Assessment of New Protected Mechanism of Liraglutide on CKD Progression

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ABSTRACT

Podocyte injury represents the main reason for proteinuria and renal disease in diabetic and non-diabetic patients. Recently Liraglutide as a GLP-1 analog is hypothesized to play a vital role in preventing kidney damage by anti-inflammatory and anti-apoptotic effect mechanisms. This study was established to evaluate the effect of Liraglutide on deterioration that happens with time in chronic kidney disease. 36 male Wister rats with a weight of 250-300 g were used in this study. All selected animals were examined and determined to be normal on general examination. Rats were divided into three groups (12rats/group): control group: negative control group injected normal saline I.P, induction group: single I.P dose (7.5 mg/kg) of doxorubicin for 14 days then administered 0.5ml/kg normal saline intraperitoneal for 28 days, and treatment group: single I.P dose (7.5 mg/kg) of doxorubicin for 14 days then administered I.P Liraglutide 200µg/kg/day freshly dissolve in 0.5ml /kg normal saline 28 days. The treatment group appeared significantly decreased ($P < 0.05$) in the pro-inflammatory expression of TNF- α as compared with the induction group, while the treatment group revealed a significant increased ($P < 0.05$) in the expression of anti-apoptotic BCL-2 in the proximal tubule of the kidney. Besides the effect of Liraglutide on renal deterioration by lowering blood glucose, the main causes of oxidative stress, Liraglutide as GLP-1 analog can reduce the consequence of proteinuria in the cases of glomerulosclerosis and tubulointerstitial damage by decreased pro-inflammatory TNF- α expression and increase expression of BCL-2 in that cell.

Keywords: Apoptosis, Podocyte injury, Renal fibrosis

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INTRODUCTION

Podocyte injury playing role in the immune and none immune pathological glomerulonephritis in renal failure¹. Excessive formation of reactive oxygen species in diabetic nephropathy is the most pathology of podocyte injury². It is collaborated system of fiber protein in the glomeruli which intact on the basement membrane surface and linked by slit diaphragms that are essential in the foot process and a crucial part of the glomerular filtering barrier to keep atypical perm selectivity^{3, 4}. Podocyte injury is the main reason for the occurrence of proteinuria

and is developed in diabetic and non-diabetic patients with renal failure⁵. It is decreased in the early diagnoses of type 1 and 2 diabetes with albumin excretion and decline podocyte injury^{6, 7}. Increased albumin in the tubule which responsible to consequence progression of kidney deleterious⁸.

One of the suggested mechanisms for the harmful effects of proteinuria is the high albumin loaded in the renal tubule stimulating epithelial cells to generate fibrogenic and pro-inflammatory

molecules⁹. Furthermore, albumin toxicities that conjugate fatty acid with albumin in the lumen viewed high levels of fatty acids as an essential factor of tubulointerstitial injury and albumin overload¹⁰. In in vitro model, incubated proximal tubule cells with a high concentration of albumin can stimulate profibrotic proinflammatory molecules TNF- α , TGF- β , MCP-1, and interleukin-8 (IL-8)¹¹. Renal fibrosis is related to chronic kidney disease (CKD), glomerulosclerosis, and tubulointerstitial fibrosis characterized by excessive deposition of protein in the extracellular matrix (ECM) of interstitial tubule and glomeruli. Recent findings confirmed that myofibroblasts is a key source of ECM in scar tissue formation¹². Myofibroblasts are α -smooth muscular tissue actin (α -SMA) situated mostly in the kidney interstitium than glomeruli while one of the most predominant contributors are fibroblasts and pericytes in the interstitium¹³.

The sequence of tubulointerstitial fibrosis includes inflammatory cell infiltration; fibroblast activation and deposition of a huge amount of extracellular matrix (ECM), tubular degeneration, and microvascular rarefaction¹⁴. At the injury site, triggered infiltrated inflammatory cells caused increased oxidative stress and fibrogenic cytokines with growth factors. The local deposition of profibrotic cytokines in the microenvironment adhering is activated of ECM-producing cells as an essential role of fibrogenesis. Consequently, continuous inflammation after injury might trigger initiator and fibrosis progression¹⁵⁻¹⁷. Profibrotic cytokine effects on myofibroblast by expressing α SMA, as well as create a huge amount of ECM. Therefore, cytokines stimulate myofibroblasts and fibroblasts proliferation which both responsible for deposition ECM in the interstitial space of kidneys¹⁸. fibronectin either type I and III collagens in the interstitial matrix is fibroblasts¹⁹.

In the animal study models, podocyte injury induced by doxorubicin as a speculative design for glomerulosclerosis and tubulointerstitial fibrosis which increased proteinuria after 48 hour from induction and peaked after 14 day beside significant increase of serum creatinine and blood urea²⁰. Glucagon-like peptide-1 (GLP-1) work as hormone secreted from small intestine and responsible for glycemic control by insulin secretion and gene expression of insulin in response of GLP-1 receptor activation in pancreatic cell²¹. Recently, studies discovered that GLP-1 receptor is found in many

different tissues such as kidney and GLP-1 analogs have anti-inflammatory, anti-oxidative, and neurogenerative protective mechanism through animal experimental, by influence inflammatory pathway and protect against apoptosis sequence^{22,23}. Liraglutide represents one of the GLP-1 analogs that could has renal protective effect. This drug considers freely soluble in aqueous of basic solution and this solubility decreased with low PH which is low soluble at PH 2.5(≤ 0.8 mg/mL)²⁴. Aim of this study was to evaluate the effect of Liraglutide on deterioration that happen with time in chronic kidney disease through anti- inflammatory and anti -apoptotic protected mechanism.

MATERIALS AND METHODS

Thirty-six Wister rats with age over eight weeks were used and placed in the animal house of Pharmacy College in Mustansiriyah University after applied to the ethical committee. Every four animals were kept in the plastic cage with dimensions (20x25x35cm) and colored for differentiation. Acclimatization of animals at room temperature 20-22 °C and normal dark/light for 1 week, besides, the animal were checked by general examination before beginning. Liraglutide pen 6mg/ml (VICTOZA) and doxorubicin vial 50 mg/25ml (EBEWE company) are obtained from the market and used in this experiment. Rats were divided into three groups (12 rats/group):

Control group: injected normal saline I.P

Induction group: injected single I.P dose (7.5 mg/kg) of doxorubicin for 14 days induced glomerulonephritis by podocyte injury then administered 0.5ml/kg normal saline intraperitoneal for 28 days.

Treatment group: injected single I.P dose (7.5 mg/kg) of doxorubicin for 14 days then administered I.P Liraglutide 200 μ g/kg/day freshly dissolve in 0.5ml /kg normal saline 28 days.

At the end of the experiment, the animals were anesthetized by a cocktail of 100 mg/kg ketamine plus 10 mg/kg xylazine in the I.M route which was used in the previous study and show optimal results²⁵. Kidneys were removed and placed in the formalin 10%. It was prepared by paraffin block section (4 μ m) thickness and placed in the water bath at (45 C) for stretch, removed by positive slide charge, and dried at 37 c in the oven²⁶. Immunohistochemistry staining of *TNF- α* and *BCL-2*

were done to assess the anti-inflammatory and anti-apoptotic effects of Liraglutide. Semiquantitative staining score was utilized depending on the positive

percentage cell. It was based on the scoring system for TNF- α ²⁷ and BCL-2²⁸, as seen in Table 1.

Table 1: Immunohistochemical score system for TNF- α and BCL-2 ^{27, 28}

Score	Percentage of TNF- α expressed	Percentage of BCL-2 expressed
0	No staining	No staining
1	Less than 25%	Less than 33%
2	26-50 %	34 -70 %
3	51-75 %	More than 70%
4	More than 76 %	

❖ TNF- α expressed depended on the epithelial cell of tubule then secreted from infiltrated inflammatory cells.

❖ BCL-2 scored for expression on proximal tubule

Statistical Analysis

Data were analyzed by multiple software programs like Medcalc, Microsoft excel 2010, and statistical packages for social sciences (Spss-23). It included Mean $\pm$ Standard error, mean value compared between groups by ANOVA followed by Tukey test²⁹. P-value expressed as significant when it was equal to or less than 0.05 and highly significant when it was equal to or less than 0.01³⁰.

RESULTS

Regarding immune staining intensity of TNF alpha, the induction group without treatment observed a significant increased ($P < 0.05$) in the expression of TNF alpha in the epithelial cell of tubule when compared with other groups while the treatment group by Liraglutide for 28 days after induction revealed a significant decreased ($P < 0.05$) in the pro-inflammatory expression of TNF alpha when compared with induction group, otherwise still a significant higher ($P < 0.05$) than the control group, Figure 1, 2.

Regarding immune staining intensity of BCL-2 expression, the induction group observed a significant decrease ($P < 0.05$) of BCL-2 expression in the proximal tubule of cortex when compared with other groups while, the treatment group by

Liraglutide for 28 days after induction has appeared a significant increased ($P < 0.05$) in BCL-2 expression when compared with induction group concerning reduce deterioration in the proximal tubule by anti-apoptotic activity, otherwise still a significantly lower ($P < 0.05$) than control group, normally found in healthy animals, (Figure 3, 4).

DISCUSSIONS

Tumor necrosis factor- α is a pro-inflammatory cytokine as well as antitumorigenic which induced tumor cell apoptosis, it was produced by immune cells like macrophages and lymphocytes, also epithelial and endothelial cells according to recent studies³¹. In the kidney, TNF- α is produced from many sources such as infiltrated inflammatory cells, epithelial cells of the tubule, especially in proximal and podocytes. It is a multivariate effect and is practiced as intermediaries of diverse biological activities from cell proliferation, apoptosis to modify immunity caused by inflammation³².

TNF- α has an active role in kidney damage partly diminished renal perfusion in blood poisoning, stimulate immune cells to release cytokines which caused swelling and cellular death³³. Tubular damage increased tubular protein load, TNF- α and epithelial cell excretion of the tubules leads to the recruitment of inflammatory cells like cytokines

MCP-1, IL-6, and others which is responsible for the accumulation of ECM in the interstitial region and timely lead to fibrosis ^{34, 35}.

Fig 1: Bar chart displays the differences in the mean of TNF alpha expression score for all studied groups, data expressed as mean $\pm$ SEM, ^a Significantly different when compared with group A (control) at $P < 0.05$, ^bSignificantly different when compared with group B (induction) at $P < 0.05$, ^csignificantly different when compared with group C (treatment) at $P < 0.05$

Fig 2: Microphotograph of immunohistochemical (IHC) staining of TNF- α of rat kidney as: A- control group which received IP saline for 28 days showing + ve brown cytoplasmic of the epithelial cell of tubule with 25% (score 1) of field; B- induction group which induced glomerulonephritis by doxorubicin then received only IP saline for 28 days showing + ve brown cytoplasmic of the epithelial cell of tubule

with more than 76% (score 4) of field; C- treatment group which induced glomerulonephritis by doxorubicin then received IP of liraglutide 200 ug /kg/day for 28 days showing + ve brown cytoplasmic of the epithelial cell of tubule with more than 26% (score 2) of field. magnification(200x)

Fig 3: Bar chart displays the differences in the mean of BCL-2 expression score for all studied groups, data expressed as mean $\pm$ SEM., a Significantly different when compared with group A(control) at $P < 0.05$, b Significantly different when compared with group B(induction) at $P < 0.05$, c Significantly different when compared with group C(treatment) at $P < 0.05$

Figure 4: Microphotograph of immunohistochemical (IHC) staining of BCL-2 of rat kidney for: A- control group which received IP saline for 28 days showing + ve brown in proximal tubule with more than 34% (score 2) of field; B- induction group which induced glomerulonephritis by doxorubicin then

received only IP saline for 28 days showing + ve brown in proximal tubule with less than 33% (score 1) of field; C- treatment group which induced glomerulonephritis by doxorubicin then received IP of Liraglutide 200 ug /kg/day for 28 days showing + ve brown of the proximal tubule in 50% of cases with more than 34% (score 2) of field. With magnification (200x)

In this study, the animal induction group by doxorubicin without treatment showed a significant increase in TNF- α among epithelial cells of the tubule especially in the proximal. In contrast, the Liraglutide treatment group appears to be effective as an anti-inflammatory by reducing the expression of TNF- α in the epithelial cells which consider an inflammatory mediator for 28 days with a single daily dose. This result corresponds with Wang X et al. study (2018) which established that liraglutide reduces the expression of the gene, TNF- α and other inflammatory markers in animal induced by mesangial proliferative glomerulonephritis³⁶ agree with Ye Y et al. study (2019) who reported that renoprotective effect of liraglutide by inhibition TNF- α as an inflammatory factor in renal injury induced by high fats diet³⁷. Furthermore, in the STZ diabetic model of Zhou SJ et al. which used liraglutide as treatment³⁸ and exenatide therapy by with Sancar-Bas S et al.³⁹, both of them attenuate renal injury by reducing TNF- α and other inflammatory markers which stimulated diabetic nephropathy. Also, many studies showed an important role of TNF- α in renal disease, which deficiency showed reduced inflammation and albuminuria in high fat induced renal injury models⁴⁰. In some genetic diseases, TNF- α lacks macrophage that reduces renal inflammation in the diabetic nephropathy model⁴¹. Clinical evaluate increased levels of circulated TNF receptor in chronic kidney disease progression^{42,43}. Recently studies confirmed the inhibition of TNF- α by using pentoxifylline, which medically alleviates GFR reduction in diabetic nephropathy⁴⁴. Tubulointerstitial fibrosis is the final feature of inflammation and apoptosis in tubular epithelial injury⁴⁵. Also, previous studies explaining albumin toxicity like tubules and the long-term exposure of tubular cells may leads to apoptosis and ultimately fibrosis⁴⁶⁻⁴⁸. Normally, apoptosis is necessary for structural changes of tissues and unnecessary removal of cells, it was an important role in the formation of organisms during the lifetime and in cell response⁴⁹. BCL-2 playing vital role in the regulation of intrinsic pathway of apoptosis⁵⁰. This process is corrosive and needs energy so primarily seen in the mitochondrial cells in the epithelial cell of the tubular, especially the proximal and distal tubules but not in the glomerular⁵¹. In progressive kidney disease, a

significant link between proteinuria level as well as tubular degeneration has already been detected to be a more serious effect of apoptosis in proximal epithelial cells. In the cell line, albumin has reduced the expression of anti-apoptotic BCL-2 by increasing the Pax protein as pro-apoptotic in the proximal tubule of humans⁵². Other studies suggest the role of BCL-2 expression in the epithelial cell of the tubule to associate with apoptosis and interstitial fibrosis as a result of proteinuria and other oxidative stress^{53,54}. Doxorubicin model used to chronic kidney progression by reducing anti-apoptotic BCL-2 in the tubule, it was important to study the effect of Liraglutide therapy^{55,56}. In the current study, Liraglutide showed a significant increase of BCL-2 expression especially in the proximal tubule, and reduce tubular atrophy as a result of the anti-apoptotic effect by continuous protein loading on tubule by doxorubicin. In recent years, the efficacy of Liraglutide can be detected from apoptosis by upregulation of bcl-2 through GLP-1R in different tissues and protect neuron from apoptosis after ischemia induction⁵⁷. It was confirmed anti-apoptotic activity of Liraglutide by ameliorate endothelial dysfunction⁵⁸ promote osteoblast proliferation in the bone⁵⁹ protect from diabetic in high fat diet model⁶⁰ and prevent cardiotoxicity by chemotherapy agent doxorubicin⁶¹.

CONCLUSION

Liraglutide is effective in reducing inflammation and apoptosis associated with chronic renal development with GLP-1R recovery in the epithelial cell of the tubule as well renoprotection by glycemic control which is indirectly effective.

RECOMMENDATION

1. Study the effect of Liraglutide on different stimulation of diabetic nephropathy either protect or prevent progression.
2. Study the effect of Liraglutide on the intrinsic and extrinsic pathway of apoptosis

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CONFLICT OF INTEREST

Nil

AUTHORS' CONTRIBUTIONS

LHH, AAMG, and RIB carried out the literature review and draft the manuscript. LHH and AMZ participated in the collection of data and arranged it in tabular form. All authors read and approved the final manuscript.

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