteinuric nephropathy to attenuate the progression of CKD such as DN. Unfortunately, the mechanism(s) of such pleiotropic action by this yellow pigment remains to be investigated. And the instability and low bioavailability greatly limits the clinical application of curcumin [117].

The compounds targeting multifactorial pathogenesis in the development and progression of DN with the molecular scaffold of pyridone are extensively studied, and are patented with IC_{50}s ranging from 0.05 to 100 mM against mouse embryonic fibroblast cell line (NIH3T3) proliferation.

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Pirifenidone (PD) (Table 1), a widely applied antifibrotic drug in the clinic, was marketed in 2008 mainly for idiopathic pulmonary fibrosis [118]. The further study of the mechanism of PD proved that PD was in possession of anti-inflammatory and antioxidative stress properties, which inhibited NOX and directly scavenged ROS in a dose-dependent manner [119]. In nephrectomized rats, PD improved macrophage infiltration of tubular and interstitial cells and reduced renal fibrosis in early- and late-stage. PD improved proteinuria and downregulated inflammatory cytokines such as TNF-α, IL-6 and CCL2. In addition, PD could inhibit fibrotic cytokines such as TGF-β and CTGF [120]. Studies in 17-week-old db/db mice have shown that the administration of PD for 4 weeks significantly reduced mesangial matrix expansion and expression of renal matrix genes but not albuminuria, which reminded us that PD is renoprotective in diabetic kidney disease and can exert its fundamentally antfibrotic effects in part via regulating RNA processing [121]. However, the current treatment of PD for DN is still in clinical trials. A Phase II clinical trial involving 77 patients was completed at San Diego Medical Center of California University in 2009 to determine whether administration of PD to type 1 and type 2 diabetic patients with advanced kidney disease will lead to preservation of kidney function. The results proved the treatment of PD (1200 mg/day) increased average glomerular filtration by about 9% after one year compared with the decrease of 4% in placebo. By the end of the trial, hemodialysis had occurred in four patients in the placebo group (26 patients in total), whereas none of the PD group entered the dialysis phase [122].

Fluorofenidone (AKF-PD) (Table 2) is a 'me-better' drug, which was designed and synthesized by Hu *et al.* and showed high inhibitory activity against the NIH3T3 cell line. In the angiotensin-II-induced fibrotic rat proximal tubular epithelial cells, AKF-PD blocked NOX-dependent oxidative stress, downregulated the level of TGF-β and alleviated fibrosis symptoms [123]. Animal experiments showed that the administration of AKF-PD to 10-week-old db/db mice (the DN model had been formed) could significantly reduce the 24 h urinary albumin excretion (127 μg/150 mg in drug group *vs* 240 μg/mg in the model group) and the thickening of glomerular mesangial matrix after 12 weeks [124]. AKF-PD treatment of rats with obstructive nephropathy caused by unilateral ureteral obstruction (UUO) ameliorated renal interstitial fibrosis by suppressing expression of type I and type III collagen probably by the inhibition of TGF-β, CTGF and PDGF. Moreover, AKF-PD appeared to be more efficient in decreasing CTGF-positive cells than PD [125]. Furthermore, compared with PD, the LD_{50} for AKF-PD is only 30% of that of PD despite their same curable effect [126]. However, there are considerable drawbacks of PD and AKF-PD such as the low efficiency (1200 mg/day), which is because the C-5 methyl is easily metabolized to an inactive carboxyl group *in vivo*, leading to the short half-life. To be encouraged, the latest reported compound ZHC-104 (Table 2) with an IC_{50} of 0.3 mM could prolong the half-life considering its C-5 trifluoromethyl, a group that is not metabolized [127]. Further research is still ongoing. During another research program, the unexpected product ZHW001 (Table 2) was obtained, which exhibited 46-times higher potent inhibitory effect on NIH3T3 cell proliferation than that of AKF-PD and is now applied for further modification [128]. Considering the attractive achievement of drug discovery targeting multifactorial pathogenesis, it gives us reasons to believe that the fast progress of the treatment of DN will come.

Concluding remarks and future directions

Many channels have contributed to the development and progression of DN. Simple blood glucose or blood pressure control is not enough to prevent DN. Drugs aimed at risk factors such as ACEIs or ARBs failed to alleviate or reverse DN whether single use or as combination therapy. Intense efforts dedicated to the pathogenesis of DN provided numerous targets such as oxidative stress, inflammation and fibrosis. In preclinical and early clinical trials of DN, the pharmaceutical agents affecting these pathways provided exciting results [129]. Specific NOX inhibitors and small-molecule anti-inflammatory agents have generated intense interest from scientific researchers. However, evolving clinical data are required to drive technological advances in the field. Of note, the anti-multifactorial pathogenesis drugs such as PD or AKF-PD block multiple pathways of DN and provide synergistic effects and additional regulatory effects. PD has been successively approved in Japan, India, Mexico, Europe and China for the treatment of idiopathic pulmonary fibrosis (IPF), which prompts us to develop anti-multifactorial pathogenesis drugs for the treatment of fibrosis, such as DN. Progress in the knowledge of pathogenesis of DN, optimization and identification of novel, potent anti-DN agents should pave the way for greater insight into the treatment of DN.

Conflicts of interest

The authors have declared no conflicts of interests.

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Please cite this article in press as: Lv, M. et al. Therapeutic strategies of diabetic nephropathy: recent progress and future perspectives, Drug Discov Today (2014), http://dx.doi.org/10.1016/j.drudis.2014.10.007