Endocrine pharmacology

Berberine as a promising anti-diabetic nephropathy drug: An analysis of its effects and mechanisms

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ABSTRACT

Diabetic nephropathy is a progressive kidney disorder and is pathologically characterized by thickened glomerular and tubular basement membranes, accumulation of the extracellular matrix and increased mesangial hypertrophy. Growing evidence has suggested that diabetic nephropathy is induced by multiple factors, such as dyslipidemia, hyperglycemia, hemodynamic abnormalities and oxidative stress, based on genetic susceptibility. Berberine (BBR; \([\text{C}_{20}\text{H}_{18}\text{NO}_{4}]^{+}\)), an isoquinoline alkaloid, is the major active constituent of \textit{Rhizoma coptidis} and \textit{Cortex phellodendri}. Recent studies have demonstrated that berberine has various pharmacological activities, including lowering blood glucose, regulating blood lipids and reducing inflammation in addition to its antioxidant activity. These findings suggest that berberine has potential applications as a therapeutic drug for diabetic nephropathy, and has significant research value. However, the possible mechanisms have not been fully established. The purpose of this paper is to investigate the renoprotective mechanisms of berberine in diabetic nephropathy and highlight the importance of berberine as a potential therapeutic reagent for diabetic nephropathy treatment.

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Contents

1. Introduction ........................................................................................................................................................................ 103
2. Renal protective effects and mechanisms ................................................................................................................................. 104
2.1. Regulating metabolic abnormalities: hyperglycemia and dyslipidemia .............................................................................. 104
2.2. Anti-oxidative stress and ameliorating insulin resistance .............................................................................................................. 105
2.3. Relieving the hemodynamic abnormalities in diabetic nephropathy ....................................................................................... 107
2.4. Anti-inflammatory activity and mechanisms of berberine in diabetic nephropathy ................................................................. 107
2.5. Modulating signal transduction pathways in diabetic nephropathy .............................................................................................. 108
  2.5.1. Effects of berberine on the NF-κB pathway in diabetic nephropathy .................................................................................... 108
  2.5.2. Effects of berberine on the PGE\(_2\)-EP receptor pathway in diabetic nephropathy ................................................................. 108
  2.5.3. Effect of berberine on the aldose reductase-polyol pathway in diabetic nephropathy .......................................................... 109
  2.5.4. Effects of berberine on the aldose reductase-polyol pathway in diabetic nephropathy .......................................................... 109
  2.5.5. Effects of berberine on the SphK1-S1P-AP1 pathway in diabetic nephropathy ................................................................. 109
  2.5.6. Effects of berberine on the MAPK pathway in diabetic nephropathy .................................................................................... 110
3. Conclusion .................................................................................................................................................................................. 111
Conflict of interest ........................................................................................................................................................................ 111
Acknowledgments .......................................................................................................................................................................... 111
References ..................................................................................................................................................................................... 111

1. Introduction

Diabetic Nephropathy (DN), a type of capillary vessel syndrome, is one of the most common and severe chronic complications of diabetes mellitus. The principal pathological changes of DN include
accumulation of the glomerular extracellular matrix (ECM), glomerular hypertrophy, glomerular basement membrane (GBM) thickening and glomerulosclerosis (Catania et al., 2007), which may devolve into chronic renal insufficiency and become the chief cause of end-stage renal disease. Berberine (BBR; \([\text{C}_{20}\text{H}_{18}\text{NO}_{4}]{^+}\)), a type of isoquinoline alkaloid with a long history of medicinal application, is the major active constituent of Rhizoma coptidis and Cortex phellodendri. Recent studies have demonstrated that berberine exhibits multiple pharmacological activities, including lowering blood glucose, regulating blood lipids, antioxidant activity, reducing inflammation and increasing insulin sensitivity, thus ameliorating insulin resistance (Yin et al., 2008). These findings suggest that berberine has wide clinical and research applications and prospects as a therapeutic drug in treating DN. However, the concrete mechanisms responsible for the renal protective effects of berberine have not yet been fully explored and are still pending further investigations. Therefore, this paper aims to summarize the beneficial effects of berberine on renal functions and its possible mechanisms in diabetic nephropathy.

2. Renal protective effects and mechanisms

2.1. Regulating metabolic abnormalities: hyperglycemia and dyslipidemia

Over a decade ago, research incontrovertibly showed the link between hyperglycemia and diabetic complications. The landmark trials and a number of other clinical studies strongly support the hypothesis that long term hyperglycemia is the most critical element that mediates the progressive tissue damage and functional decline characterizing diabetic complications, especially in DN. Therefore, DN related glucose metabolism research has gained interest recently. Hyperglycemia causes the accumulation of sorbitol in kidney cells, results in hyperosmolality pressure and destroys cellular structure, thus decreasing the activity of the Na\(^{+}\)-K\(^{+}\)-ATPase, which has negative effects on physiological kidney functions of kidney, including glomerular filtration and tubular resorption (Kikkawa et al., 2003). Long-term hyperglycemia induces insulin resistance and oxidative stress, which, in turn, forms a positive-feedback effect on high glucose regulation. Increased oxidative stress cooperates with the activated polyol pathway to stimulate the protein kinase C (PKC) pathway, while insulin resistance activates signaling pathways, such as the P38 and extracellular signal-regulated kinase (ERK) pathways, to increase the expression of transforming growth factor-\(\beta\) (TGF-\(\beta\)), fibronectin, and type I collagen and type IV collagens in glomerular mesangial cells. This contributes to the deposition of ECM and leads to glomerulosclerosis and tubular interstitial fibrosis. Hyperglycemia also causes advanced glycosylation end-products, which are located outside of the cells, to affect the production of type IV collagen and the formation of ECM and increase several growth factors and other cytokines including TGF-\(\beta\), vascular endothelial growth factor (VEGF) and connective tissue growth factor (CTGF). These actions stimulate the classical TGF-\(\beta\)/Smad pathway, and result in renal tubule damage and cause interstitial fibrosis. Additionally, hyperglycemia promotes the expression glucose transporters (GLUTs), such as GLUT1/4. The increased GLUTs, in turn, induce the abnormalities in glucose metabolism and cause renal injury (Konrad et al., 2001). Research has shown that berberine can significantly diminish the nuclear translocation of NF-kB p65 and high glucose-induced c-jun phosphorylation (Fig. 1A). Berberine can also block high glucose-induced TGF-\(\beta\) gene expression and the transcriptional and DNA-binding activities of activator protein-1 (AP-1). Berberine inhibits these changes in signaling pathway key factors, and then decreases the mRNA levels of intracellular adhesion molecule-1, TGF-\(\beta\), fibronectin, and downstream inflammatory mediators in the fibrotic response in the diabetic kidney. This also reduces the synthesis of ECM, inflammatory mediators and other pro-fibrogenic factors, which results in decreased ECM deposition and reduced inflammatory reactions, eventually attenuating the degree of glomerulosclerosis and renal fibrosis (Jiang et al., 2011). Additionally, berberine inhibits the G1-S phase transition under high glucose conditions in mesangial cell cycle progression. In this process, berberine reverses the high glucose-induced downregulation of p21\(^{\text{Waf1/Cip1}}\) and p27\(^{\text{Kip1}}\) and results in mesangial cell cycle arrest at the G1 to S phase transition, indicating that berberine negatively regulates high glucose-induced cell proliferation and hypertrophy, which are responsible for ECM accumulation and the development of glomerulosclerosis (Lan et al., 2014). These alterations could delay the development of DN. Moreover, berberine promotes the expression of GLUT4, which is activated by p38 mitogen-activated protein kinase (p38MAPK), and improves glucose uptake and utilization, to prevent DN development. In addition, research demonstrated that berberine can reduce glucose-6-phosphatase activity and decrease hydrogen binding to nicotinamide adenine dinucleotide phosphate to form reduced nicotinamide adenine dinucleotide phosphate, ultimately reducing lipogenesis and diminishing the level of glycogenolysis and gluconeogenesis, to prevent renal injury (Xia et al., 2011). The exact mechanisms of berberine induced hypoglycemic effects are complex and remain unknown. Further quantitative research on these questions is needed. Moreover, the hypoglycemic activity of medicinal plants or plant derivatives needs extensive research, as the number of diabetic patients is continuously on the rise.

Aside from hyperglycemia, epidemiological investigation found that dyslipidemia is also a pivotal factor that affects DN development. The preferential phagocytosis of the modified low-density lipoprotein reportedly plays a significant role in the cytomorphosis of mesangial foam cells. Glomerular mesangial cells express scavenger receptors, which participate in the formation of oxidized low-density lipoprotein and the uptake of the modified glycosylated protein, leading to the activation of these cells as macrophages. The intra-mesangial-recruited macrophages may further oxidize the low-density lipoprotein to cause a vicious self-perpetuating cycle, leading to progressive renal damage. Moreover, gradual accumulation of modified lipoproteins within the mesangium activates the secretion of various adhesion molecules and chemotactic factors in glomerular mesangial cells, such as macrophage colony stimulating factor and intracellular adhesion molecule-1, resulting in the pathogenesis of tubular fibrosis and glomerulosclerosis, particularly in the DN environment (Tarabra et al., 2009). Furthermore, activated macrophages in the kidney mesangium will increase the expression of proinflammatory factors, accelerate oxidative stress and the release of proliferative cytokines, such as TGF-\(\beta\) and platelet-derived growth factor-\(\beta\), and contribute to the production of ECM proteins, thereby promoting mesangial expansion as described in the DN. One added point is that the uptake of modified low density lipoprotein by mesangial macrophages will stimulate the synthesis of eicosanooids, including leukotrienes and thromboxanes, which lead to potentially malignant alterations in intra-glomerular hemodynamics. Additionally, the tubular uptake and metabolism of the lipid component of filtered lipoproteins result in the local expression of chemokines and cytokines, and, thus, promote interstitial inflammation in overt DN (Yang et al., 2014a, 2014b). Meantime, hyperlipidemia induces excess sterol regulatory element-binding proteins (SREBP) to elevate the levels of blood cholesterol and triglycerides, leading to ECM deposition, and eventually provokes glomerulosclerosis, renal fibrosis and even end-stage renal disease (Cvetkovic et al., 2009). To date, some studies have found that berberine stimulated the adenosine monophosphate activated protein kinase (AMPK) signaling pathway by promoting the phosphorylation of acetyl-CoA carboxylase (ACC) in fat and liver cells in a concentration-dependent manner (Fig. 1B). Activated


AMPK also affected the activities of HMG-CoA reductase and ACC to suppress the expression of lipid synthesis genes, such as fatty acid synthase, SREBP1c, and peroxisome proliferator activated receptor γ, in cells, which significantly decreased triglyceride and cholesterol synthesis and secretion. This gradually normalized blood lipid levels and decreased the release of TGF-β1 and VEGF (Kong et al., 2004). These changes decrease ECM accumulation and alleviate glomerular sclerosis and renal tubule fibrosis. The activated AMPK pathway also promoted free fatty acids oxidation and decreased lipid formation to alleviate the dangers of dyslipidemia in DN (Kahn et al., 2005). The anti-oxidation effect of berberine concurrently depresses the generation of dyslipidemia-stimulated oxidative stress and decreases mesangial cell cycle transition from G1 to S phase, thus regulating high glucose-induced cell proliferation and hypertrophy. The regulatory effects of berberine on dyslipidemia remind us that berberine could be used as a drug to targeting dyslipidemia in DN, which suggests that berberine and other similar traditional Chinese medicines should garner more attention in research.

2.2. Anti-oxidative stress and ameliorating insulin resistance

The etiopathogenesis of DN is extremely complicated and remains controversial. However, research increasingly suggests that oxidative stress may play critical roles in the onset and development of DN. In DN, oxidative stress is typically promoted by excess nutrients, such as free fatty acids and hyperglycemia, which can lead to the generation of reactive oxygen species and cytokines, thus promoting the formation of ECM proteins accumulation. Meanwhile, berberine directly reduces the synthesis of lipids through suppressing HMG-CoA reductase activity. The regulatory actions of berberine on dyslipidemia remind us that berberine could be used as a drug to targeting dyslipidemia in DN, which suggests that berberine and other similar traditional Chinese medicines should garner more attention in research.

Fig. 1. Regulating the metabolic abnormalities: hyperglycemia and dyslipidemia. (A) Berberine decreases blood glucose to reduce kidney inflammation and glomerulosclerosis not only by inhibiting the NF-κB and c-Jun pathway, but also by up-regulating the reduced p21Waf1/Cip1 and p27 Kip1, which results in the arrest of mesangial cell cycle transition from G1 to S phase, thus regulating high glucose-induced cell proliferation and hypertrophy. Abbreviations: ICAM-1: intercellular cell adhesion molecule-1; G6PD: glucose-6-phosphate dehydrogenase. (B) Berberine not only accelerates the free fatty acid oxidation, thus reducing lipoproteins accumulation by stimulating the AMPK pathway, but also increases LDL receptor mRNA stability by activating the ERK and JNK/c-jun pathway to reduce the levels of reactive oxygen species and cytokines, thus decreasing ECM proteins accumulation. Meanwhile, berberine directly reduces the synthesis of lipids through suppressing HMG-CoA reductase activity. Abbreviations: ACC: acetyl-CoA carboxylase; TG: triglyceride; CHO: cholesterol; FAS: fatty acid synthetase.
the excessive oxidative stress mediates the overproduction of angiotensin II, which may accelerate the progress of hypertension in the glomeruli and enhance the glomerular filtration rate. Together, they promote proteinuria and cause GBM thickening, which promote the development of DN (Kamiyama et al., 2013). In addition, oxidative stress activates intracellular signaling pathways, such as JNK and PKC, and stimulates transcription factors such as nuclear factor NF-κB and AP-1. The high expression of corresponding activity products leads to the increased deposition of ECM and reduced matrix degradation, which result in glomerulosclerosis and renal fibrosis (Wu et al., 2009). Recently, the inhibitory effects of berberine on oxidative stress are observed in both cells cultured in high glucose and diabetic animal models (Fig. 2A). The level of superoxide dismutase mRNA expression can be up-regulated by berberine, and it plays an important role in berberine’s activity against oxidative stress (Moghaddam et al., 2014). Moreover, berberine is reported to increase the levels of sirtuin 1, a deacetylase with antioxidant activity. During oxidative stress, sirtuin 1 induces the deacetylation of forkhead box O transcription factors and increases the transcription of their target genes, including superoxide dismutase. It is possible that berberine increased superoxide dismutase expression via the Sirtuin 1/Forkhead box O pathway, but additional research is still needed to confirm this hypothesis. Recent research indicates that berberine suppresses oxidative stress by inhibiting the nuclear factor erythroid-2-related factor-2 (Nrf2) pathway. The inhibitory effect of berberine on Nrf2 depends on the activation of the AMPK, PI3K/Akt and P38 pathways, as blocking these pathways could diminish the effect of berberine on Nrf2. Berberine activates these pathways, and then induces nuclear translocation of Nrf2, which will activate the expression of antioxidant enzymes such as superoxide dismutase and glutathione, and reduce the degree of oxidative stress (Hsu et al., 2012). It is worth noting that DN is a complicated metabolic disease characterized by both impaired insulin secretion and insulin sensitivity. Insulin resistance is an important precursor of hyperglycemia and hyperinsulinemia, which leads to glucose metabolic disturbance, inflammation and the stress response and results in further damage of islet β-cells. This positive feedback accelerates the progress of glomerular hypertension in nephropathy and enhances the glomerular filtration rate to promote proteinuria and cause GBM thickening (Zhou et al., 2009). At present, impaired insulin signaling and complicating hyperinsulinemia will elevate blood pressure and vasoconstriction, cause glomerular vasodilatation and glomerular hypertension to enhance the permeability of glomerular capillary. This results in glomerular hypertrophy and promotes the ECM accumulation outside the kidney cells, which helps to accelerate the process of renal fibrosis. Additionally, hyperinsulinemia will increase blood sodium sensitivity and glomerulus internal pressure, leading to microalbuminuria. The pathologic changes caused by insulin resistance promote the occurrence and development of DN. Research has shown that berberine inhibits IKKβ phosphorylation at Ser181 and NF-κB p65 to down-regulate the activation of NF-κB and largely depress the NF-κB pathway, thus decreasing the expression of IL-6 and TNF-α mRNA in adipose cells (Fig. 2B). Thereafter, the levels of the corresponding inflammatory factor and cytokines dropped to improve the inhibitory effect of GLUT-4 and promote glucose

Fig. 2. Antioxidant and ameliorating the insulin resistance. (A) Berberine increases the levels of antioxidant enzymes by mediating the signaling pathway, such as the AMPK, P38/Akt, P38 and SIRT1/Forkhead box O, which eventually relieves the symptoms of diabetic nephropathy. Abbreviations: RAAS: renin-angiotensin-aldosterone system; SIRT1: sirtuin 1; FOXO: Forkhead box O. (B) Berberine up-regulates the quantities of adiponectin and visfatin, which helps to normalize and improve the insulin resistance. Meanwhile, the enhanced insulin sensitivity and debilitated insulin resistance would effectively ameliorate diabetic nephropathy. Abbreviations: IRS-1: insulin receptor substrate; IkB-α: IkB kinase-α.
utilization and indirectly ameliorate insulin resistance (Choi et al., 2006). Meanwhile, the reduced levels of IL-6 increase the tyrosine phosphorylation of insulin receptor substrate 1 and stimulate the insulin pathway, thus enhancing insulin sensitivity to directly improve insulin resistances (Klover et al., 2005). In addition, berberine could also improve insulin resistances by activation of adipokines. A report revealed that berberine increases the expression and production of visfatin and adiponectin mRNAs and proteins expression and production of visfatin and adiponectin within a tested dose range in a dose-dependent pattern. The activated adiponectin stimulated the AMPK pathway to inhibit free fatty acid oxidation in the liver and skeletal muscle and decrease hepatic glucose output, while recombinant visfatin enhanced glucose uptake in 3T3-L1 adipocytes and L6 muscle cells to indirectly ameliorate insulin resistances (Fukuhara et al., 2005). These beneficial alterations suggest that berberine possesses a certain amount of anti-insulin resistance effects in DN, which delays the development of diabetes. There have also been reports that berberine ameliorates insulin resistance through inhibiting oxidative stress and endoplasmic reticulum stress. However, its pathogenesis is unclear and needs to be confirmed during the subsequent experiments.

2.3. Relieving the hemodynamic abnormalities in diabetic nephropathy

Hemodynamic abnormalities such as increased systemic and intraglomerular pressure as well as activation of various vasoactive hormone pathways are involved in the pathogenesis of DN. These factors act independently to activate intracellular pathways, such as the MAPKs, PKC, NF-κB pathways and various growth factors, including the prosclerotic cytokines TGF-β1, CTGF, and the angiogenic growth factors permeability enhancing growth factor and VEGF, to promote glomerular pathological changes including ECM accumulation and thickened GBM, which are considered as hallmarks of DN (Wolf, 2004). Meanwhile, increased glomerular capillary pressure induces an enhanced transcapillary hydraulic pressure gradient and an increased glomerular plasma flow. These pathologic changes result from a decreasing resistance in both the afferent and efferent arterioles, where the former is more dilated than the latter (Ravid, 2006). The glomerular hypertension affects the reabsorption of water and salt to form shear stress, and the mechanical strain is and is linked to hyperfiltration and hyperperfusion. Then, the abnormalities above ultimately enhance proteinuria, glomerulosclerosis and tubulointerstitial fibrosis. Then the progressive structural and functional injuries eventually translate into DN. With berberine treatment, the level of NF-κB decreased, and the IκB-α level is partially restored. Then, the level of activated NF-κB, which translocates to the nucleus, decreases to reduce the expression of its target genes. The corresponding levels of downstream proteins, including intracellular adhesion molecule-1, TGF-β1 and fibronectin are all down-regulated (Liu et al., 2010). These alterations will decrease shear stress and mechanical strain and diminish ECM accumulation in glomerular mesangial cells, monocytes and glomeruli, which lead to a significant decrease of glomerular capillary pressure, GBM thickening and tubulointerstitial fibrosis, eventually ameliorating the progressive structural and functional changes. Additionally, the formation of advanced glycosylation end-products and oxidative stress in renal tissue is suppressed by administration of berberine and is one important mechanism indicated that berberine could delay the progression of DN by relieving the hemodynamic abnormalities (Wu et al., 2012).

2.4. Anti-inflammatory activity and mechanisms of berberine in diabetic nephropathy

In addition to hyperglycemia, dyslipidemia, hemodynamic abnormalities, chronic low-grade inflammation and the innate immune system activation also have significant functions in DN pathogenesis. Increasing experimental evidence and clinical data show that the inflammatory cell proliferation and inflammatory cytokines levels are often increased in DN. Impaired glomeruli not only increase microalbumin production, but also secrete immune cytokines together with tubular epithelial cells, which results in renal interstitial monocyte/macrophage infiltration and aggravates the inflammatory response. In these circumstances, the activated glomerular mesangial cells secrete type IV collagen, laminin and fibrin, leading to glomerulosclerosis and renal fibrosis. Over time, these abnormalities will form DN and further evolve to end-stage renal disease (Navarro-Gonzalez et al., 2011). Inflammation contributes to the development of DN, but does not exist independently. Therefore, it is necessary to explore a timely and appropriate anti-inflammatory treatment due to its vital role in DN. The anti-inflammatory action of berberine is observed both in vivo and in vitro (Fig. 3). Research shows that berberine reduces the infiltration of inflammatory cells and cytokines in animals with DN (Deng and Xie, 2014). Furthermore, berberine improves the ratios of anti-inflammatory and proinflammatory cytokines, such as IL-10/IL-1α, IL-10/IL-6 and IL-10/TNF-α. In addition to evidence from animal models and cultured cells, the anti-inflammatory effect of berberine has also been identified in clinical studies (Zhang et al., 2008). It is reported that berberine significantly reduces the level of IL-6 in patient serum at a dose of 1 g/day for 3 months. Berberine suppresses inflammation through complex mechanisms. Apart from its antioxidant activity, the AMPK pathway is closely related to the anti-inflammatory efficacy of berberine. Blocking AMPK could abolish the inhibitory effect of berberine on proinflammatory cytokines including cyclooxygenase-2 and iNOS, in macrophages. iNOS has a close relationship with insulin resistances, while cyclooxygenase-2 promotes the synthesis of prostaglandins, which are important mediators for DN pathogenesis (Jeong et al., 2009). Moreover, the anti-inflammatory action of berberine has a close relationship with its inhibitory effect on the MAPK pathway, and the inhibitory effect is dependent on the AMPK activation in macrophages. However, controversial results exist about these regulatory effects. As members of a complex signaling network, berberine treatment could induce the activation of AMPK and P38, which in turn promote the nuclear translocation of NFκB and inhibit the production of proinflammatory cytokines. The roles of these relationships in the pharmacological effects of berberine need further study. The NF-κB pathway plays a key role in controlling inflammation and is a critical target for the anti-inflammatory activity of berberine as well (Hsu et al., 2013). Berberine administration greatly reduces IKK-β activation through phosphorylation at Ser181. In addition, the inhibitory effect of berberine on IKK-β required a cysteine residue at position 179 of IKK-β. As inhibitory IκB-α is phosphorylated by IKK-β and then degraded, inhibition of IKK-β by berberine results in the stabilization of IκB-α and blocks the nuclear translocation of NF-κB. In kidney cells, the inhibitory effect could reduce the production of pro-inflammatory cytokines (Wan et al., 2013). Meanwhile, the Rho GTPase is a member of the superfamily of small GTP binding proteins with multiple biological functions and has been conclusively shown to regulate the NFκB signaling pathway in diabetic rats. Additionally, berberine inhibited the classical NFκB pathway by suppressing Rho GTPase. Moreover, the inhibitory effect of berberine on the Rho GTPase relied on its antioxidant activity (Xie et al., 2013). Like NFκB, AP-1 is critical for the development of inflammation. Administration of berberine to macrophages or epithelial cells greatly attenuated the DNA binding activity of AP-1 and reduced the production of cytokines including monocyte chemotactic factor protein-1 and cyclooxygenase-2 (Schonthal et al., 2011).
2.5. Modulating signal transduction pathways in diabetic nephropathy

2.5.1. Effects of berberine on the NF-κB pathway in diabetic nephropathy

Of all the influential factors, the signaling pathway mechanism is a critical element in the pathogenesis of DN. A growing number of studies show that berberine ameliorates renal dysfunction by acting on several signaling pathways in diabetes. Research finds that the NF-κB signaling pathway is activated in the renal tissue of diabetic mice and berberine inhibits activation of the NF-κB pathway. In this field, berberine exerts its inhibitory effect on IκB protein degradation and increases IκB synthesis in glomerular mesangial cells (Ni et al., 2015). Additionally, berberine suppresses the increased expression of p65 in the nucleus and decreases it in cytoplasm, which leads to the inhibition of the NF-κB pathway. These changes will result in decreasing the transcription and translation of many inflammatory mediators, such as TGF-β1 and intracellular adhesion molecule-1, to accelerate ECM accumulation and decrease the number of inflammatory cells and mononuclear macrophage infiltration into glomeruli and renal interstitium. Berberine then ameliorates the symptoms of GBM thickening, glomerulosclerosis and tubulointerstitial fibrosis, the hallmarks for progressive DN (Mezzano et al., 2004).

2.5.2. Effects of berberine on the PGE2-EP receptor pathway in diabetic nephropathy

PGE2 is an inflammatory mediator that has pleiotropic effects on signaling cascades in the disease via specific G protein-coupled receptors (GPCRs). Based on previous studies, the enhanced proliferation of glomerular mesangial cells and remarkable renal injury often coexist with the activation of Gαi and inhibition of Gαs and cAMP in diabetes. Excessive PGE2 has particularly been shown to be associated with an increased glomerular filtration rate in the early stages of DN and subsequent glomerular hypertrophy, proteinuria as well as renal injury. In our previous studies, berberine treatment reduced the levels of IL-6 and PGE2 in the renal cortex (Tang et al., 2014b) (Fig. 4A). Also, we note that the increased expression of EP1 is down-regulated, while reductions in EP4 are up-regulated in the berberine-treated rats compared with DN rats. Meanwhile, the expression of the Gαs protein was up-regulated and the Gαi protein was down-regulated. Then, increased cAMP markedly suppressed the secretion of TGF-β1 and CTGF (Yang et al., 2014a, 2014b). TGF-β1 mediates the inflammatory response and promotes the synthesis of collagens and fibronectin, while CTGF predominantly accelerates the deposition of ECM and glomerular mesangial cells proliferation. Therefore, we have reason to believe that berberine treatment could reduce ECM accumulation, decrease the inflammatory response, and suppress glomerular mesangial cell proliferation, thus ameliorating the symptoms of GBM thickening, glomerulosclerosis and renal fibrosis by inhibiting the PGE2-GPCR-cAMP signaling pathway (Tang et al., 2013).

2.5.3. Effect of berberine on the RhoA/ROCK pathway in diabetic nephropathy

RhoA/ROCK signaling has been widely implicated as an important contributor to diabetes and kidney disease. Many studies have already identified that the RhoA/ROCK pathway is activated in glomerular mesangial cells and diabetic kidney, which significantly accelerated the renal inflammation and damage process (Kolavennu et al., 2008). Research shows that the production of
reactive oxygen species, hydrogen peroxide and malondialdehyde are effectively abrogated, thus markedly inhibiting the phosphorylation of the myosin phosphatase targeting subunit (MYPT) and RhoA membrane translocation after berberine treatment. This is followed by ROCK down-regulation in the renal cortex. Inhibition of the RhoA/ROCK pathway will significantly reduce the levels of intracellular adhesion molecule-1 and TGF-β1, two well-known inflammatory factors. Meanwhile, the inhibited RhoA/ROCK pathway also partially reduces the levels of fibronectin and type IV collagen protein to decrease ECM accumulation in kidney cells to mitigate the renal fibrosis and glomerulosclerosis (Komers et al., 2011). Therefore, we hypothesize that inhibiting the RhoA/ROCK pathway with berberine in vivo and in vitro may contribute to the underlying mechanisms of berberine as an important therapeutic strategy for DN treatment.

2.5.4. Effects of berberine on the aldose reductase-polyol pathway in diabetic nephropathy

Under hyperglycemic conditions, accelerated flux is said to promote the process of DN through the polyol-pathway. In this pathway, the excessive glucose levels increase the rate of oxidative phosphorylation as well as stimulate the polyol pathway (Dunlop, 2000). Aldose reductase is a major regulatory enzyme in substrate flux through the polyol pathway. Activation of aldose reductase and the polyol pathway trigger signal transduction cascades, such as MAPK, PKC, cAMP response element binding protein (CREB), and the transcription factors AP-1. This also results in the overexpression of cytokines and proteins, including TGF-β, TNF-α and fibronectin, leading to excessive deposition of ECM and mesangial expansion, eventually accelerating the DN progress (Price et al., 2004). Berberine treatment significantly inhibited aldose reductase activity and both the mRNA and protein expression of aldose reductase are diminished, which indicates that berberine suppresses a rate-limiting enzyme in the polyol pathway and leads to inhibition of the polyol pathway (Fig. 4B). This is followed by the decreased accumulation of sorbitol and fructose. We predict that the inhibitory effect must have relationships with the inhibition of aldose reductase and the polyol pathway. The accumulation of a variety of materials will decrease cell permeability and increase the activity of the Na⁺-K⁺-ATPase, as previously shown. All of these changes slow the progressive accumulation of ECM components in the mesangium and gradually renew renal functions (Srivastava et al., 2005). Thus, both increased aldose reductase activity and the polyol pathway are implicated in the pathogenesis of DN, and berberine may have a positive therapeutic effect in the management of DN through inhibiting the aldose reductase-polyol pathway.

2.5.5. Effects of berberine on the SphK1-S1P-AP1 pathway in diabetic nephropathy

Recently, sphingosine 1-phosphate (S1P), a potent mitogenic/migratory signaling lipid catalyzed by sphingosine kinase 1 (SphK1), has been linked to the progression of DN. Research shows that SphK1 is abundantly expressed and activated in the diabetic
kidney, and the SphK1/AP-1 pathway plays a pivotal role in matrix accumulation in glomerular mesangial cells (Awad et al., 2011). This study suggests that the SphK1-S1P-AP1 pathway should draw considerable attention because of its potential pathogenic role in the development of DN. During hyperglycemia, AP-1 transcription is increased, which results in the activation of the SphK1-S1P pathway, followed by elevated SphK1 and S1P/S1P2 expression in different types of kidney cells. These proteins up-regulate the expression of α-SMA, TGF-β1 and fibronectin, mimic TGF-β1 to activate the Smad signaling cascade, and are responsible for glomerular mesangial cells hypertrophy and the progression of renal fibrosis (Wang et al., 2005). Berberine treatment decreases AP-1 transcription (Fig. 4C). Meanwhile, researchers are surprised to find that the expression of SphK1 is markedly attenuated, and sequentially accompanied by a decrease in S1P and S1P2. At this point, the SphK1-S1P signaling pathway is largely suppressed, and, consequently α-smooth muscle actin (α-SMA) expression is significantly attenuated, which alleviates glomerular mesangial cell hypertrophy (Huang et al., 2013). Furthermore, research finds that berberine inhibits the p38MAPK phosphorylation, accompanied by significant decreases in the levels of phospho-CREB, fibronectin, and collagen. In DN, research finds that berberine inhibits the p38MAPK phosphorylation, accompanied by significant decreases in the levels of phospho-CREB, fibronectin, and collagen. In DN, research finds that berberine reduces hyperglycemia-induced fibronectin expression and collagen synthesis in glomerular mesangial cells partly through inhibiting p38MAPK pathway (Li et al., 2009a, 2009b) (Fig. 5).

2.5.6. Effects of berberine on the MAPK pathway in diabetic nephropathy

The p38MAPK pathway is mainly activated by increased reactive oxygen species and inflammatory cytokines, which subsequently leads to cell proliferation and apoptosis during the development of DN. A study suggests that phosphorylated p38MAPK is mainly expressed in glomerular mesangial cells, and the overexpression of phospho-p38MAPK-positive cells in patients with DN reflects the correlation to tubulointerstitial lesions. Moreover, the p38MAPK pathway also participates in collagen synthesis. Once activated, it phosphorylates several transcription factors, including CREB, at serine and threonine residues. Then, phospho-CREB translocates into the nucleus and binds to the cAMP response element located in upstream genes, and subsequently regulates the expression of fibrosis cytokines and ECM proteins, such as fibronectin and collagens. In DN, research finds that berberine reduces hyperglycemia-induced fibronectin expression and collagen synthesis in glomerular mesangial cells partly through inhibiting p38MAPK pathway (Li et al., 2009a, 2009b) (Fig. 5). Meanwhile, research demonstrates that ERK plays a key role in intracellular signaling to integrate the transcription of genes involved in numerous cellular responses in DN. In different renal cells, Raf-1 kinase phosphorylates and activates the MAPK/ERK kinase (MEK-1/2), a dual-specificity kinase that phosphorylates the Threonine and Tyrosine residues of various isoforms of MAPK, leading to the activation ERK-1/2. The renin-angiotensin-aldosterone system and VEGF expression are then
activated, which results in glomerular mesangial cell proliferation and potential damage to the kidney (Turner et al., 2001). Research also shows that the ameliorative effects of berberine on kidney injury and glomerular mesangial cells proliferation may be explained by decreasing the expression of the MEK/ERK-dependent transcription factors c-Fos and Egr-1, which play regulatory roles in growth factor activation and cell cycle re-entry (Liang et al., 2006) (Fig. 5). In addition, berberine inhibits the activity of JNK in a dose-dependent and time-dependent fashion, which regulates the expression of cyclooxygenase-2 and delays the process of vascular sclerosis and glomerulosclerosis to ameliorate the progression of DN (Fig. 5). Despite these effective functions in DN, the detailed molecular mechanisms and the specific pharmacological targets were not clearly demonstrated. Therefore, topics in further research are seeking possible specific therapeutic targets and the characteristic changes in downstream pathways so that berberine could play a key role in treating DN while targeting the above pathways.

3. Conclusion

Berberine has been widely used in the treatment of DN and has achieved great effects. However, the specific mechanisms have not been completely established and were only confined to anti-oxidative stress, inhibiting aldose reductase, etc. With the continuous advance of quantitative proteomics and the development of experimental techniques, a better understanding of the mechanisms and physicochemical properties of berberine will provide sufficient theoretical supports for us to promote the clinical application of berberine by identifying its possible therapeutic targets and learn more about the occurrence and development of DN.

Conflict of interest

The authors declare that they have no conflict of interest.

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