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Renoprotective Effects of Metformin

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Abstract
Background/Aims: It has become clear that metformin exerts pleiotropic actions beyond its glucose-lowering agent effect. In this review, we summarise the state of the art concerning the potential renoprotective effects of metformin in vitro, animal models and clinical nephrology. ***Methods:*** A literature search was performed in PUBMED, ScienceDirect, between January 1957 and March 2017 using the following keywords: "metformin," "nephroprotection," "renoprotection," "survival," "renal failure," "chronic kidney diseases," "fibrosis," "polycystic kidney disease" and "microalbuminuria." ***Results:*** A recent review of 17 observational studies concluded that metformin use appeared associated with reduced all-cause mortality in patients with CKD. Metformin has been shown to exert positive effects on the kidney in vitro and animal models representing different types of renal diseases, from acute kidney injury to chronic kidney disease. A retrospective cohort study from the Scientific Registry of Transplant Recipients indicated that metformin was associated with lower adjusted hazards for living donor and deceased donor allograft survival at 3 years posttransplant, and with lower mortality. ***Conclusion:*** Based on experimental evidence and some relevant clinical observations, metformin seems to be a promising drug in the treatment of progressive renal damage. RCT studies are the next essential step.

Introduction

Chronic kidney disease (CKD) is one of the most common metabolic diseases in all communities; according to the US Annual Data Report of Renal Data System of 2015, the overall prevalence of CKD in the general population varies between 3.5 and 14%, depending on the population studied and particularly based on the "yes" or "no" strictly following the proposed KDIGO guidelines concerning diagnosis of CKD [1]. Older age, diabetes, hypertension, cardiovascular disease and obesity are associated with CKD [2].

Type 2 diabetes mellitus is a highly prevalent chronic disease and diabetic nephropathy is one of the most important complications of diabetes mellitus [2]. Metformin, a biguanide drug, is still the first-line medication for the treatment of type 2 diabetes mellitus [3]. Recent stud-

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ies suggest that metformin in addition to its efficacy in treating type 2 diabetes, may by the activation of the AMPK singnalling, can also have therapeutic efficacy in other renal pathological conditions [4].

This review presents the epidemiology and the presumed mechanisms responsible for the renoprotection of metformin in vitro, in vivo models and in particular clinical conditions.

Metformin's Effect on Survival in Humans

Metformin exerts benign pleiotropic actions beyond its effects as glucose-lowering agents in the treatment of diabetes mellitus. Beneficial effects of metformin on survival rate in different clinical settings as well as in experimental animal models have been shown in several studies.

In a recent comprehensive meta-analysis of 17 observational studies, metformin use was associated with reduced all-cause mortality in patients with CKD CHF (chronic heart failure), or CLD (chronic liver disease), and fewer heart failure readmissions in patients with CKD or CHF were observed in patients with metformin therapy [5].

Metformin slowed age-related comorbidities (e.g., cardiovascular diseases (CVD), cancer, depression, dementia, and frailty-related diseases) and decreased mortality in old men with type 2 diabetes (T2D) [6]. In critically ill patients with type 2 diabetes admitted in medical or surgical intensive care units, use of metformin in their medical history was associated with reduced 30-day mortality [7].

Metformin therapy was associated with lower rates of mortality in ambulatory patients affected by both diabetes and heart failure [8] and reduced risk of cardiac failure morbidity and mortality in diabetic patients [9]. In a group of 401 diabetic patients with advanced low ventricular ejection fraction, one-year survival in metformin-treated (n = 99) and patients not receiving metformin treatment was 91 and 76%, respectively. In comparison to the metformin user, non-metformin-treated patients were at significantly increased risk for the combined end point of death or urgent transplantation [10]. Likewise, metformin therapy reduced the mortality of heart failure patients with new-onset diabetes mellitus [11]. These studies support an observational study indicating that thiazolidinediones and metformin are not associated with increased mortality rate and may improve outcomes in older patients with diabetes and heart failure [12].

In a large population of 19,691 patients having diabetes with established atherothrombotic symptoms, the mortality rates were lesser in metformin users vs. patients not on metformin therapy (6.3 vs. 9.8%). This association with lower mortality was consistent among elder subgroups or patients with a history of congestive heart failure or patients having a moderate form of CKD (eGFR of 30–60 mL/min/1.73 m^2) [13].

In an observational cohort study (involving 2,206 patients with type 2 diabetes), treatment with metformin alone or in combination with other hypoglycaemic agents were associated with a decreased risk of all-cause hospitalisations and reduced all-cause mortality compared with regimens without metformin [14]. Intensive glycaemic control with metformin was cost- and life-saving in overweight type-2 diabetes patients (0.43 life-years gained per patient) over the 11-year-period in a study conducted by Swiss investigators [15]. However, in some studies, it has been demonstrated that in older adults with 2 or more chronic conditions (atrial fibrillation, coronary artery disease, CKD, depression, diabetes, heart failure, hyperlipidaemia, hypertension, and thromboembolic disease) metformin therapy was not associated with reduced mortality [16].

The UK Prospective Diabetes Study (UKPDS) Group demonstrated that metformin therapy could reduce the risks for any diabetes-related endpoint in diabetic patients by 32%, diabetes-related death by 42% and all-cause mortality by 36% when compared to treatment with sulfonylurea or insulin [17]. Metformin has solid cardiovascular protective effects beside its antihyperglycaemic actions leading to lower rate of mortality in diabetic patients [18]. A 4-year follow up study of 51,675 patients from the Swedish National Diabetes Register of metformin therapy compared with any other antidiabetic treatment. Metformin showed lower risk than insulin for CVD and all-cause mortality and slightly lower risk for all-cause mortality compared with other oral hypoglycemic agents, in this huge cohort followed for 4 years. Patients with renal impairment showed no increased risk of CVD, all-cause mortality or acidosis/serious infection. In clinical practice, the benefits of metformin use clearly outbalance the risk of severe side effects [19]. In a retrospective, observational study comprising more than 90,000 patients (76,811 patients were prescribed metformin monotherapy, mean follow-up of 2.9 years, 15,687 sulphonyl urea monotherapies, mean follow-up 3.1 years) all-cause mortality was reported higher in patients prescribed sulphonyl urea compared with metformin monotherapy [20]. This study was in line with a former study showing that metformin therapy, alone or in combination with sulfonylurea, was associated with reduced

all-cause and cardiovascular mortality compared with sulfonylurea monotherapy among new users of these agents [21] or in subjects with heart failure and type 2 diabetes [22–24]. Thiazolidinedione's prescription in AMI showed higher risk of readmission for heart failure after myocardial infarction in comparison to the metformin therapy within 1 year after the administration of AMI [25].

Renoprotective Effect of Metformin

Kidney Fibrosis

Several excellent reviews have been published on the pathophysiology of kidney fibrosis [26]. The progression of CKD towards end-stage kidney disease is illustrated by the loss of kidney cells and replacement by extracellular matrix (ECM) independently of the etiology of primary underlying disease. Grgic et al. [27] recently demonstrated that selective epithelial injury can drive the formation of interstitial fibrosis, capillary rarefaction and potentially glomerulosclerosis, substantiating a direct role for damaged tubule epithelium in the pathogenesis of CKD.

As a consequence, CKD is followed by glomerulosclerosis and tubulo-interstitial fibrosis caused by an imbalance between excessive synthesis and reduced breakdown of the ECM [28]. Several molecules and cells are intertwined with the progression of renal fibrosis (e.g., angiotensin II, transforming growth factor-β, epithelial-mesenchymal transition, wingless/int-1 [WNT] signalling) leading to proliferation and activation of (myo) fibroblasts. Tubulo-interstitial renal fibrosis is characterised as a progressive damaging connective tissue deposition on the kidney parenchyma [29].

Effect of Metformin on Kidney Fibrosis

The most relevant studies dealing with potential protection by metformin of fibrosis in cell culture, animal models and humans and the multiple biochemical alterations that are involved in reduction of fibrogenesis by metformin are summarised in Table 1 [30–43].

Different animal and in vitro models were studied to verify the effects of metformin on the biomarkers and the parameters used in fibrosis.

Besides the morphological analysis and immunohistochemistry, studied parameters were mainly AMPK and target of rapamycin (mTOR) activity biomarkers and TNF-α, α-SMA, TGF-β1, fibronectin, vimentin, e-cadherin (Table 1).

In general, metformin can attenuate tubulo-interstitial fibrosis and epithelial mesenchymal transition in in vitro and in vivo models through the activation of AMPK and downregulation of transforming growth factor-β1. In addition, metformin inhibits activation of ERK signalling and attenuated the production of ECM proteins and collagen deposition. Metformin can reverse angiotensin II-induced increased expression of fibronectin, collagen I, activated ERK signalling and TGF-beta1 in renal fibroblasts cultures.

Polycystic Kidney Disease

Pathophysiology of Polycystic Kidney Disease

The overactivity of both mammalian mTOR and cystic fibrosis transmembrane conductance regulator plays crucial roles in the expansion of renal cysts in autosomal dominant polycystic kidney disease (ADPKD) [44]. Loss-of-function mutations in either PKD1 or PKD2 genes, which encode polycystin-1 (TRPP1) and polycystin-2 (TRPP2), respectively, can lead to ADPKD [45]. Other molecules and signalling pathways like the renin-angiotensin-aldosterone system, vasopressin and cyclic adenosine monophosphate, epidermal growth factor and insulin-like growth factor tyrosine kinases, vascular endothelial growth factor, extracellular signal-related kinase, tumour necrosis factor-α, cyclin-dependent kinases, caspases and apoptosis, and cyclic adenosine monophosphate-activated protein kinases are implicated as well in cyst growth [46].

Effect of Metformin on Polycystic Kidney Disease

Agents reversing the aforementioned signalling pathways and autophagy inducers such as mammalian mTOR inhibitors, cyclin-dependent kinase inhibitors, metformin, curcumin, and triptolide can be considered in the treatment of PKD [46]. It has been demonstrated that AMP-activated kinase (AMPK) can suppress the activity of each of the proteins playing a pathogenesis role of PKD [47]. In addition, cystic fibrosis transmembrane conductance regulator and mammalian mTOR are both negatively regulated by AMPK. In in vitro and ex vivo models of renal cystogenesis, metformin showed significant arrest of cystic growth and produces a significant decrease in the cystic index in 2 mouse models of ADPKD [41]. Hence, AMPK activators such as metformin may have a potential role to play in the clinical management of ADPKD [44].

Metformin- and Gentamicin-Induced Nephrotoxicity

Gentamicin causes (i) the induction of the mitochondrial permeability transition, which is leading to the release of cytochrome c, outflow and reduction of pyridine

Table 1. Metformin (M) effects on renal fibrosis

Year	Author	Cell or Kidney	Method	Metformin (M) dose/ concentration	Effects (citation)	Studied parameters
2016	Wang et al. [28]	Kidney	Male Sprague–Dawley rats were treated with M at a dose of 125 μg/kg/day I.P., 3 days prior to ischaemia-reperfusion (IRI) injury, or from 3 days IRI to 12 weeks after reperfusion (continuous administration of M)	125 μg/kg/ day	"Continuous administration of metformin significantly attenuated tubule-interstitial fibrosis and EMT in rats, potentially via activation of AMPK and downregulation of TGF-β1."	Renal function, histology, and expressions of IL-6, TNF-α, a-SMA, TGF-β1, vimentin, and E-cadherin
2016	Shen et al. [29]	Kidney Normal rat kidney fibroblasts (NRK-49F)	Unilateral ureteral obstructed male C57BL/6J mice (UUO) treated with M for 7 days – renal fibroblast NRK-49F cells pre-treated with 1 mM M for 1 h followed by the administration of 1 micromol Ang II.	200 mg/kg body weight per day – 1 mM (for in vitro study)	"Administration of M inhibited the activation of ERK signalling and attenuated the production of extracellular matrix (ECM) proteins and collagen deposition in the obstructed kidneys. In cultured renal fibroblasts, Ang II increased the expression of fibronectin and collagen I and also activated ERK signalling and TGF in a time-dependent manner. Pre-treatment of the cells with M blocked Ang II–induced ERK signalling activation and ECM overproduction. M prevents renal fibrosis, possibly through the inhibition of ERK signalling."	Transforming growth factor-β (TGF-β) and p-ERK – Fibronectin (FN), collagen I (Col-I), and TGF-β
2015	Thakur et al. [30]	Kidney Primary human renal proximal tubular epithelial cells (HRPTECs) – murine PTECs, rat kidney interstitial fibroblast cells, TGF-beta1 transgenic mouse model	Renal proximal tubular epithelial cells were pre-treated with 1 mmol/L M for 30 min before TGF-β1 treatment for 24 h	1 mmol/L	"In vivo relevance indicated by experiments in the kidney cortex of TGF-β1 transgenic mice in which a significant decrease in AMPK and tuberin phosphorylation on activating sites is associated with an increase in mesenchymal markers and decrease in E-cadherin. Activation of AMPK with AICAR or M markedly reduced TGF-β1-induced fibronectin and an SMA expression and prevented the decrease in E-cadherin expression. The study provides a strong rationale for the use of AMPK activators to treat TGF-beta-associated fibrotic kidney diseases independent of their origin."	AMPK activation (confirmed by enhanced phosphorylation of AMPK on Thr172 and pack on Ser79) – fibronectin, a-smooth muscle actin), E-cadherin, p-AMPKa (Thr172), p-Tuberin (Ser1387), AMPKa1, AMPKa2, AMPKa and tuberin antibodies

Table 1. (continued)

Year	Author	Cell or Kidney	Method	Metformin (M) dose/ concentration	Effects (citation)	Studied parameters
2015	Cavaglieri et al. [31]	Kidney	Five- to 7-month-old adult male C57Bl/6 mice treated with M by gavage in unilateral ureteral obstruction	200 mg/kg/day	"M reduced expression of extracellular matrix proteins and profibrotic factor TGFβ in obstructed kidneys, measured by immunohistochemistry. Interstitial fibroblast activation was evident in obstructed kidneys and ameliorated by M. M treatment, initiated 1 day before unilateral ureteral obstruction surgery attenuates development of renal inflammation and fibrosis."	AMPK activity – α-SMA – TNFα and VCAM1 – Collagens and fibronectin – collagen Iα2 mRNA and collagen IIIα1 mRNA
2015	Lu et al. [32]	Primary-cultured Balb/c mice renal fibroblasts	Activation of AMPK in primary cultured renal fibroblasts; cells were treated with M 10 mM for different time points (0, 0.5, 1, 6, 12 h)	(10 mM)	Activation of AMPK by M reduced TGF-β1-induced collagen type I production by suppression of Smad3-driven CTGF expression. Activation of AMPK might be a novel strategy for the treatment of chronic kidney disease (CKD) partially by inhibition of renal interstitial fibrosis.	Phosphorylation of Smad3 and production of CTGF, collagen type I.
2014	Declèves et al. [33]	Kidney	M (0.3 g/kg), was administered 24 h prior to ischaemia to male adult Wistar rats	300 mg/kg	M increased macro-autophagic protein LC3 and normalised p62/SQSTM1 expression and mTOR activity. Ischaemia-reperfusion increases in Beclin-1 and PINK1 expressions, consistent with increased mitophagy, were also mitigated with AMPK agonists. Stress-responsive and apoptotic markers expression increase in ischaemia-reperfusion and are significantly attenuated with agonist administration, as are early indicators of fibrosis.	AMPK and mTOR activities (AMPK activity was expressed as p-AMPK expression per total AMPK expression. The increase in phosphorylated p70S6K is indicative of increased mTORC1 activity) – LC3-I protein, Beclin-1, PINK1.
2015	Kim [34]	HK-2 cells, a human proximal tubular cell line	Cells were incubated with TM (0.2 M) or TG (0.2 M) with or without M, compound C, and AICAR for 24 h – Male mice (C57BL/6) treated with M by gavage for 3 days in mouse model of ER stress-induced acute kidney injury and for 14 days in the mouse model of UUO-induced progressive kidney injury.	Cell culture (M 1 mM) – animal study (300 mg/kg/day)	M (the best known clinical activator of AMPK) suppressed TM- or TG-induced ER stress, as shown by the inhibition of TM- or TG-induced upregulation of glucose-related protein (GRP)78 and phosphorylated eukaryotic initiation factor-2α through induction of heme oxygenase-1. M inhibited TM- or TG-induced epithelial-mesenchymal transitions as well. M reduced GRP78 expression and renal fibrosis in a mouse model of unilateral ureteral obstruction. AMPK may serve as a promising therapeutic target by reducing ER stress and renal fibrosis.	Small interfering (si)RNA experiments, and immunohistochemically staining -smooth muscle actin (-SMA), E-cadherin, GRP78, and monocyte chemotactic protein-1 – CHOP, home oxygenase (HO)-1, total eIF2, phosphospecific eIF2 (Ser51) – GRP78 and alpha – smooth muscle actin, E-cadherin.

Table 1. (continued)

Year	Author	Cell or Kidney	Method	Metformin (M) dose/ concentration	Effects (citation)	Studied parameters
2013	Satriano [35]	Kidney	Male Wistar rats underwent kidney surgery (remnant kidnery) then treated with M by gavage for 7 days.	250 mg/kg/day	Induction of AMPK activity with either M or 5-aminoimidazole-4-carboxamide ribonucleotide increases AMPK activity in this model and also corrects kidney metabolic inefficiency, improves kidney function, and ameliorates kidney fibrosis and structural alterations.	Histologic analysis, AMPK activity,evaluation of the activated, phosphorylated state of the enzyme and total AMPK expression – eGFR and QO2/TNA.
2013	Lee et al. [36]	HK-2 cells, a human proximal tubular cell line	Proximal tubular cells were incubated with TGF-beta, angiotensin II, aldosterone, high glucose and albumin for 3 days and then treated with M (1 mM) for 2 days.	1 mM	The epithelial-mesenchymal transition (EMT) is a novel mechanism that promotes renal fibrosis. Transforming growth factor-β (TGF-β), angiotensin II, aldosterone, high glucose, and urinary albumin are well-known causes of EMT and renal fibrosis. M (the best known clinical activator of AMPK) suppressed EMT induction through inhibition of ROS via induction of heme oxygenase-1 and endogenous antioxidant thioredoxin. AMPK activation may serve as a promising therapeutic target in the prevention and/or treatment of tubulo-interstitial fibrosis induced by TGF-β, angiotensin II, aldosterone, high glucose, and urinary albumin.	Smooth muscle actin (-SMA) and E-cadherin – reactive oxygen species (ROS), Nox4, actin
2011	Song et al. [37]	Kidney	Male Sprague-Dawley diabetic rats were gavaged with M (55.33 mg/[kg × day] for 35 days)	55.33 mg/(kg × day), 35 days	The blood glucose, TGF-beta1 and Smad3 expression in kidney of rats were significantly lower than that of model group. The kidney weight/body weight of rats in sericin treatment group, sericin prevention group and metformin group were significantly lower than that of model group. Sericin can inhibit activation of TGF-beta1/Smad3 signal pathway in kidney of DN rats. Decrease of glomerulosclerosis and renal interstitial fibrosis. The therapeutic and preventive effects of sericin on DN are comparable to effects of M.	TGF-beta1/Smad3 signal pathway

Table 1. (continued)

Year	Author	Cell or Kidney	Method	Metformin (M) dose/ concentration	Effects (citation)	Studied parameters
2011	Takiyama et al. [38]	Kidney Human renal proximal epithelial tubular cell cultures (HRPTECs)	Male Zucker diabetic fatty (ZDF; Gmi-fa/fa) rats were treated from 9 to 39 weeks with M or insulin HRPTECs were incubated with 0.01–1 mmol/L of M for 4 h.	250 mg/kg/ day	HIF-1α enhanced the epithelial-to-mesenchymal transition (EMT) in vitro and that genetic ablation of renal epithelial HIF-1a inhibited the development of tubulo-interstitial fibrosis. M inhibited hypoxia-induced HIF-1α accumulation and the expression of HIF-1-targeted genes in (human renal proximal tubular epithelial cells) HRPTECs – M significantly decreased ATP production and oxygen consumption rates, which subsequently led to increased cellular oxygen tension. Finally, M, but not insulin, attenuated tubular HIF-1α expression and pimonidazole staining and ameliorated tubular injury in ZDF rats. – hypoxia-induced HIF-1α accumulation in diabetic nephropathy could be suppressed by the antidiabetic drug, M, through the repression of oxygen consumption. These data provide a novel mechanism of effects of M to improve microalbuminuria in diabetic nephropathy, by protecting from hypoxia-induced renal fibrosis by attenuating the expression of HIF-1α	Morphological analysis and immunohistochemistry – human plasminogen activator inhibitor (PAI)-1 Serpine gene, the human SLC2A1 gene, the human HIF-1α gene, the human vascular endothelial growth factor (VEGF) gene, and the 18S gene – hypoxia-induced HIF-1alpha protein – HIF-1α – AMPK-α1 or AMPK-α2 protein – PAI-1, VEGF, and Glut-1 mRNA – p-AMPK and p-ACC, and p-mTOR
2011	Takiar et al. [39]	Kidney Madin Darby canine kidney (MDCK) type II cells	In in vitro MDCK, cells were incubated with 1.0 mM M for 2-4 h up to 24 h – MDCK cells in a 3D collagen matrix forming spontaneous cysts in the presence of forskolin and IBMX and treated with 1.0 mM M for 20 days. – For example, vivo kidneys were removed from C57/B6 mice then embryonic kidney coincubated with 8-Br-cAMP and M for 4 days – for in vivo C57BL/6 mice (8-week-old) were treated i.p. with M or with vehicle for 3 days with different dosage of M in one procedure and in other study with 300 mg/kg up to 18 days.	1.0 mM in vitro) 50, 125, 250, 375, 500, 650 mg/kg 300 mg/kg/ day	M stimulates AMPK, resulting in inhibition of cystic fibrosis, CFTR and the mTOR pathways,inducing significant arrest of cystic growth in both in vitro and ex vivo models of renal cystogenesis. M reduces cyst size. M produces a significant decrease in the cystic index in two mouse models of autosomal dominant polycystic kidney disease. AMPK activation is slowing renal cystogenesis as well as the potential for therapeutic application of M in the context of autosomal dominant polycystic kidney disease.	pAMPK, panAMPK, pACC, β-actin, CFTC, p70 S6K. total S6K, histology, and cystic Index

Table 1. (continued)

Year	Author	Cell or Kidney	Method	Metformin (M) dose/ concentration	Effects (citation)	Studied parameters
2011	Louro et al. [40]	Kidney Male diabetic GK rats between 5 and 6 months of age were treated orally with drinking water.		60 mg/kg/ day	Insulin and M were able to improve glycoxidation, fibrosis and kidney inflammationy parameters.	Monoclonal anti-antibodies (CML, TGF-β1 and IL-1β) and mouse anti-actin monoclonal antibody. – The pro-inflammatory markers, TNF-α and IL-6 Histological assessment of renal injury

nucleotides due to their consumption in the DNA repair process by poly (ADP-ribose) polymerase (PARP) and (ii) high production of reactive oxygen species (ROS) leading to breakage of DNA [47]. Morales et al. [50] demonstrated in rats that metformin prevented gentamicin-induced nephropathy through a mitochondria-dependent pathway, normalizsng oxidative stress and restoring mitochondrial functional integrity [47]. In a rabbit model, metformin showed a strong nephroprotective effect at 40 mg/kg/day of gentamicin [48]. In addition, post-treatment or co-treatment with metformin can prevent the rise of serum BUN and serum creatinine induced by gentamicin, attenuating the damage score [49]. Metformin lowers the activity of N-acetyl-beta-D-glycosaminidase, together with a reduction of lipid peroxidation, thereby boosting the antioxidant systems and improving mitochondrial homeostasis [50].

Metformin and Aristolochic Acid and Streptozotocin-Nicotinamide-Induced Diabetic Nephropathy-Induced Nephropathy

It is recognised that aristolochic acid-induced nephrotoxicity is related to the accumulation of methylglyoxal and N(ε)-(carboxymethyl)lysine. Metformin can reduce the activity of renal semi carbazide-sensitive amine oxidase, which is a key enzyme involved in the generation of methylglyoxal. In fact, methylglyoxal scavenging by metformin reduces aristolochic acid nephrotoxicity [51]. Metformin treatment showed a significant renoprotective effect against streptozotocin-nicotinamide-induced diabetic nephropathy in rats. However, the concomitant administration of metformin and coenzyme Q10 showed a better renoprotective effect than coenzyme Q10 or metformin alone [52]. It is also reported that metformin lessens high glucose-induced oxidative stress by the modulation of p38 mitogen-activated protein kinase expression, which may contribute to its renoprotective abilities in diabetes [53].

Metformin Protective Effects on Podocytes and Some Other Models of Chronic Kidney Injury

Diabetic nephropathy is featured by the loss of human podocytes expressing organic cation transporter 1, which is the major uptake transporter of metformin. Metformin can reverse hyperglycaemia-induced reduction of AMPK phosphorylation and mTOR activation in podocytes. Metformin modulates apoptosis and cell signalling of human podocytes under high glucose conditions through the activation of AMPK and inhibition of mTOR signalling [54]. Diabetic rats treated with 150–500 mg/kg metformin for 8 weeks had a dose-dependent significant reduction in urinary albumin and nephrin concentrations, glomerular basement membrane thickness, and the foot process fusion rate compared with the control T2DM model rats [55]. Urinary albumin and podocalyxin (PCX) were markedly increased and there is a significant alteration in renal glomerular structure in type 2 diabetic rats. Treatment of such rats with different doses of metformin restored all these changes to a varying degree associated with its role in restoring PCX expression and inhibiting urinary excretion of PCX in a dose-dependent way [56]. It is shown that the exposure of cultured podocytes to metformin (10–75 μM) affects purinergic signalling through the inhibition of ecto-ATPase leading to an increase of the extracellular ATP concentration and activation of P2 receptors and consequent modulation of the podocyte metabolism through AMPK and NAD(P)H oxidase, ameliorating podocyte functioning [57].

Increase in urinary and renal 8-hydroxydeoxyguanosine levels and podocyte loss was shown in the diabetic model of rats [58]. These abnormalities were improved by metformin, providing a protective effect on glomerular podocytes at a dose of 300 mg metformin/kg/day for 8 weeks and 350 mg metformin/kg/day for 17 weeks [59]. In mice, independent of the expression of OCT1/2 and AMPK-β1, metformin (500 mg/kg/day) has a beneficial effect in early stages of renal disease induced by unilateral ureteral obstruction [59]. Pre-treatment with metformin at a dose of 25–100 mg/kg for 7 days shows protective effects in an ischaemia-reperfusion model induced in rats as indicated by improved kidney function, less development of fibrosis and structural alterations [60]. Metformin (250 mg/kg/day, for one week to one month) prevents severe kidney failure, vascular calcification and high bone turnover disease in a rat model for CKD /mineral and bone disorders (adenine administration) [61]. In an in vitro study, metformin had shown a preventive effect on vascular calcification via AMPK-eNOS-NO pathway [62].

In a male Sprague-Dawley rat crystal formation model, metformin at the dose of 200 mg/kg/day for 8-week attenuated oxidative stress, a causal factor and key promoter of urolithiasis, associated with renal tubular epithelium cell injury. Kidney crystal formation and adherence to the epithelium in the ethylene glycol (EG) + metformin treated group was decreased significantly compared with the EG-treated group [63]. Metformin shows increased protein abundance of inner medullary urea transporter UT-A1 and aquaporin 2 in tolvaptan-induced nephrogenic diabetes insipidus rats, thereby improving urinary concentration capacity [64].

Renoprotective Effect of Metformin in Human in Retrospective and Clinical Studies

In a population (n = 469,688 patients) open cohort study using a large UK primary care database, the risk of severe complications of diabetes including, blindness, hyperglycaemia, hypoglycaemia, amputation, and severe kidney failure, was significantly decreased in metformin use compared to non-use [65]. Metformin initiation has been shown to be associated with a lower risk of decline in kidney function or death compared to sulfonylureas independent of changes in body mass index and systolic blood pressure and glycated haemoglobin over time [66].

It was suggested that oral metformin therapy might be used instead of intravenous insulin for glycaemic control in traumatised critically ill patients and may reduce the microalbuminuria to creatinine ratio [67]. In type 2 diabetic patients, favourable effects of metformin on blood pressure, lipid profile, metabolic control, and insulin resistance can significantly decrease the urine albumin excretion rate with no changes in renal hemodynamics [68].

A retrospective cohort study from the Scientific Registry of Transplant Recipients linked data for all incident kidney transplants (2001–2012) with national pharmacy claims (n = 46,914; Fig. 1). Recipients having one or more pharmacy claims for a metformin-containing product (n = 4,609) were compared with those having one or more claims for a non-metformin glucose-lowering agent (n = 42,305) [69]. Metformin was associated with lower adjusted hazard ratios (HRs) at 3 years post-transplant for living donor (0.55 [0.38–0.80]; p = 0.002) and deceased donor allograft survival (0.55 [0.44–0.70]; $p < 0.0001$), and with lower mortality.

The Relationship between Metformin and Lactic Acidosis

The fear of lactic acidosis with metformin still influences treatment strategies, particularly in patients, with moderate and severe kidney disease. The term "metformin-associated lactic acidosis" (MALA) first appeared in the literature in 1977, and has been used to describe almost all cases of lactic acidosis observed in a metformin-treated patient ever since. Metformin intoxication (overdose) and metformin accumulation in the setting of acute kidney failure are typical situations where treatment with metformin can cause lactic acidosis. In most cases, however, metformin therapy may be merely concomitant and may not have a causal role at all.

Literature reports concerning the prevalence of MALA rarely provide sufficient details of the clinical context such as the metformin dose and duration, renal function over time, a careful analysis of the clinical setting, the availability of assay data and time registration for the blood metformin concentration. Analysing the largest available pharmacovigilance database showed that 3 key criteria: a high lactate concentration, a low pH, and available metformin concentration, were met in just 10.4% of cases [70]. Hence, it is usually impossible to distinguish between lactic acidosis in the context of metformin accumulation (i.e., acute kidney failure or intoxication) and lactic acidosis caused by systemic conditions (sepsis, cardiac failure, haemorrhage, etc.) in a patient taking metformin.

In the general population of type 2 diabetes, there have been several large studies that have systematically exam-

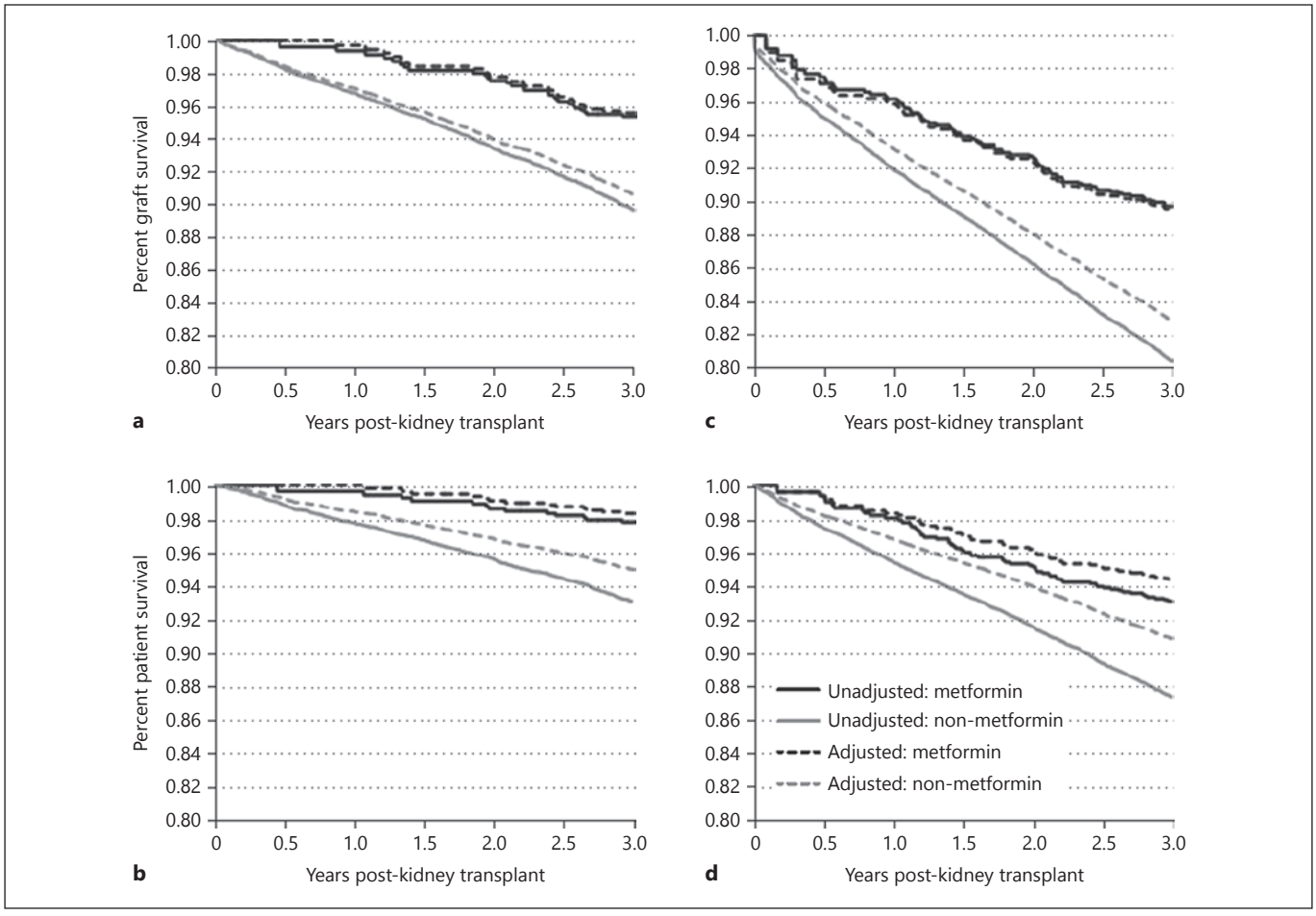

Fig. 1. Allograft survival (**a**) and patient survival (**b**) in living donor kidney recipients; allograft survival (**c**) and patient survival (**d**) in deceased donor kidney recipients. Time-zero indicates date of transplant with survival left-censored until the date of the first diabetic medication fill of any type. Use of metformin-containing medication was treated as a single time-varying covariate [67].

ined the risk of MALA. In the comprehensive updated Cochrane meta-analysis, Salpeter et al. [71] pooled data from of 347 cohort studies and trials comparing metformin vs. placebo and vs. other anti-diabetic drugs in the treatment of type 2 diabetes. Cases of fatal or non-fatal lactic acidosis were not observed after 70,490 patient-years of follow-up in the metformin group and 55,451 patient-years of follow-up in the non-metformin group. The estimated upper limits of true incidence of lactic acidosis were 4.3 and 5.4 per 100,000 patients-years in the metformin and non-metformin group respectively. Although exclusion of participants with kidney dysfunction may have resulted in the low observed MALA rates, 43% of the 334 trials that were pooled did not exclude patients with kidney disease at baseline.

Ekström et al. [19] studied a cohort of 51,675 patients from the Swedish National Registry, and found that a higher risk of MALA was not observed in patients with CKD [19]. Metformin, compared with any other treatment, showed reduced risks of acidosis/serious infection (adjusted HR 0.85, 95% CI 0.74–0.97) and all-cause mortality (HR 0.87, 95% CI 0.77–0.99) in patients with eGFR 45–60 mL/min/1.73 m^2, and no increased risks of all-cause mortality, acidosis/serious infection or CVD were found in patients with eGFR 30–45 mL/min/1.73 m^2. Similarly, in a study of 50,048 type 2 diabetic patients from the UK General Practice Research Database, occurrence of lactic acidosis was rare (6 cases total) and did not differ between those who received metformin vs. other oral anti-diabetic agents [72].

Two recent studies have added controversy. However, Eppenga et al. [73] analyzed data from 223,968 patients using metformin and 34,571 using other oral agents between 2004 and 2012, using a UK general practice database. The primary outcome was lactic acidosis defined by clinical

code, lactate level greater than 5 mmol/L, or both. The overall incidence rate was 7.4 vs. 2.2 per 100,000 person-years among metformin users vs. non-users. The authors concluded that the risk of lactic acidosis or elevated lactate level was significantly higher in metformin-treated patients with moderate to severe CKD as compared with those using other therapies, a risk compounded at higher doses. Inzucchi et al. [74] in their review pointed towards several relevant limitations of this study. While the overall incidence rate for lactic acidosis was low (35 events over 337,590 patient-years of follow-up), the incidence rate with worsening severity of kidney function was non-significant. With such small number of events, conclusions cannot be made.

A recent study of diabetic kidney disease patients by Hung et al. [75] using historical data from Taiwan provides insight into the potential toxicities of metformin in a setting where use of this anti-diabetic agent was previously unrestricted. Until 2009, metformin could be prescribed to all patients in Taiwan irrespective of kidney function up to end stage renal failure. In this study, investigators examined 12,350 type 2 diabetic patients with stage 5 CKD from the Taiwan National Health Insurance Database who had an ICD-9 code for CKD and were prescribed an erythropoietin-stimulating agent (ESA; in whom coverage was restricted to those with creatinine levels of >6 mg/dL (>530 μmol/L) and anemia, CKD 5). Among this source population, 1,005 of patients were metformin users and 11,345 were non-users. In rigorous analyses that matched 813 metformin users to 2,439 non-users using propensity scores, metformin users demonstrated a 35% higher mortality risk compared to non-users: adjusted HR 1.35 (95% CI 1.20–1.51), but no significant increase in lactate levels was observed. Hung et al. [75] did not provide data for the duration of metformin treatment, the number of anti-diabetes drugs used, the HbA1C concentrations and the number of comorbidities [76]. Nevertheless, the message of this paper consists a strong warning towards the use of Metformin in CKD 5 patients characterised by their very fragile clinical situation.

Lastly, a recent systematic review [74] of 65 studies rigorously examined the risk of lactic acidosis in moderate to severe CKD patients over the period of 1950–2014 and concluded the following:

1. The risk of lactic acidosis is essentially nil in the context of clinical trials, including those that did not specify kidney disease as an exclusion criterion.

2. The incidence of lactic acidosis in the setting of metformin therapy is low, and the drug is not necessarily responsible when lactic acidosis occurs in patients taking this medication.

3. MALA risk may have been underestimated due to confounding by indication; and conversely, ascertainment of MALA using lactate levels may have overestimated risk.

4. A conservative synthesis of these data is that as long as kidney function is stable and the patient is observed closely, metformin is unlikely to measurably increase the risk of lactic acidosis in patients with moderate CKD (i.e., eGFR 30–60 mL/min/1.73 m^2).

Most countries, scientific associations considered CKD as a contraindication for the use of metformin in these patients. The recent authorisations from the European Medicines Agency and the US Food and Drug Administration for the relaxed use of metformin in patients with diabetes and CKD stage 3A and B (eGFR 59–30 mL/min/1.73 m^2) allow to administer metformin to patients across CKD stages 1–3, but not in 4 and 5.

Very recently a clinical evaluation of metformin in patients with CKD (stages 3A, 3B and 4) was finalised. The study consists of 3 parts: (i) a dose-finding study in CKD1–5 diabetic patients, (ii) chronic administration of metformin doses adjusted to the renal function to patients with CKD3A, 3B, and 4, and (iii) assessed pharmacokinetic parameters in patients with moderate-to-severe CKD. This study addressing these issues and providing recommendations for optimal use of metformin in CKD 3A, 3B and 4 is currently under revision for publication.

Conclusion

There is no doubt that metformin has a number of relevant pleiotropic effects on various systems/organs particularly the kidney. Of interest is its protective effect on eGFR and less development of interstitial fibrosis observed in several experimental models of chronic progressive renal damage (remnant kidney, obstruction, toxins, nephrocalcinosis).

In clinical medicine, metformin improves the survival of CKD patients as well as the graft survival of living and cadaveric kidney donor transplantation.

Randomised clinical trials are needed to support the results obtained in animals and man concerning the renoprotection effect of metformin on the rate of progression of chronic kidney diseases.

Disclosure Statement

The authors have no conflicts of interest to declare.

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