Reno - protective effects of GLP-1 receptor agonist and antiplatelets in experimentally – induced diabetic kidney disease in male albino rats

Thesis

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SUMMARY

In the present study, we observed that chronic uncontrolled hyperglycemia following STZ administration led to the development of diabetic nephropathy (DN). We have reported that DN was attenuated by both metformin (70mg/kg/day) or dulaglutide (**1.**5mg/kg BW/week) and combined to cliostazol (5mg/kg BW /day). The renoprotective effect of these drugs was demonstrated by evaluating various factors such as renal function parameters and biochemical, histopathological, inflammatory and oxidative stress markers. Furthermore, the expression of eNOS and NFKB was determined to delineate the mechanistic pathway responsible for its nephroprotection.

Despite the marked advances in the therapeutic maneuvers for chronic kidney diseases such as medications, dialysis and renal replacement therapy. The prevalence of Chronic Kidney Disease are progressively increasing and reaching epidemic levels. With a increasing risk of fatal complications leading to elevated rats of morbidity and mortality especially with sever cases, early intervention can significantly delay the pathogenesis and mark-edly improve the prognosis.

Diabetic Nephropathy is one of the fatal microvascular complications of type 2 diabetes mellitus, and it is one of the leading causes of death in diabetic patients, this is because once proteinuria occurs; the rate of decline of renal function in diabetic nephropathy is higher than the rate of decline in other renal diseases leading to accelerated renal damage with histological and structural changes and rapid deterioration in the renal functional parameters. In addition to the poor sensitivity of the standard

renal functional biomarkers, specially serum urea and creatinine. So, accurate new markers are needed for the early diagnosis and risk stratification of diabetic nephropathy.

Metformin, approved as the most widely accepted first-line of treatment to lower blood glucose levels in type 2 diabetic patients. In addition to its anti-diabetic actions, recent studies revealed additional nephroprotective effects of metformin in vitro and in vivo. Metformin diminish apoptosis in different experimental renal diseases. Also it reduce albuminuria in diabetic patients. These effects were mediated through the AMPK/mTOR signaling axis. These data enhance the renoprotective effects of metformin in diabetic nephropathy.

The renal benefits of dulaglutide, as decreasing in GFR and reduction in albuminuria, are more dominate in DKD with macroalbuminuria at baseline. Dulaglutide, also decreased weight gain and controled hypoglycaemia. GLP1 agonists protect the kidney by reducing oxidative stress and inflammation. also dulaglutide has a renoprotective effect a part from its regulation of blood glucose and blood pressure in patients with advanced CKD, a renal vascular effect, an anti-inflammatory effect or a reduction in glomerular atherosclerosis, or from other undiscovered effects of GLP1 receptor agonists, remains to be established.

The administration of cilostazol in streptozotocin (STZ)-induced diabetic rats relieves oxidative stress, regulates the expression of NF-κB and TGF-β and thus ameliorates the onset of DN , cilostazol delays DN progression, improves mitochondrial morphology and mtDNA copy number changes, and delays cell apoptosis. In mesangial cells, cilostazol

decreases oxidative stress, apoptosis, and DN-related cytokines. These data support its role as adjuvant treatment for DN

This study was carried out on 6 main groups (each contains 10 rats) of adult male albino rats. The first of them is the normal control group received no medications. All the other groups were type 2 diabetic. The 2nd group was diabetic rats received no treatment. The 3rd diabetic group received metformin at dose (70 mg/kg/day), it was administrated daily for 8 weeks. The 4th diabetic group received dulaglutide at a dose of (**1.**5mg/kg BW /week), it was administrated for 8 weeks. The 5th diabetic group received both metformin at a dose of (70 mg/kg/day) and cliostazol at dose (5mg/kg BW /day) orally. Both were administrated for 8 weeks. The 6th diabetic group received both dulaglutide at a dose of (**1.**5mg/kg BW /week) and cilostazol at dose (5mg/kg BW /day) orally. Both were administrated for 8 weeks.

The parameters used to evaluate the diabetic nephropathy and metformin, dulaglutide and cliostazol treatment consequences were serum insulin, glucose and HbA1c. The kidney function was evaluated by the serum urea, creatinine and albumin.

1. HDL- cholesterol, LDL- cholesterol and triglycerides.

   Also TGFβ, collagen IV, FN, SOD,GSH ,FOXO1, PKB and NO in the rat serum by ELISA were measured.

   2-While the eNOS and NFKB gene expression was measured by Quantitative real time PCR in the kidney tissues.

3- Histopathological examination of kidney tissues.

The obtained results of this study could be summarized as follow:

- The induction of type 2diabetes of the rats resulted in hyperglycemia and insulin resistance manifested by a significant increase in the serum levels of glucose, insulin and HbA1c and it was also associated with significant increase in the serum creatinine, serum urea and serum albumin and significant increase in serum LDL- cholesterol and triglycerides.

Significant increase in serum levels of TGFβ, collagen IV, FOXO1, FN, SOD and significant decrease in serum levels of HDL- cholesterol, NO,GSH and PKB levels. The examination of eNOS showed significant decrease while NFKB gene showed significant increase in the examined tissues.

- On studying the nephroprotective effects of metformin by a dose (70 mg/kg) there were a significant decrease in the serum levels of glucose, insulin, HbA1c, urea , creatinine and albumin. Significant decrease in serum levels of TGFβ, collagen IV, FOXO1, FN, SOD, LDL- cholesterol and triglycerides.

While there was a significant increase in the serum levels of HDL-cholesterol, NO, GSH and PKB. Also, the examination of eNOS showed significant increase while NFKB gene showed significant decrease in the examined tissues.

- On studying the nephroprotective effects of dulaglutide by a dose (**1.**5mg/kg BW /week) there were a significant decrease in the serum levels of glucose, insulin, HbA1c, urea, creatinine, and albumin. Significant decrease in serum levels of TGFβ, collagen IV, FOXO1, FN, SOD, LDL- cholesterol and triglycerides.

While there was a significant increase in the serum levels of HDL-cholesterol, NO, GSH and PKB. AlsoThe examination of eNOS showed significant increase while NFKB gene showed significant decrease in the examined tissues.

- On studying the nephroprotective effects of both metformin and cilostazol by a dose (5mg/kg) there were a significant decrease in the serum levels of glucose, insulin, HbA1c, urea, creatinine and albumin. Significant decrease in serum levels of TGFβ, collagen IV, FOXO1, FN, SOD, LDL- cholesterol and triglycerides.

  While there was a significant increase in the serum levels of HDL-cholesterol, NO, GSH and PKB. Also, the examination of eNOS showed significant increase while NFKB gene showed significant decrease in the examined tissues.

- On studying the prophylactic effect of both dulaglutide and cilostazol by a dose (5mg/kg), there were a significant decrease in the serum levels of glucose, insulin, HbA1c, urea, creatinine and albumin. Significant decrease in serum levels of TGFβ, collagen IV, FOXO1, FN, SOD, LDL- cholesterol and triglycerides.

  While there was a significant increase in the serum levels of HDL-cholesterol, NO, GSH and PKB. Also, the examination of eNOS showed significant increase while NFKB gene showed significant decrease in the examined tissues.

- By comparing the treated groups together, the dulaglutide group showed significant decrease in the serum levels of insulin, HbA1c, creatinin,albumin , TGFβ, collagen IV, SOD and FOXO1also NFKB gene showed significant decrease than metformin treated group. While there was a significant increase in the serum levels of NO and HDL- cholesterol., having the nearest levels to normal control group in most of the parameters, so we could consider it the best combination group in our study.

- By comparing the combined treated groups together, both metformin and cliostazol group showed significant decrease in the serum levels of LDL- cholesterol and SOD than metformin alone treated group. While there were a significant increase in the serum levels of GSH, also NFKB gene showed significant decrease than metformin treated group.

- By comparing the combined treated groups together, both dulaglutide and cilostazol group showed significant decrease in the serum levels of glucose, albumin, LDL- cholesterol, TGS, collagen IV, SOD than dulaglutide alone treated group. While there was a significant increase in the serum levels of NO., having the nearest levels to normal control group in most of the parameters, so we could consider it the best combination group in our study.

- Histopathological examination of DN, there is expansion of mesangium and thickening of the basement membrane of glomeruli. The renal tubules exhibit hypertrophy initially in the early stages, but eventually interstitial fibrosis with tubular atrophy sets in, together with arteriolar hyalinosis along with infiltration with macrophages and T lymphocytes in the late stages. Ultra-structurally, there is loss of podocyte as well as reduced endothelial cell fenestration. Moreover, the structural change is also accompanied by functional loss, treatment with metformin or dulaglutide showed improvement but these changes need more time than 8 weeks to return close to normal again.

From the above results we concluded that the pathogenesis of diabetic kidney disease is mediated even partially through hyperglycemia, inflammation, oxidative stress, fibrosis and apoptosis and that metformin or dulaglutide combined with cilostazol have a more renoprotective effect.