■ R E V I E W

Cellular and molecular mechanisms of metformin: an overview

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A B S T R A C T

Considerable efforts have been made since the 1950s to better understand the cellular and molecular mechanisms of action of metformin, a potent antihyperglycaemic agent now recommended as the first-line oral therapy for T2D (Type 2 diabetes). The main effect of this drug from the biguanide family is to acutely decrease hepatic glucose production, mostly through a mild and transient inhibition of the mitochondrial respiratory chain complex I. In addition, the resulting decrease in hepatic energy status activates AMPK (AMP-activated protein kinase), a cellular metabolic sensor, providing a generally accepted mechanism for the action of metformin on hepatic gluconeogenesis. The demonstration that respiratory chain complex I, but not AMPK, is the primary target of metformin was recently strengthened by showing that the metabolic effect of the drug is preserved in liver-specific AMPK-deficient mice. Beyond its effect on glucose metabolism, metformin has been reported to restore ovarian function in PCOS (polycystic ovary syndrome), reduce fatty liver, and to lower microvascular and macrovascular complications associated with T2D. Its use has also recently been suggested as an adjuvant treatment for cancer or gestational diabetes and for the prevention in pre-diabetic populations. These emerging new therapeutic areas for metformin will be reviewed together with recent findings from pharmacogenetic studies linking genetic variations to drug response, a promising new step towards personalized medicine in the treatment of T2D.

INTRODUCTION

The prevalence of T2D (Type 2 diabetes) has reached epidemic proportions worldwide and promotes the risk of cardiovascular diseases and early mortality. Prevention and management of T2D has become a major public health challenge around the world. Metformin (1,1-dimethylbiguanide), a biguanide derivate, is the most widely prescribed drug to treat hyperglycaemia in individuals with T2D and is recommended, in conjunction with lifestyle modification (i.e. diet, weight control and physical activity), as a first-line oral therapy in the recent guidelines of the ADA (American Diabetes Association) and EASD (European Association of the Study of Diabetes) [1,2]. This recommendation was based on clinical studies from the UKPDS (UK Prospective Diabetes Study), a multi-centre randomized controlled trial of different therapies for T2D [3]. This landmark study reported that intensive glucose control with metformin appeared to decrease the risk of diabetes-related end points and death in overweight diabetic patients, and was associated with less weight gain and fewer hypoglycaemic attacks when compared with insulin and sulfonylureas. The reduction in cardiovascular mortality by metformin compared with any other oral diabetes agent or placebo was confirmed by meta-analysis including more than 30 clinical trials [4,5].

Despite being introduced clinically in the 1950s (although it was only available in the U.S.A. from 1995), the exact mechanism of action of metformin has not been fully elucidated. Recent clinical trials suggest that metformin, in addition to its efficacy in treating T2D, may also have therapeutic potential in other conditions, including diabetic nephropathy, cardiovascular diseases, polycystic ovary disease and the prevention or treatment of cancer. The present review will document the different mechanisms of the action of metformin described to reverse these disturbances both in diabetic and non-diabetic patients.

ANTI-HYPERGLYCAEMIC ACTION OF METFORMIN

Metformin is currently the drug of first choice for the treatment of T2D, being prescribed to at least 120 million people worldwide. Metformin is regarded as an antihyperglycaemic agent because it lowers blood glucose concentrations in T2D without causing overt hypoglycaemia. Metformin is also frequently described as an insulin-sensitizer, leading to reduction in insulin resistance and a significant decrease in plasma fasting insulin levels. The improvement in insulin sensitivity by metformin could be ascribed to its positive effects on insulin receptor expression and tyrosine kinase activity

[6]. Metformin may also exert its beneficial metabolic actions in part through the modulation of multiple components of the incretin axis. Indeed, Maida et al. [7] have recently reported that metformin acutely increases plasma levels of GLP-1 (glucagon-like peptide-1) and induces islet incretin receptor gene expression through a mechanism that is dependent on PPAR (peroxisome-proliferator-activated receptor)-α. However, a growing body of evidence from clinical studies and animal models suggests that the primary function of metformin is to decrease hepatic glucose production [8], mainly by inhibiting gluconeogenesis [9,10]. Several mechanisms have been proposed to explain this inhibitory action on hepatic gluconeogenesis, including changes in enzyme activities [11–13] or a reduction in hepatic uptake of gluconeogenic substrates [14]. The preferential action of metformin in hepatocytes is due to the predominant expression of OCT1 (organic cation transporter 1), which has been shown to facilitate cellular uptake of metformin [15]. Consistent with this, accumulation of metformin in the liver has been shown to be higher than in other tissues, reaching high micromolar concentrations in the periportal area [16]. Furthermore, deletion of the *Oct1* [*Slc22a1* (solute carrier family 22 member 1)] gene in mice dramatically reduces metformin uptake in hepatocytes, and human subjects carrying polymorphisms of the *SLC22A1* gene display an impaired effect of metformin in lowering blood glucose levels [15].

Although the molecular target of metformin has been elusive for several years, Zhou et al. [17] reported that the activation of AMPK (AMP-activated protein kinase) was intimately associated with the pleiotropic actions of metformin. AMPK is a phylogenetically conserved serine/threonine protein kinase viewed as a fuel gauge monitoring systemic and cellular energy status, and which plays a crucial role in protecting cellular functions under energy-restricted conditions. AMPK is a heterotrimeric protein consisting of a catalytic α-subunit and two regulatory subunits, β and γ, and each subunit has at least two isoforms. AMPK is activated by an increase in the intracellular AMP/ATP ratio resulting from an imbalance between ATP production and consumption. Activation of AMPK involves AMP binding to regulatory sites on the γ-subunits. This causes conformational changes that allosterically activate the enzyme and inhibit dephosphorylation of Thr^{172} within the activation loop of the catalytic α-subunit. AMPK activation requires phosphorylation on Thr^{172} by upstream kinases, identified as the tumour suppressor STK11 (serine/threonine kinase 11)/LKB1 and CaMKKβ (Ca^{2+}/calmodulin-dependent protein kinase β), which is stimulated further by the allosteric activator AMP [18]. Moreover, it has been recently shown that ADP, and therefore the ADP/ATP ratio, could also play a regulatory role on AMPK by binding to specific domains in the γ-subunit [19,20]. Activated AMPK switches cells
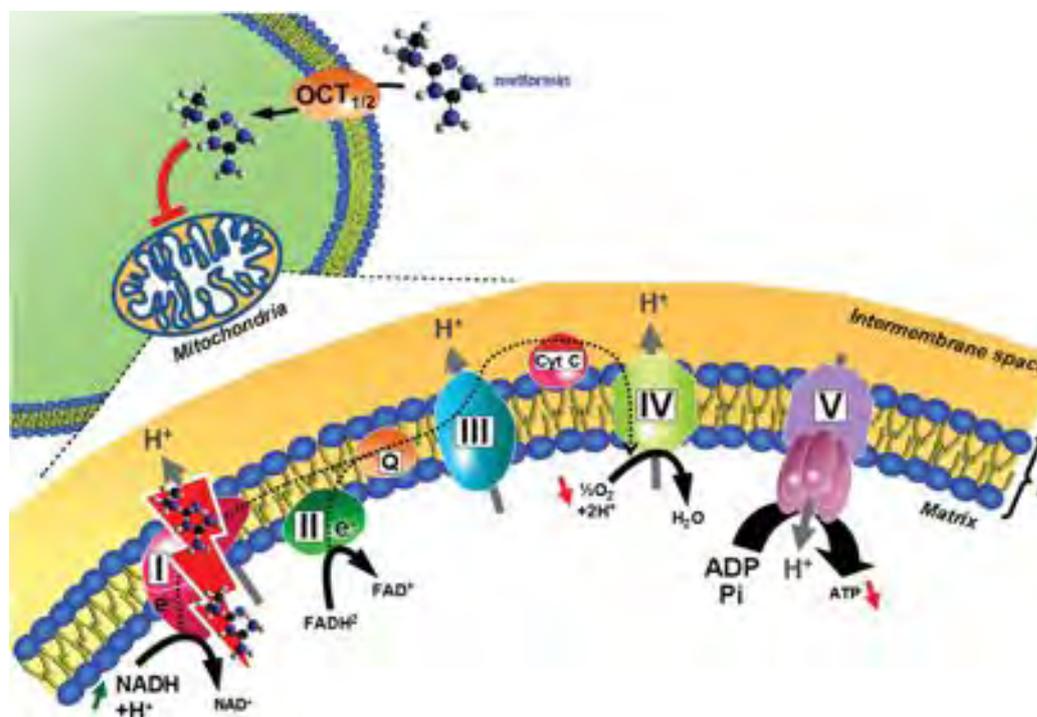
Figure 1   The mitochondrial respiratory chain complex 1 is the primary target of metformin

Owing to its high acid dissociation constant ($pK_a = 12.4$), metformin exists in a positively charged protonated form under physiological conditions and, as a result, can only partially cross the plasma membrane by passive diffusion. Thus its intracellular transport is mediated by different isoforms of OCTs depending on the tissue under consideration (e.g. OCT1 in the liver or OCT2 in the kidney). Once inside the cytosolic compartment, mitochondria then constitute the primary target of metformin. The positive charge of metformin has been proposed to account for its accumulation within the matrix of energized mitochondria, driven by the membrane potential, whereas the apolar hydrocarbon side chain of the drug could also promote binding to hydrophobic structures, especially the phospholipids of mitochondrial membranes [31]. Although the exact mechanism(s) by which metformin acts at the molecular level remains unknown, it has been shown that the drug inhibits the mitochondrial respiratory chain specifically at the level of complex I without affecting any other steps in the mitochondrial machinery. This unique property of the drug induces a decrease in NADH oxidation, proton pumping across the inner mitochondrial membrane and oxygen consumption rate, leading to lowering of the proton gradient and, ultimately, to a reduction in proton-driven synthesis of ATP from ADP and P_i.

from an anabolic to a catabolic state, shutting down the ATP-consuming synthetic pathways and restoring energy balance. This regulation involves phosphorylation by AMPK of key metabolic enzymes and transcription factors/co-activators modulating gene expression [18]. As a result, glucose, lipid and protein synthesis, as well as cell growth, are inhibited, whereas fatty acid oxidation and glucose uptake are stimulated.

Metformin most probably does not directly activate either LKB1 or AMPK, as the drug does not influence the phosphorylation of AMPK by LKB1 in a cell-free assay [21]. Rather, there is evidence that AMPK activation by metformin is secondary to its effect on the mitochondria, the primary target of the drug. One of the most significant breakthroughs in the understanding of the cellular mechanism of metformin was indeed made in the early 2000s by two independent research groups reporting for the first time that this member of the biguanide family induced mild and specific inhibition of the mitochondrial respiratory chain complex I (Figure 1). The initial observation was made in both perfused livers and isolated hepatocytes from rodents [22,23], but was later expanded to other tissues, including skeletal muscle [24], endothelial cells [25], pancreatic β-cells [26] and neurons [27]. Although the exact mechanism(s) by which metformin inhibits the respiratory chain complex I remains unknown, we have shown that this unique drug effect does not necessitate AMPK and is also found in human primary hepatocytes [28]. In addition, it appears that the mitochondrial action of metformin requires intact cells [22,29,30] and is prevented, at least in hepatocytes, neither by inhibition of NOS (nitric oxide synthase) or by various ROS (reactive oxygen species) scavengers [22]. In addition, the maximal inhibitory effect of metformin on complex I activity is also lower than the reference inhibitor rotenone ($\sim$40% with metformin compared with 80% with rotenone), suggesting that, owing to different physico-chemical properties, their respective site of action differs on one or several of the subunits of the respiratory chain complex I. For instance, the positive charge of metformin was proposed to account for its accumulation

within the matrix of energized mitochondria, driven by the membrane potential ($\Delta\psi$) [23]. Furthermore, the apolar hydrocarbon side chain of the drug could also promote its binding to hydrophobic structures, especially the phospholipids of mitochondrial membranes [31]. Interestingly, it has been shown that metformin, by contrast with rotenone, also exerts an inhibitory effect on mitochondrial ROS production by selectively blocking the reverse electron flow through the respiratory chain complex I [32,33]. Further investigations are still required to clarify the mechanism(s) by which metformin modulates the respiratory chain complex I in such a unique way. It is also worth mentioning that metformin probably also exerts some non-mitochondrial effects, since it has been shown to affect erythrocyte metabolism, a cell lacking mitochondria, by modulating membrane fluidity [34,35].

Taken together, the activation of AMPK by metformin in the liver, and probably in other tissues, is the direct consequence of a transient reduction in cellular energy status induced by the mild and specific inhibition of the respiratory chain complex I by the drug [28] (Figure 1). In line with this, methyl succinate, a substrate of the respiratory chain complex II bypassing the inhibition of complex I, has been shown to antagonize the metformin-induced AMPK activation in a pancreatic β-cell line [26]. Furthermore, Hawley et al. [36] have recently reported that AMPK activation by metformin is abolished in a cell line stably expressing AMPK complexes containing an AMP-insensitive $\gamma 2$-subunit mutant, demonstrating that increased cytosolic AMP indeed triggers the activation of the kinase by the drug.

AMPK involvement in the antidiabetic effect of metformin was initially supported by a study showing that the glucose-lowering effect of the drug was greatly decreased in mice lacking hepatic LKB1 [37]. LKB1/AMPK signalling has been reported to regulate the phosphorylation and nuclear exclusion of CRTC2 [CREB (cAMP-response-element-binding protein)-regulated transcription co-activator 2; also referred to as TORC2 (transducer of regulated CREB-binding protein 2)] [37]. CRTC2 has been identified as a pivotal regulator of hepatic glucose output in response to fasting by directing transcriptional activation of the gluconeogenic programme [38]. Non-phosphorylated CRTC2 translocates to the nucleus, where it associates with phosphorylated CREB to drive the expression of PGC-1α (PPAR-γ co-activator-1α) and its subsequent gluconeogenic target genes PEPCK (phosphoenolpyruvate carboxykinase) and G6Pase (glucose-6-phosphatase). Phosphorylation of Ser[171] on CRTC2 by AMPK and/or AMPK-related kinases, including SIKs (salt-inducible kinases), is critical for determining the activity, cellular localization and degradation of CRTC2, thereby inhibiting the gluconeogenic programme [37,38]. However, since CRTC2 is O-glycolysated at Ser[171]

in insulin-resistance states, making phosphorylation impossible [39], it is unlikely that metformin regulates gluconeogenesis through CTRC2 phosphorylation. A possible alternative mechanism for the inhibitory action of metformin on TORC2-mediated gluconeogenesis has been recently proposed (Figure 2), involving an increase of hepatic SIRT1 (sirtuin 1) activity, an NAD$^+$-dependent protein deacetylase, through AMPK-mediated induction of NAMPT (nicotinamide phosphoribosyltransferase), the rate-limiting enzyme for NAD$^+$ biosynthesis [40]. SIRT1 has been reported to deacetylate CRTC2, resulting in the loss of protection from COP1 (constitutive photomorphogenesis 1)-mediated ubiquitination and subsequent degradation [41]. This probably occurs in parallel with another mechanism for the action of metformin which involves the disassembly of the CREB–CBP (CREB binding protein)–CRTC2 complex at the PGC-1α and PEPCK promoters [42]. The regulation of gluconeogenic gene expression by metformin appears to be dependent on the phosphorylation of CBP at Ser[436], but not CRTC2, through AMPK-induced atypical PKC (protein kinase C) ι/λ activation [42]. In addition, a variety of transcription factors have been shown to participate in the metformin-induced inhibition of gluconeogenic genes in the liver (Figure 2). Kim et al. [43] demonstrated that metformin regulates hepatic gluconeogenesis through an AMPK-mediated up-regulation of the orphan nuclear receptor SHP (small heterodimer partner), which operates as a transcriptional repressor. SHP inhibits CREB-dependent hepatic gluconeogenic gene expression via direct interaction with CREB and competition with CRTC2 binding in the CREB–CBP complex [44]. Takashima et al. [45] have proposed a role for KLF15 (Krüppel-like factor 15) in the metformin-induced inhibition of genes coding for both gluconeogenic and amino-acid-catabolic enzymes, these later being potentially implicated in the regulation of gluconeogenesis through the control of gluconeogenic substrate availability. Metformin suppressed KLF15 gene expression and promoted its ubiquitination for proteasomal degradation. Restoration of KLF15 expression only partially rescued the inhibitory effect of metformin on hepatic glucose production, indicating that other factors also contribute to metformin action [45].

Understanding the mechanism of action of metformin is complicated further by our recent study establishing that both LKB1 and AMPK activities are dispensable for metformin-induced inhibition of glucose output or gluconeogenesis [46]. We reported that a reduction in hepatic energy status, but not AMPK activation, constitutes the critical factor underlying the effects of metformin on the regulation of hepatic glucose output [46]. As the rate of hepatic glucose production is closely linked to hepatic energy metabolism (six ATP equivalents are required per molecule of glucose synthesized), disruption of the main energy supply in hepatocytes (mitochondrial oxidative
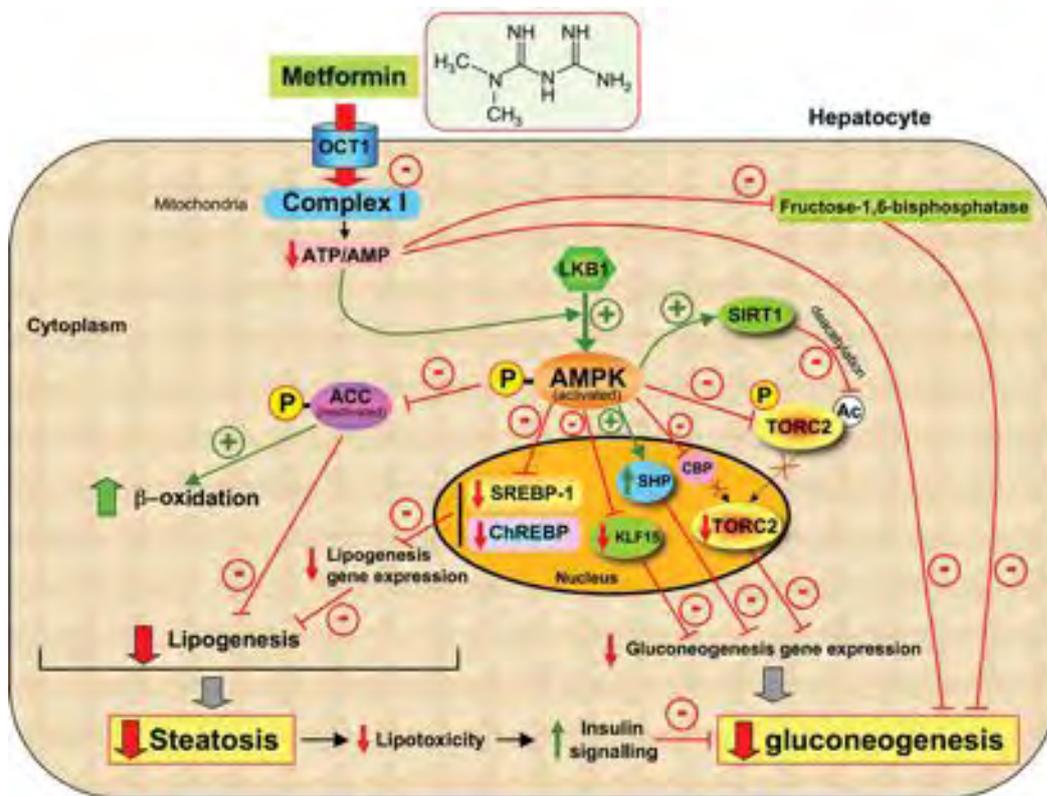
Figure 2   Potential molecular mechanisms of the action of metformin on hepatic gluconeogenesis

After hepatic uptake through OCT1, the mitochondria is the primary target of metformin, exerting specific and AMPK-independent inhibition of respiratory chain complex I. The resultant mild decrease in energy status leads to an acute and transient inhibition of the energy-consuming gluconeogenic pathway. In addition, through AMPK-dependent and -independent regulatory points, metformin can lead to the inhibition of glucose production by disrupting gluconeogenesis gene expression. In parallel, the LKB1-dependent activation of AMPK triggered by ATP depletion could reduce hepatic lipogenesis and exert an indirect effect on hepatic insulin sensitivity to control hepatic glucose output. Ac, acetylated.

phosphorylation) through inhibition of the respiratory chain complex I would have a profound effect on the flux through gluconeogenesis (Figure 2). In addition, as AMP tends to increase whenever ATP falls, this could also provide an alternative explanation for the acute inhibition of gluconeogenesis by metformin via allosteric regulation of key enzymes in this pathway, such as FBPase (fructose-1,6-bisphosphatase) [47]. Of particular note is the metformin-induced inhibition of glucose production independently of transcriptional repression of gluconeogenic genes. Interestingly, forced expression of key gluconeogenic genes through PGC-1α overexpression did not decrease the metformin-induced reduction in glucose output, but was again associated with a significant depletion of energy stores [46]. These results indicate that metformin could acutely suppress gluconeogenesis via a transcription-independent process and that changes in gene expression are therefore not the exclusive determinant in the regulation of glucose output (Figure 2). Interestingly, suppression of hepatic glucose production by metformin in insulin-resistant high-fat fed rats is dependent on an inhibition of the substrate flux

through G6Pase and not on a decrease in the amount of the protein [13], supporting the notion that the action of metformin is related to a reduction in the gluconeogenic flux, rather than a direct inhibition of gluconeogenic gene expression.

In addition to the suppression of endogenous glucose production, metformin has been shown to be beneficial in improving lipid metabolism. Evidence that metformin improved fatty liver disease by reversing hepatic steatosis in *ob/ob* mice [48,49] and in rodents fed on a high-fat diet [50] has been demonstrated, and has also been reported in some clinical studies [51,52]. Metformin-induced reduction in hepatic lipid content is consistent with an increase in both fatty acid oxidation and inhibition of lipogenesis, presumably mediated by AMPK activation [17,48,53]. Indeed, AMPK co-ordinates the changes in the hepatic lipid metabolism and thus regulates the partitioning of fatty acids between oxidative and biosynthetic pathways [18]. Thus AMPK activation by metformin induces the phosphorylation and inactivation of ACC (acetyl-CoA carboxylase), an important rate-controlling enzyme for the synthesis of malonyl-CoA,

which is both a critical precursor for the biosynthesis of fatty acids and a potent inhibitor of mitochondrial fatty acid oxidation [17]. In human hepatoma HepG2 cells, metformin enhances ACC phosphorylation and induces the reduction of triacylglycerol (triglyceride) levels, which can be supported by increased fatty acid oxidation and/or decreased fatty acid synthesis [53]. In addition, AMPK suppresses the expression of lipogenic genes such as FAS (fatty acid synthase), S14 (spot 14) and ACC by direct phosphorylation of transcription factors [ChREBP (carbohydrate-response-element-binding protein) and HNF4 (hepatocyte nuclear factor 4)] and co-activators (p300) [54–59]. Metformin participates in the regulation of lipogenesis gene expression by down-regulating SREBP (sterol-regulatory-element-binding protein)-1c gene expression [17] and by inhibiting its proteolytic processing and transcriptional activity upon AMPK-mediated phosphorylation at Ser^{372} [60]. A recent study by Kim et al. [61] reported that metformin induces AMPK-mediated TR4 (thyroid hormone receptor 4) phosphorylation and repression of SCD1 (stearoyl-CoA desaturase 1) expression, a rate-limiting enzyme involved in the biosynthesis of mono-unsaturated fatty acids from saturated fatty acids.

Fatty liver disease is strongly associated with insulin resistance, and the apparent inhibition of hepatic glucose production by metformin may be secondary to the primary improvement of hepatic steatosis and insulin resistance. This hypothesis may also offer a potential explanation for the loss of an effect of metformin on blood glucose levels in liver-specific LKB1-knockout mice fed on a high-fat diet [37]. Thus impaired metformin-induced AMPK phosphorylation could fail to reduce hepatic lipid levels and to improve insulin sensitivity in liver-specific LKB1-deficient mice, impeding normalization of blood glucose levels.

ACTION OF METFORMIN ON DIABETIC NEPHROPATHY

Despite a large panel of antidiabetic agents, it is assumed that between 20 and 40 % of patients with diabetes ultimately develop diabetic nephropathy. Diabetic nephropathy is a microvascular complication of diabetes, others including diabetic retinopathy and diabetic neuropathy. Because metformin is excreted by the kidney, the reduction in renal clearance of metformin is considered as an important risk factor for lactic acidosis. As a consequence, it is recommended that the dose of metformin is reviewed if the serum creatinine exceeds 130 μmol/l (or estimated glomerular filtration rate is <45 ml/min per 1.73 m²) and to stop metformin treatment if the creatinine level increases above 150 μmol/l (or estimated glomerular filtration rate is below 30 ml/min per 1.73 m²) [1].

However, some recent findings have accumulated suggesting that metformin could favour protection against the deleterious consequences of hyperglycaemia in the kidney. Some of these findings come from rodent models of diabetes, such as Zucker diabetic fatty rats. In this model, Takiyama et al. [62] demonstrated that metformin treatment (250 mg/kg of body weight per day) for 9–39 weeks ameliorated tubular injury associated with hyperglycaemia, whereas no protective effect was observed with insulin. The authors showed that metformin specifically reduced HIF-1 (hypoxia-inducible factor-1) expression (and its specific target genes) not only by reducing ATP synthesis, but also by a fall in oxygen consumption in renal cells. Interestingly, the beneficial effect of metformin is preserved after knockdown of the α-subunit of AMPK and can not be reproduced by AICAR (5-amino-4-imidazolecarboxamide riboside; an AMPK activator) or rapamycin {an mTORC1 [mTOR (mammalian target of rapamycin) complex 1] inhibitor} [62]. This suggested that, in this case, metformin acts independently of AMPK by decreasing renal oxygen consumption. Because chronic hypoxia and the consequent increase in HIF-1 expression are now considered as key events during the initiation and progression of diabetic nephropathy and kidney fibrosis, the management of renal chronic hypoxia becomes a new therapeutic strategy for the prevention of diabetic nephropathy. It has been also demonstrated that metformin prevents gentamicin-induced acute renal failure, presumably by decreasing ROS-mediated lipid peroxidation [63], and decreases the TGFβ (transforming growth factor β)-induced EMT (epithelial–mesenchymal transition), a key event in the development of the tubulointerstitial fibrosis during diabetic nephropathy [64].

It is now well accepted that hyperglycaemia increases ROS production in diabetic podocytes, contributing to the development of diabetic nephropathy. Until now, all of the strategies used to decrease ROS production in the diabetic kidney have failed. Interestingly, Piwkowska et al. [65] showed that metformin activates AMPK and decreases the NADPH oxidase activity, ultimately leading to a decrease in ROS production in cultured podocytes. As a consequence, the control of ROS production by metformin could be a new optimal strategy for the management of diabetic nephropathy. The deleterious role of lipotoxicity in kidney has been recognized in Goto–Kakizaki [66] and OLETF rats [67], two rodent models of T2D. In the latter, diabetic nephropathy was correlated with kidney triacylglyerol content, and metformin reduced fat content by decreasing SREBP-1, FAS and ACC expression in the kidney [67]. The reduction in renal lipotoxicity by metformin could thus be a new strategy for the prevention of diabetic nephropathy. Another intriguing effect of metformin is a reduction in cystic growth in the dominant polycystic

kidney disease mouse model [68]. This beneficial effect has been explained by AMPK activation by metformin and subsequent inhibition of both the CFTR (cystic fibrosis transmembrane conductance regulator) and mTOR pathways, demonstrating that the drug can modulate multiple molecular pathways in the kidney.

The reduction in kidney damage in animal models of T2D has to be confirmed in clinical studies. Taken together, a careful revision of clinical recommendations, especially the contra-indications of metformin use, may be required, in agreement with recent literature reviews suggesting that metformin treatment could probably be extended to all diabetic patients [69,70]. Thus, because the risk of lactic acidosis is extremely low and the use of metformin results in cardiovascular, renal and survival benefits, the use of metformin should be revised accordingly to a definitive glomerular filtration rate cut-off or adaptation of the dose of metformin by determination of its plasma levels [71].

ACTION OF METFORMIN ON THE CARDIOVASCULAR SYSTEM

IHD (ischaemic heart disease) remains the leading cause of death in the patients with T2D [72]. Importantly, the UKPDS longitudinal trial demonstrated that metformin reduced diabetes-related death by 42 % (95 % confidence interval, 9–63 %; $P =$ 0.017) and all-cause mortality by 36 % (95 % confidence interval, 9–55 %; $P =$ 0.011). Interestingly, in that study, metformin was used as a primary prevention and its beneficial effect was rapidly observed after a median duration of the study of 10.7 years [3]. This conclusion has been replicated in other clinical or epidemiological studies [73,74]. Thus the use of metformin as the first-line antidiabetic treatment in T2D patients was not only justified by the antihyperglycaemic effect of the drug, but also by the reduction in the mortality rate in this population. The mechanisms of such a beneficial effect are not clearly understood, but results accumulated concerning some of the potential mechanisms of action of metformin in the heart include the promotion of myocardial preconditioning, the reduction in cardiomyocyte apoptosis during ischaemia, the adaptation of cardiomyocyte metabolism during ischaemia or the protection against the development of heart failure.

Experimental evidence suggests that metformin reduces cardiac ischaemia/reperfusion injury. Indeed, Yin et al. [75] have shown that metformin treatment improves cardiac function (preserved left ventricular ejection fraction) and reduces the infarct size after a myocardial infarction in Sprague–Dawley rats. By contrast with sham-operated rats, the metformin-treated group were less insulin-resistant and had altered myocardial AMPK phosphorylation status during cardiac remodelling.

Myocardial preconditionning is now recognized as a protective mechanism that induces a reduction in infarct size and the consequent risk of heart failure. Induction of such a mechanism has been demonstrated in a rat model of neonatal streptozotocin-induced T2D treated without or with metformin for 3 days before myocardial ischaemia/reperfusion injury [76]. In that study, metformin-induced preconditionning was supported by a reduction in infarct size in the treated group.

Cardioplegic-induced hypoxia/reoxygenation injury results in cardiomyocyte apoptosis. In cardiomyocytes, metformin attenuated the production of pro-apoptotic proteins, increased the anti-apoptotic proteins and reduced the percentage of apoptotic cardiomyocytes [77]. This effect was correlated with the activation of AMPK and was reproduced by AICAR, another AMPK activator. Another property of metformin (which seemed fundamental to reduce the risk of heart failure) is the adaptation of cardiac metabolism during myocardial ischaemic conditions. The healthy heart obtains 60–90 % of its energy for oxidative phosphorylation from fatty acid oxidation [78], whereas a failing heart has been demonstrated to shift the balance towards an increase in glucose uptake and utilization [79]. Because utilization of fatty acid utilizes more oxygen per unit of ATP generated than glucose, the promotion of a metabolic shift from fatty acid oxidation to glucose utilization may improve ventricular performance and slow the progression of heart dysfunction [78,79]. Thus, in a volume-overload model of heart failure in rats (aortocaval fistula), Benes et al. [80] demonstrated that metformin normalized serum NEFAs (non-esterified fatty acids) and modified the cardiac lipid/glucose oxidation ratio, suggesting a metabolic adaptation induced by the drug.

Another new area of intense research is the possibility of using metformin in patients with a history of heart failure. Metformin is classically contra-indicated in patients with heart failure because this condition increases the risk of lactic acidosis. Surprisingly, recent evidence suggests that this contra-indication could be revised [81]. Indeed, metformin alone or in combination with a sulfonylurea reduced the mortality and morbidity of T2D patients with heart failure in comparison with sulfonylurea monotherapy [82–84]. This unexpected result has been found in the REACH (Reduction of Atherothrombosis for Continued Health) Registry which included 19691 T2D patients with established atherothrombosis [85]. The mortality rate was significantly lower in patients treated with metformin, including those in whom metformin use was not now recommended (i.e. a history of congestive heart failure, patients older than 65 years and patients with an estimated creatinine clearance of 30–60 ml/min per 1.73 m^2). The cardiovascular protection in metformin-treated T2D patients appears not to be dependent on a reduction

in HbA$_{1c}$ (glycated haemoglobin) levels, since it was not observed with other oral antidiabetic drugs [86], suggesting that metformin has specific properties on cardiovascular outcomes. Even if further studies are needed to better understand this specific point at the molecular level, some original mechanisms have already been proposed. Thus dysregulated autophagy has been described as a key mechanism for the development of diabetic cardiomyopathy and heart failure. Interestingly, treatment with metformin restored impaired autophagy in OVE26 diabetic mice and prevented heart damage in this model [87]. This effect is probably dependent on cardiac AMPK activation, since metformin is inefficient in cardiac-specific AMPK-dominant-negative transgenic diabetic mice. In addition, Gundewar et al. [88] have demonstrated that metformin significantly improves left ventricular function and survival in a murine model of heart failure (myocardial ischaemia induced by left coronary artery occlusion). The authors showed that metformin significantly improved myocardial cell mitochondrial respiration and ATP synthesis by an underlying mechanism requiring the activation of AMPK and its downstream mediators eNOS (endothelial NOS) and PGC-1α [88]. Similar prevention of both heart failure and mortality by metformin was observed in a dog model of heart failure [89]. In this case, other AMPK activators, such as AICAR, have equivalent effects compared with those of metformin, suggesting that myocardial AMPK activation is also required. A large proportion of T2D patients have chronic high blood pressure, which is known to induce cardiac hypertrophy and fibrosis. Metformin inhibits cardiac hypertrophy in a rat model of pressure overload (transaortic constriction) via a reduction in AngII (angiotensin II)-induced protein synthesis and enhanced phosphorylation of AMPK and eNOS, leading to a subsequent increase in NO production. All of these effects were abolished by compound C, a non-specific AMPK inhibitor [90].

Beyond its specific effects in the heart, the reduction in mortality by metformin also invited the question of how endothelial dysfunction and atherogenesis could be prevented by the drug. Endothelial dysfunction, as characterized by an impairment in endothelium-dependent relaxation and reduced NO bioactivity, is a critical step for atherogenesis. In addition, vascular NO inhibits platelet aggregation and adhesion and can also reduce leucocyte adhesion to the vessel wall (see [91] for a review). Schulz et al. [92] have demonstrated that AMPK phosphorylates and activates eNOS in cultured endothelial cells, stimulates NO synthesis in response to several agonists and increases endothelium-dependent vasodilation in animal models. Taken together, such findings have suggested an anti-atherogenic role for the AMPK system. High glucose leads to endothelial ROS overproduction, which promotes endothelial dysfunction. Metformin decreased

intracellular ROS production in aortic endothelial cells by inhibiting both NADPH oxidase and the respiratory chain complex I [93]. Furthermore, activated AMPK reduces hyperglycaemia-induced mitochondrial ROS production by the induction of MnSOD (manganese superoxide dismutase) and the promotion of mitochondrial biogenesis through the activation of the PGC-1α pathway in HUVECs (human umbilical vein endothelial cells) [94]. Lastly, activated AMPK largely offsets the adverse effects of palmitate on endothelial superoxide production and NF-κB (nuclear factor κB) activation. Recently, two additional vascular targets of metformin have been described: AGEs (advanced glycation endproducts), and cell-adhesion molecules [soluble ICAMs (intercellular adhesion molecules) and the soluble VCAMs (vascular cell-adhesion molecules)]. AGEs are important contributors of diabetic complications by promoting cellular oxidative stress and inflammation. It has been reported that metformin can reduce the synthesis of AGEs and their specific cell receptor expression independently of its antihyperglycaemic effects [95]. Although done *in vitro*, this suggests that metformin can directly modulate the glycation process. In addition, excessive plasma levels of ICAM-1 and VCAM-1 are linked with an increase in cardiovascular events. Interestingly, as for AGE, metformin decreases ICAM-1 and VCAM-1 levels in T2D patients independently of its normoglycaemic property [96]. These studies support the notion that activated AMPK has a beneficial effect on endothelial function by suppressing oxidative stress in endothelial cells [97]. Taken together, these findings suggest that metformin has complex properties on endothelial function, ROS production and cardiomyocyte functionality.

ACTION OF METFORMIN ON POLYCYSTIC OVARY SYNDROME

PCOS (polycystic ovary syndrome) is a common endocrinopathy, affecting at least 5–15 % of reproductive-aged women [98]. The revised diagnostic criteria of PCOS associated menstrual disturbance and/or hyperandrogenism and/or polycystic ovary on ultrasound [99]. It is now recognized that insulin resistance is a common but not an imperative feature in PCOS. As a consequence, insulin sensitizers have been proposed as a pharmaceutical option in overweight women with PCOS and insulin resistance. Recently, a meta-analysis of 31 clinical trials demonstrated that metformin treatment may increase ovulation, improve menstrual cyclicity and reduce serum androgen levels in these patients [98]. These beneficial effects of metformin are based on the alleviation of excess insulin acting upon the ovary and through direct ovarian effects. Insulin was shown to directly stimulate several steroidogenic enzymes in the ovary, such as CYP

(cytochrome P450) 17, 3β-HSD (3β-hydroxysteroid dehydrogenase) and the StAR (steroidogenic acute regulatory protein) protein. By improving insulin sensitivity, metformin reduces CYP17 activity [100]. Furthermore, metformin suppresses androstenedione production by a direct effect on ovarian theca cells and decreases FSH (follicle-stimulating hormone)-stimulated 3β-HSD, StAR, CYP11A1 and aromatase activities in both rat granulosa cells and women with PCOS (with a reduction in basal and FSH-stimulated progesterone and oestradiol levels as a consequence) [100]. The molecular pathways whereby metformin acts directly on the ovary remain elusive. It has been demonstrated that metformin treatment increased AMPK activity in rat granulosa cells, leading to subsequent reduction of steroid synthesis [101]. However, it is still unclear whether this effect is AMPK-dependent or not. Pharmacogenetic aspects of the action of metformin have to be taken into account in the effect of the drug on PCOS. Indeed, results from the PP-COS (Pregnancy in PCOS) trial indicated that a polymorphism in the LKB1 gene is associated with a significant decreased chance of ovulation in PCOS patients treated with metformin [102]. Interestingly, metformin has been shown to reduce the risks of abortion in women with PCOS at high risk of pregnancy and neonatal complications by increasing some factors needed for implantation and pregnancy safekeeping, such as IGFBP-1 [IGF (insulin-growth factor)-binding protein-1] and glycodelin levels, or uterine artery blood flow [100]. By contrast, metformin reduces factors increasing a risk of abortion, such as endometrial androgen receptor expression, PAI-1 (plasminogen-activator inhibitor-1) levels and plasma ET-1 (endothelin-1). Most of these effects are probably mediated by the metformin-induced improvement in insulin sensitivity. From a clinical point of view, metformin administration should be considered as an initial intervention in (overweight or obese) PCOS patients, especially when oral contraception is contra-indicated or when insulin resistance is present.

ACTION OF METFORMIN ON CANCER

Recent prospective and case-control studies conducted on large cohorts have confirmed that T2D is associated with a significantly increased risk of cancer mainly affecting the breast, colon, prostate, kidney and pancreas [103]. This increased risk has been attributed to the growth-promoting effect of chronic elevated plasma insulin levels [104]. Insulin resistance and the resultant hyperinsulinaemia might indeed promote carcinogenesis directly through the insulin receptor or indirectly by increasing the levels of IGFs, steroid sex hormones, inflammatory processes and disrupting adipokines homoeostasis [104]. However, additional explanations for this association may be proposed, such as the role of

persistent elevated plasma glucose levels [105]. Given the epidemiological evidence between T2D and an increased risk of cancer, the impact of metformin therapy on cancer risk and cancer-related mortality has been evaluated in the first pilot case-control study with a cohort of 12 000 T2D patients [106]. Metformin therapy was associated with a reduced risk of cancer (odds ratio of any exposure to metformin was 0.79). Furthermore, the authors found a dose–response relationship between the duration of exposure to metformin and cancer incidence [106]. Similarly, more recent retrospective and observational studies have reported a reduced incidence of neoplastic diseases and cancer mortality in T2D patients treated with metformin [107–109]. Importantly, metformin use has been associated with a significant decrease in the relative risk of specific cancers, such as prostate, pancreas and breast cancers [110–112]. These observations are consistent with *in vitro* and *in vivo* studies showing the antiproliferative action of metformin on various cancer cell lines [113] and several cancers in animal models (Table 1).

Although the underlying mechanisms are not yet completely elucidated, the association between metformin and a reduced risk of cancer in T2D patients may simply be explained through the action of metformin on the improvement in blood glucose and insulin levels [114]. Accordingly, prevention of tumour growth in animal models with diet-induced hyperinsulinaemia is attributable to reductions in circulating insulin levels [115,116]. Given that hyperinsulinaemia is associated with increased levels of IGF-1, it is possible that the metformin-lowering effects on serum insulin and IGF-1 levels might explain, at least in part, its therapeutic efficacy (Figure 3). This hypothesis is particularly relevant in light of recent studies showing that calorie restriction, which lowers insulin and IGF-1 levels, induces a dramatic decrease in the incidence of cancer in rodent models [117]. However, a decrease in insulinaemia is not always correlated with metformin efficacy as shown in PTEN (phosphatase and tensin homologue deleted on chromosome 10)$^{+/-}$, HER-2/neu and APC (adenomatous polyposis coli)$^{min/+}$ mouse tumour models, indicating an insulin-independent antitumoral action of metformin [118–120]. Hence metformin appears to have a direct action on tumour growth both *in vitro* and *in vivo* by a mechanism involving activation of the LKB1/AMPK pathway and subsequent modulation of downstream pathways controlling cellular proliferation (Figure 3). AMPK knockdown by siRNA (small interfering RNA) or AMPK inhibitors partially revert the antiproliferative action of metformin in breast and ovarian cancer cells [121–123]. Furthermore, the antitumoral action of metformin was significantly reduced in mice displaying a decrease in LKB1 expression [119]. The antineoplastic activity of metformin via AMPK activation is mediated through the inhibition of

Table 1   Protective effect of metformin in animal models of cancer

IP, intraperitoneal. This Table was reproduced from B. Viollet et M. Foretz : Metformine et cancer. Du diabète au cancer : de nouvelles perspectives thérapeutiques pour la metformine. *Médecine des maladies Métaboliques*, vol. 5, no. 1, février 2011, pp. 29–37. © 2011 Elsevier Masson SAS. Tous droits réservés.

Cancer model	Tumour type	Treatment	Route	Reference
Xenograft	Pancreas	250 mg/kg of body weight	IP	[127]
	Lung	50 mg/kg of body weight	Oral	[158]
	Colon	250 mg/kg	IP	[142]
	Prostate	200 μg/ml; 1 mg/day	Oral; IP	[134]
	Breast	2 mg/ml	Oral	[138]
	Leukaemia	250 mg/kg of body weight	IP	[124]
	Cancer stem cells	100 μg/ml	IP	[159]
Chemically induced	Breast	50–500 mg/kg of body weight	Oral	[160]
	Lung	250 mg/kg of body weight	IP	[161]
	Colon	250 mg/kg of body weight	IP	[162]
	Pancreas	320 mg/kg of body weight	Oral	[115]
HER2/neu mice	Breast	100 mg/kg of body weight	Oral	[118]
PTEN$^{+/-}$ mice	All	300 mg/kg of body weight	Oral	[119]
APC$^{Min/+}$ mice	Gastrointestinal polyps	250 mg/kg of body weight	Oral	[120]

mTORC1 signalling, leading to inhibition of protein synthesis and cell proliferation [121,123,124]. AMPK inhibits mTORC1 at multiple levels through the phosphorylation of TSC2 (tuberous sclerosis 2) on Ser^{1345}, leading to the accumulation of Rheb-GDP (the inactive form), and the phosphorylation of raptor (regulatory associated protein of mTOR) on Ser^{722} and Ser^{792}, which disrupts its association with mTOR and thereby prevents mTORC1 activation. However, recent studies have revealed the existence of an alternative AMPK-independent pathway, potentially mediated by RAG GTPase, by which metformin inhibits mTORC1 signalling [125]. Of particular note is the inhibition of IGF-1-induced mTOR activity by thiazolidinediones, another class of antidiabetic drugs which activate AMPK [18], indicating that activation of the kinase could further attenuate signalling pathways downstream insulin and/or IGF-1 receptors, particularly at the level of mTOR [126]. Furthermore, it is of interest that the metformin-induced activation of AMPK disrupted cross-talk between insulin/IGF-1 and GPCR (G-protein-coupled receptor) signalling pathways in pancreatic cancer cells [127]. Another mode of action of metformin might be through an AMPK-mediated regulation of fatty acid synthesis. Indeed, cells derived from prostate, breast and colon cancers constitutively overexpress FAS, a key enzyme for *de novo* fatty acid biosynthesis, which has been associated with a malignant phenotype. Interestingly, it has been observed that a reduction in FAS and ACC expression by AMPK activation diminishes the viability and growth of prostate cancer cells [128]. Another potential mechanism is based on the positive impact of metformin on chronic inflammation [129], a major contributory factor to cancer development and progression. Emerging

results showing the capacity of AMPK to inhibit the inflammatory responses [130] suggest that metformin may also target the inflammatory component present in the microenvironment of most neoplastic tissues, leading to tumour reduction. In addition, inhibition of neoplastic angiogenesis by metformin might also participate in the reduction of tumour growth [131]. Consistently, metformin has been shown to significantly decrease the levels of VEGF (vascular endothelial growth factor) and PAI-1 [132].

Although these results suggest a pivotal role of LKB1/ AMPK signalling, the antineoplastic action of metformin could also be independent of AMPK activation. Indeed, metformin was reported to decrease the expression of the oncoprotein HER2 (*erbB-2*) in human breast cancer cells via a direct and AMPK-independent inhibition of p70^{S6K1} (p70 S6 kinase 1) activity [133]. Metformin also exerts its anticancer effect through induction of cell-cycle arrest in prostate cancer cell lines via a decrease in cyclin D1 protein expression [134] and an increase in REDD1 (regulated in development and DNA damage response 1) expression in a p53-dependent manner [135]. In breast cancer cells, metformin-induced cell-cycle arrest was due to enhanced binding of CDK2 (cyclin-dependent kinase 2) by p27^{Kip} or p21^{Cip} in addition to cyclin D1 down-regulation and AMPK activation [136]. In addition to the inhibition of cancer cell proliferation, metformin has been shown to promote cell death of some cancer cells through the activation of apoptotic pathways by both caspase-dependent and caspase-independent mechanisms [137,138]. The caspase-independent pathway involved the activation of PARP [poly(ADP-ribose) polymerase], and correlates with enhanced synthesis of PARP and nuclear translocation of AIF (apoptosis-inducing factor),
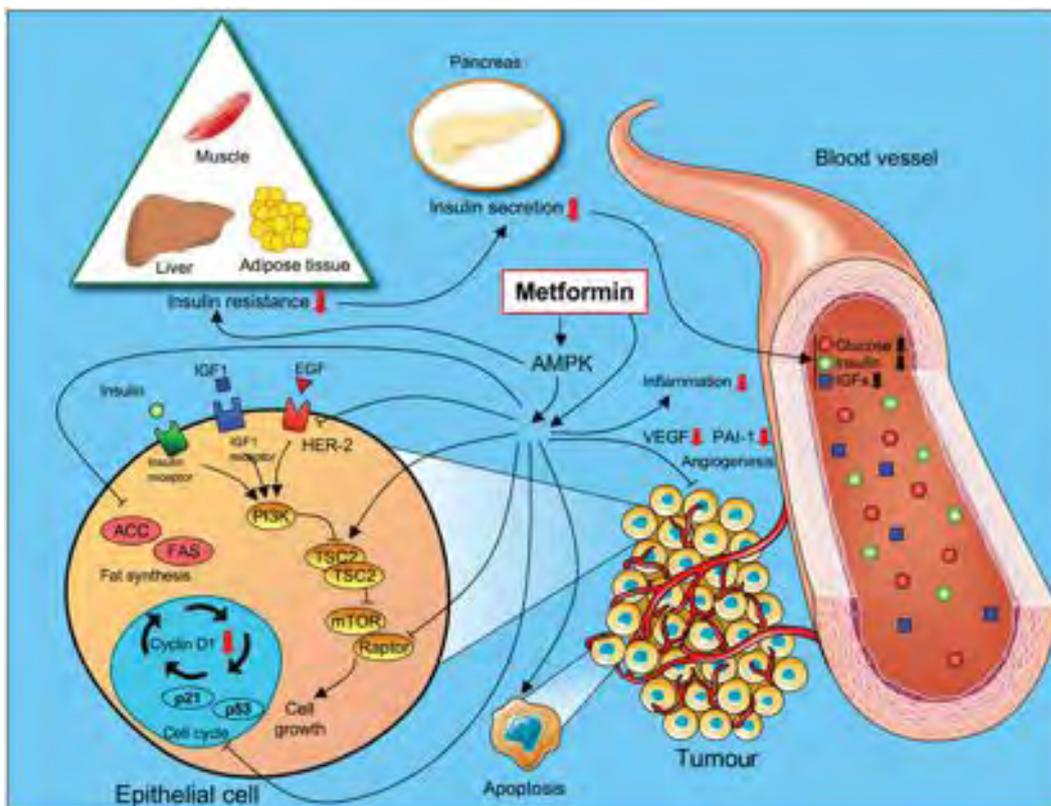
Figure 3   Control of cell proliferation and tumour growth by metformin

The anti-neoplastic action of metformin appears to be exerted by several pathways. Metformin inhibits the growth of cancer cells by the reversal of hyperglycaemia, insulin resistance and hyperinsulinaemia, resulting in reduced levels of glucose, insulin and IGFs and the activation of growth signalling pathways through their respective receptors. The antitumour effects of metformin appear to be regulated by both AMPK-dependent or -independent mechanisms, leading to inhibition of mTOR signalling, cell cycle by a decrease in cyclin D1 level, stimulation of p53/p21 axis, fatty acid synthesis, angiogenesis and inflammation. EGF, epidermal growth factor; PI3K, phosphoinositide 3-kinase; This Figure was reproduced with permission from B. Viollet et M. Foretz : Metformine et cancer. Du diabète au cancer : de nouvelles perspectives thérapeutiques pour la metformine. *Médecine des maladies Métaboliques* vol. 5, no. 1, février 2011, pp. 29–37. © 2011 Elsevier Masson SAS. Tous droits réservés.

which plays an important role in mediating cell death [139]. Additionally, it has been shown that the metformin-stimulated apoptosis of colon cancer cells was associated with a loss of p53-dependent enhancement of autophagy and glycolysis, an effect stimulated by nutrient deprivation. In contrast, metformin promotes apoptosis of prostate cancer cells in a p53-dependent manner in the presence of 2-deoxyglucose [140].

Metabolic adaptations are critical in maintaining the survival of cancer cells that are often under a variety of stress stimuli, such as hypoxia and lack of nutrients. To successfully meet their high metabolic demand, it is crucial that cancer cells engage proper adaptive responses to provide sufficient ATP supply and support survival. A study has revealed that AMPK activation promotes the survival of cells metabolically impaired by glucose limitation in part through p53 activation [141]. It has been suggested that metformin could inhibit the growth of cancer cells by decreasing the cellular energy status and force a metabolic conversion that cancer cells are unable to execute. Indeed, loss of p53 impairs the ability of cancer cells to respond to metabolic changes induced by metformin and to survive under conditions of nutrient deprivation [142]. Similarly, LKB1-deficient cells were more sensitive to metformin-induced energy stress when cultured at low glucose concentrations and were unable to compensate for the decreased cellular ATP concentration causing cell death [116]. A recent study has demonstrated that the combination of metformin and 2-deoxyglucose inhibited mitochondrial respiration and glycolysis in prostate cancer cells, leading to a massive ATP depletion and affected cell viability by inducing apoptosis [140].

NEW THERAPEUTIC PERSPECTIVES

Gestational diabetes

The risk of GD (gestational diabetes) is increasing in obese women and is associated with adverse pregnancy

outcomes [143]. Results accumulated from case-control studies [144] or the Metformin in Gestational Diabetes Trial [145] suggested that women treated with metformin had less weight gain and improved neonatal outcomes compared with those treated with insulin. Although no significant adverse events were observed when metformin was administered during pregnancy, its use in overweight women with GD has to be confirmed by additional studies and new guidelines.

Diabetes prevention

The DPP (Diabetes Prevention Program) was a clinical trial comparing the efficacy of lifestyle modifications and metformin on glucose homoeostasis in 3234 pre-diabetic patients [146]. In this study, metformin was efficient in significantly reducing (-31%) the development of T2D. Even if a reduction in body weight through physical activity and hypocaloric diet use was unanimously recognized as a cornerstone for a global prevention of T2D, the use of metformin in pre-diabetic population looks promising, but has to be evaluated in additional studies.

Regulation of the circadian clock

Mammalian behaviour, including spontaneous loco-motion, sleeping, eating and drinking, is influenced by a circadian system composed of a central clock in the brain and subsidiary oscillators existing in peripheral tissues. Circadian rhythms are regulated by the alternating actions of activators and repressors of transcription, in particular CLOCK (circadian locomotor output cycles kaput), BMAL1 [brain and muscle ARNT (aryl hydrocarbon receptor nuclear translocator)-like 1], PER (Period) and CRY (Cryptochrome) [147]. Um et al. [148] have proposed a molecular mechanism by which metformin causes a dramatic shift in the circadian phase of peripheral tissues. It was indeed shown that metformin-induced AMPK activation promoted the phosphorylation of Ser^{386} of CK1 (casein kinase 1), one of the key circadian regulators, thereby enhancing the CK1-mediated phosphorylation of PER2, leading to its degradation and ultimately to the shortening of the period length. Accordingly, PER2 accumulates to higher levels in organs of mice lacking the catalytic α2-subunit of AMPK [148]. Recent evidence indicates that dysregulation of circadian functions could underlie, at least in part, the development of obesity and insulin resistance. Caton et al. [40] recently examined the effect of metformin on the dysregulation of clock genes in adipose tissue of obese db/db mice and in mice fed on a high-fat diet. Interestingly, metformin markedly enhanced expression of the core clock components CLOCK, BMAL1 and PER2 through induction of AMPK/NAMPT/SIRT1 signalling and was associated with a reduction in hyperglycaemia and hyperinsulinaemia in db/db mice. Taken together, the apparent beneficial association between the targeted modulation of the circadian system and the whole-body metabolic state suggests that chronotherapy could be a promising approach for the treatment of obesity and T2D.

Metformin and pharmacogenetics

Metformin is a hydrophilic base which exists at physiological pH as an organic cation ($pK_a = 12.4$). Consequently, its passive diffusion through cell membranes is very limited. Indeed, it has been shown that metformin can only negligibly permeate the plasma membrane by passive diffusion [29] and cationic transporters such OCT1/OCT2 are, to date, the main transporters identified to be involved in the intracellular internalization of the drug [15,149]. It is worth noting that most of the experiments using metformin were performed in immortalized cell lines treated with drug concentrations far above those reported to accumulate in tissues after oral administration of metformin [149]. The effect may be related to the low levels of cationic transporters at the surface of these cell lines. Interestingly, uptake of metformin into immortalized cell lines is very low and can be greatly enhanced by ectopic expression of organic ion transporter cDNAs [150,151]. Thus the key determinant for the action of metformin appears to be a balance between the concentration and time of exposure, which can in fact reflect the tissue/cell-specific abundance of organic transporters

Understanding the link between genetic variation and the response to drugs will be essential to move towards personalized medicine. Metformin requires the OCTs to be transported into the liver, the gut (OCT1) and the kidney (OCT1 and OCT2) [152]. In contrast, MATE1 (multi-drug and toxin extrusion 1) facilitates the excretion of metformin into bile and urine [152]. OCT1 and OCT2 are encoded by the *SLC22A1* and *SLC22A2* genes respectively, and MATE1 by the *SLC47A1* (solute carrier family 47 member A1) gene. In OCT1$^{-/-}$ mice, the hepatic metformin concentration in the liver and in the gut is lower than in control mice, suggesting that OCT1 is essential for the uptake of the drug in these tissues [15]. Recently, Shu et al. [15] showed that *SLC22A1* variants reducing OCT1 function increased the AUC (area under the curve) of glucose during an OGTT (oral glucose tolerance test) after metformin treatment in healthy volunteers compared with subjects with wild-type alleles. In contrast, the loss-of-function variants R61C and 420del have no consequence on the HbA$_{1c}$ level achieved during metformin treatment in T2D patients [153]. Urinary excretion of metformin is preserved in OCT1$^{-/-}$ mice, indicating that renal excretion of metformin is dependent on OCT2. This last point has been challenged by Tzvetkov et al. [154], who have demonstrated a significant OCT1 expression in the human kidney and a reduction in metformin renal clearance being dependent on OCT1 polymorphisms

(R61C, G401S, 420del or G465R). OCT2 polymorphisms are also known to modify metformin renal clearance. Indeed, 14 genetic variants of the *SLC22A2* gene were identified in which the 808G>T polymorphism was associated with a reduced tubular clearance of metformin and prevented the tubular secretion of metformin by cimetidine [155]. Other important OCT2 variants for the renal elimination of metformin have been described in healthy volunteers (see [156] for a review). Nevertheless, the clinical relevance of such variants in T2D patients remains to be determined in a large-scale studies. In addition, other candidate genes may be involved in the therapeutic response to metformin in diabetic populations. Thus, in a genome-wide association study investigating the glycaemic response to metformin, Zhou et al. [157] have found a locus associated with treatment success and containing the ATM (ataxia telangiectasia mutated) gene, a gene involved in DNA repair and cell-cycle control. Interestingly, it has been found that inhibition of ATM markedly reduced AMPK activation by metformin, indicating that ATM acts upstream of AMPK and is probably required for mediating the effect of metformin [157].

CONCLUSIONS

Metformin is currently used as an antihyperglycaemic agent. It is accepted that the main effect of this drug is to decrease hepatic glucose production through a mild inhibition of the mitochondrial respiratory chain complex I. As a consequence, the resulting transient decrease in cellular energy status promotes the activation of AMPK, a well-known cellular energetic sensor. Consequently, metformin-induced AMPK activation is believed to promote the transcriptional inhibition of the hepatic gluconeogenic programme. Nevertheless, our recent findings showing that inhibition of hepatic glucose production by metformin is preserved in liver-specific AMPK-knockout mice strongly suggests that other mechanism(s) are involved. Thus the decrease in hepatic energy status following inhibition of the respiratory chain complex I by metformin is probably the central explanation for the acute reduction in hepatic gluconeogenesis by the drug. Additionally, AMPK-dependent mechanisms linked to the action of metformin on hepatic lipid metabolism are also proposed, notably for explaining its beneficial effect on hepatic steatosis and insulin resistance, leading to the normalization of blood glucose levels. Interestingly, metformin had protective properties against diabetic complications, especially by reducing the diabetes-related death rate. Although still unclear, multiple molecular mechanisms have been proposed, including modulation of myocardial preconditioning, a reduction in cardiomyocyte apoptosis during ischaemia, a metabolic switch during ischaemia, protection against

the development of heart failure or protection of endothelial function. Most of these require AMPK activation, whereas others (such as the antioxidative properties of metformin on endothelial cells) appear to be kinase-independent. Metformin appears to have a dual impact on ovarian function in PCOS by decreasing insulin resistance and by direct ovarian effects. Finally, the risk of lactic acidosis is relatively low in comparison with the multiple benefits of metformin treatment, explaining the exponential development of its therapeutic use. New clinical indications of metformin are awaiting large-scale clinical studies before they can be recommended. Among these, the use of metformin in cancer therapy and for large-scale prevention in pre-diabetic populations look the most promising ones. Finally, new findings from phamacogenetics studies will also provide results to predict or adapt the dose of metformin in personalized medicine.

FUNDING

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