

The potential beneficial effect of exenatide on cisplatin induced nephrotoxicity in non-diabetic rats

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Abstract

Background: Cisplatin is a major antitumor drug used for treatment of solid tumors. Nephrotoxicity is its main limiting side effect. Exenatide is described as an incretin mimetic polypeptide, it acts as a GLP-1 receptor agonist and its insulinotropic activity is mediated through binding to and stimulation of GLP-1 receptors on human pancreatic β -cells. Aim: the present work was designed to study the effect of exenatide on experimentally cisplatin induced nephropathy in non-diabetic rats. Materials and Methods: Thirty two male adult Sprague Dawley rats randomly divided into four groups, each consisted of 8 animals, and distributed as follows: Group I: (control) normal group and received saline vehicle. Group II: (cisplatin) group includes rats injected intraperitoneally by a single dose of cisplatin (6 mg/kg) to induce nephrotoxicity, received no treatment and were sacrificed 7 days after cisplatin administration. Group III: (exenatide) group: rats treated by exenatide (10 mg/kg/day s.c.) for 4 weeks. Group IV: (exenatide+cisplatin) group: rats treated by exenatide (10 mg/kg/day s.c.) for 4 weeks before induction of nephrotoxicity by cisplatin. The following parameters were measured: fasting blood glucose, serum urea, serum creatinine, reduced glutathione (GSH) level in the renal tissue, tumor necrosis factor- α (TNF- α) level in the renal tissue, renal blood flow changes and histopathological changes of the kidney. Results: Pretreatment with exenatide resulted in significant reduction in serum urea, serum creatinine level and renal TNF- α level compared to cisplatin group but still significantly higher than control group with significant increase in renal GSH level and renal blood flow compared to cisplatin group but still also significantly lower than control group. Cisplatin group showing tubular degeneration with infiltration of inflammatory cells while pretreatment with exenatide showing certain improvement in general histological structure with mild tubular degeneration and less inflammatory cell infiltration. Conclusion: it can be concluded that the use of exenatide improved the biochemical and histopathological changes that occur in the renal tissue by cisplatin.

Keywords: Antioxidant; Cisplatin; Exenatide; Nephrotoxicity.

1. Introduction

The kidney has a vital role in drug elimination. It is well known that the kidney is one of the organs with high blood flow; it is supplied by 25% of cardiac output. Therefore, the kidney is exposed to high concentrations of drugs and metabolites, giving a chance of drug toxicity induction. It is therefore not surprising that 25% of all cases of acute renal failure were resulted from drug administration (Declodet and Maartens, 2011). Cisplatin is an effective antitumor drug used for treatment of solid tumors. Nephrotoxicity is considered its main limiting side effect. Twenty percent of patients receiving high-doses of cisplatin have renal dysfunction. Several investigations were carried out to reveal the mechanism of this renal cell injury, and some studies suggest that inflammation, oxidative stress and apoptosis probably explain a part of this injury (Hasiyeti et al., 2013). Exenatide is described as an incretin mimetic polypeptide, it acts as a GLP-1 receptor agonist and its insulinotropic activity is mediated through binding to and stimulation of GLP-1 receptors on β -cells of pancreas (Koole et al., 2017). Exenatide is approved as an adjunctive S.C therapy to improve glycemic control in diabetic patients not achieving adequate glycemic control while taking metformin, a sulfonylurea, a thiazolidinedione, or a combination of these oral agents (Holman et al., 2017).

To the best of our knowledge, there are no studies concerning the possible renoprotective effect of exenatide in non-diabetic rats so, the present work was designed to study the effect of exenatide on experimentally induced nephropathy in non-diabetic rats in order to reveal the ability of exenatide to protect the kidney even in absence of hyperglycemia and diabetes mellitus.

2. Material and methods

2.1. Drugs and chemicals

- Exenatide: It was purchased from Sigma-Aldrich Chemical Co. It was supplied as a sterile solution for subcutaneous injection (250 μ g/ml).

- b) Cisplatin: (Oncotec Pharma Production GmbH, Germany). It is available as vials.
- c) All drugs and other chemicals used were of the high analytical grade.

2.2. Animals

Thirty two male adult Sprague Dawley rats weighing 150-200 g were obtained from Experimental Animal Breeding Farm, (Helwan-Cairo). They were caged 8 per cage in well ventilated place at room temperature. They allowed free water and standard food (pellets specific for rat feeding obtained from animal breeding farm) for 7 days for acclimatization.

2.3. Study design

The rats randomly divided into four groups, each consisted of 8 animals, and distributed as follows:

Group I: (control) normal group and received saline vehicle.

Group II: (cisplatin) group includes rats injected intraperitoneally by a single dose of cisplatin (6 mg/kg) to induce nephrotoxicity and received no treatment. Rats were sacrificed 7 days after cisplatin administration (Saad and Al-Rikabi, 2002).

Group III: (exenatide) group: rats treated by exenatide (10 mg/kg/day s.c.) for 4 weeks (Tanaka et al., 2014).

Group IV: (exenatide+cisplatin) group: rats treated by exenatide (10 mg/kg/day) s.c. for 4 weeks before induction of nephrotoxicity by cisplatin.

The following parameters were measured: fasting blood glucose, serum urea, serum creatinine, reduced glutathione (GSH) level in the renal tissue, tumor necrosis factor- α (TNF- α) level in the renal tissue, renal blood flow changes and histopathological changes of the kidney.

2.4. Procedural details

2.4.1. Measurement of renal blood flow

At the end of experiment, after complete anesthesia, the rats were put on their back. Then the abdominal cavity of each rat was opened through a median incision and the left renal artery was exposed. Bi-directional blood flowmeter with FFT analysis (Hadeco, Japan) was used. After setting the mode of pulsed Doppler blood flowmeter, we use ultrasonic gel on the probe top and turn the volume control to the maximum. The probe pressed softly to the measurement area and at an angle of 45-60°. After hearing the optimal sounds, we wait for 5 seconds without moving the probe then press the freeze key to freeze the waveform (Haywood et al., 1981) then blood samples were taken from the heart (Parasuraman et al., 2010). Samples were incubated at 37°C until blood clotted and then centrifuged at 3000 revolution per minute (rpm) for 15 min for separation of serum and stored at -20°C for biochemical analysis of serum urea and serum creatinine. Both kidneys were removed and the upper 1/3 of each kidney was put into a buffered 4% formaline fixation solution and processed with paraffin wax for histopathological examination. Sections (5 μ m) were stained with hematoxylin and eosin (Dury and Wallington, 1967). Another portion was used to prepare renal tissue homogenate to determine tissue level of reduced glutathione (GSH) and tumor necrosis factor alpha (TNF- α).

2.4.2. Measurement of Fasting blood glucose levels (F.B.G)

The fasting blood glucose level was measured on a Beckman Glucose Analyzer II. Separation of glucose was achieved by dimedone precipitation and expressed as milligrams per deciliter (Efendic et al., 1988).

2.4.3. Measurement of serum urea

Urea in the sample is hydrolyzed enzymatically into ammonia (NH₄) and carbon dioxide (CO₂). Ammonia ions formed reacts with salicylate and hypochlorite (NaClO), in presence of the catalyst nitroprusside, to form green indophenols. The intensity of the color formed is proportional to the urea concentration in the sample (Kaplan, 1984).

2.4.4. Measurement of serum creatinine

The assay is based on the reaction of creatinine with sodium picrate. Creatinine reacts with alkaline picrate forming a red complex. The time interval chosen for measurement avoids interference from other serum constituents. The intensity of the color formed is proportional to the creatinine concentration in the sample (Murray, 1984).

2.4.5. Measurement of reduced glutathione (GSH) level in renal tissue

Kidney homogenate preparation: a portion of isolated kidney tissue from each rat was homogenized in a 100 mmol KCl buffer (7.0 p H) containing EDTA 0.3 mM, All homogenates were centrifuged at 600 g for 60 minutes at 4°C and protein content of the supernate was measured and then used for biochemical assays (Noori et al., 2009). GSH level in renal tissues was measured by colorimetric method based on the reduction of 5, 5'- dithionitrobenzoic acid (DTNB) with GSH to produce a yellow compound. The reduced chromogens directly proportional to GSH concentration and its absorbance can be measured at 405nm. Values were expressed as nmol/mg protein.

2.4.6. Estimation of tumor necrosis factor alpha (TNF- α) level in renal tissue

TNF- α concentration in renal tissues was measured by using USA & Canada. R&D Systems, Inc kit, according to manufacturer,s protocol.

2.5. Statistical analysis

Results are presented as mean \pm standard deviation (mean \pm SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) to detect significant differences between the group means. Probability (P) values of < 0.05 were considered as statistically significant.

3. Results

3.1. Biochemical results

In this work single i.p injection of cisplatin (6 mg/kg) did not produce significant change on FBG level. Exenatide group showed insignificant change in serum urea, serum creatinine level, intrarenal GSH level, intrarenal TNF- α level and renal blood flow compared to control group. Cisplatin administration resulted in significant increase ($p < 0.05$) in serum urea, serum creatinine level and renal TNF- α level with significant reduction ($p < 0.05$) in renal GSH level and renal blood flow compared to control group. Pretreatment with exenatide resulted in significant reduction ($p < 0.05$) in serum urea, serum creatinine level and renal TNF- α level compared to cisplatin group but still significantly higher than control group with significant increase ($p < 0.05$) in renal GSH level and renal blood flow compared to cisplatin group but still significantly lower than control group.

Table (1): Effect of exenatide administration (10 mg/kg/day) s.c. for 4 weeks before induction of nephrotoxicity by cisplatin [induced by i.p injection of a single dose of cisplatin (6 mg/kg)] on FBG, Serum urea, Serum creatinine, intrarenal GSH, intrarenal TNF- α & Renal blood flow.

Groups	FBG mg/dl Mean \pm SD	Serum urea Mg/dl Mean \pm SD	Serum creatinine Mg/ dl Mean \pm SD	GSH nmol/mg protein Mean \pm SD	TNF α Ug/mg protein Mean \pm SD	Renal blood flow ml/Min Mean \pm SD
Control Group	86 \pm 3.6	27.63 \pm 1.5	0.67 \pm 0.03	43.12 \pm 0.9	49.64 \pm 0.16	11.3 \pm 0.8
Cisplatin Group	84 \pm 3.2	89.35 \pm 0.17 ^a	1.94 \pm 0.02 ^a	23.59 \pm 1.2 ^a	180.45 \pm 0.18 ^a	2.5 \pm 1.4 ^a
Exenatide Group	85 \pm 2.4	28.23 \pm 1.2 ^b	0.7 \pm 0.02 ^b	41.33 \pm 1.8 ^b	49.72 \pm 0.13 ^b	10.8 \pm 0.9 ^b
Exenatide + Cisplatin Group	83 \pm 3.6	45.37 \pm 0.16 ^{a,b,c}	0.97 \pm 0.09 ^{a,b,c}	35.41 \pm 0.16 ^{a,b,c}	75.67 \pm 0.02 ^{a,b,c}	6.5 \pm 0.6 ^{a,b,c}

Data are presented as mean (M) \pm SD

a: Significant difference versus control at $p < 0.05$

b: Significant difference versus cisplatin group at $p < 0.05$

c: Significant difference versus exenatide group at $p < 0.05$

3.2. Histopathological results

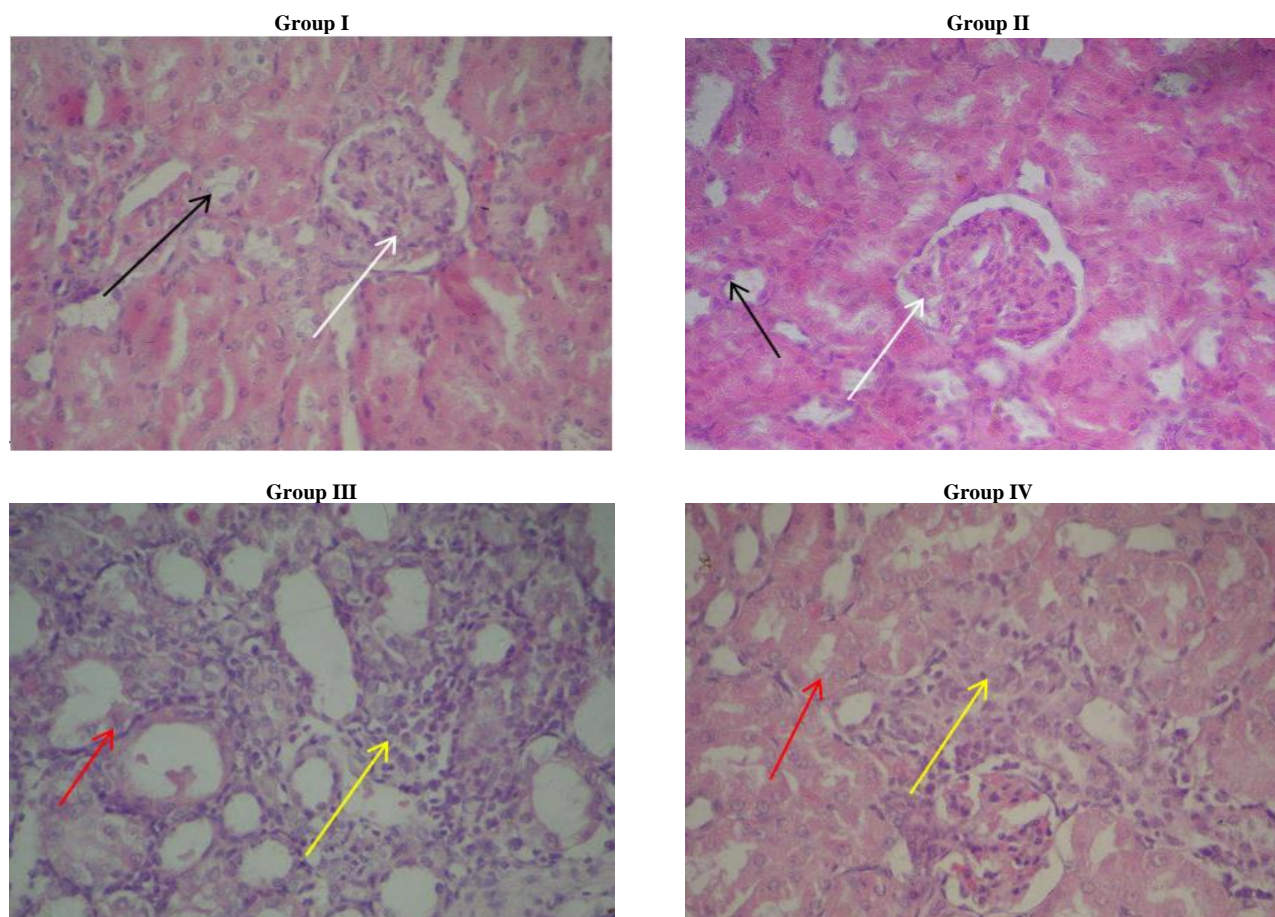


Fig. 1: Photomicrographs of Cut Sections in the Kidney of Different Groups (H&Ex40) Showing: Normal Lobulation of Glomeruli (White Arrow) & Normal Renal Tubules (Black Arrow) in Group I (Normal Control) and Group II (Exenatide Group) While in Group III (Cisplatin Group) Showing Sever

Inflammatory Cell Infiltration (Yellow Arrow) with Necrosis of Renal Tubules (Red Arrow). on the Contrary, Group IV (Exenatide + Cisplatin) Showing General Improvement of the Histopathological Picture Regarding Inflammatory Cell Infiltration (Yellow Arrow) And Necrosis of Renal Tubules (Red Arrow).

Histopathological examination revealed that both control group and exenatide group showed normal lobulation of glomeruli & normal renal tubules. None treated group (cisplatin group) showing tubular degeneration with infiltration of inflammatory cells. Rats treated with exenatide before induction of nephropathy by cisplatin showing certain improvement in general histological structure with mild tubular degeneration and less inflammatory cell infiltration Fig. (1).

4. Discussion

In this work single intraperitoneally injection of cisplatin (6 mg/kg) did not produce significant change on FBG level, this is consistent with (Bhoomika et al., 2013) while single intraperitoneally injection of cisplatin (6 mg/kg) resulted in significant increase of serum creatinine, serum urea, and this is in line with (Mohamed et al., 2014). TNF- α level and reduced glutathione (GSH) level in renal tissue were measured to demonstrate the pathophysiological mechanisms involved in cisplatin induced nephrotoxicity. Single intraperitoneally injection of cisplatin (6 mg/kg) resulted in significant increase of TNF- α level in renal tissue with significant reduction of reduced glutathione (GSH) level in renal tissue, this in line with (Ozkok and Charles, 2014).

The mechanism for cisplatin renal cell injury was demonstrated by Hasiyeti et al. (2013) who reported that cisplatin is a major antitumor drug used for the treatment of solid tumors.

The mechanism involved in cisplatin-induced nephrotoxicity includes inhibition of protein synthesis, DNA damage, mitochondrial injury and apoptotic cell death in renal tubules. Cisplatin reduces the bioavailability of nitric oxide (NO), which up regulates monocyte chemo-attractant protein-1, connective tissue growth factors and tumor necrosis factor- α , which play a central role in renal damage. Moreover, cisplatin generates excessive reactive oxygen species (ROS) which stimulate mitogen activated protein kinase leading to renal injury and inflammation. As well as glucose-6-phosphate dehydrogenase and hexokinase activity are stimulated by cisplatin resulting in increasing free radical production and decrease of antioxidant production (Pan et al., 2014). Yao et al. (2007) mentioned that conversion of cisplatin to nephrotoxic molecules in the proximal tubule cells is required for cell injury. The highest concentration of cisplatin is found in cytosol, mitochondria, nuclei, and microsomes. Cisplatin is conjugated to glutathione and then metabolized to a reactive thiol, a potent nephrotoxin. Cisplatin can form monohydrated complexes by hydrolytic reactions; the monohydrated complex is more toxic to the renal cells than cisplatin.

In this work, single i.p injection of cisplatin (6 mg/kg) resulted in significant reduction of renal blood flow, this in agreement with (Bagnis et al., 2000). This result could be explained by Tusgaard et al. (2011) who demonstrated that cisplatin resulted in inhibition of cyclooxygenase enzyme with subsequent decrease in PGE2 in the kidney this may explain how cisplatin administration resulted in reduction of renal blood flow. Exenatide is believed to exert its beneficial effect in the endocrine pancreas by maintaining optimal β -cell mass and function by increasing the β -cell gene expression. In turn, this may lead to β -cell proliferation and neogenesis, thereby inhibiting apoptosis in pancreatic islet cells (Xu et al., 2009).

Administration of exenatide before induction of nephrotoxicity by cisplatin resulted in improvement of serum urea, serum creatinine and renal blood flow, this is in line with (Wang et al., 2019) who concluded that exenatide can mitigate the renal injury in diabetic rats. as well as Marcel et al. (2016) demonstrated that exenatide administration in healthy overweight men increases GFR, ERPF and glomerular pressure, probably by reducing afferent renal vascular resistance in an NO-dependent manner. To demonstrate the possible mechanisms of exenatide improving effect on kidney parameters, TNF- α level and reduced glutathione (GSH) level in renal tissue were measured. Treatment with exenatide (10 mg/kg/day) s.c for 4 weeks before induction of nephrotoxicity by cisplatin resulted in improvement of TNF- α level in renal tissue this result is in agreement with Chaudhuri et al. (2012) who reported that exenatide exerts a rapid anti-inflammatory effect at the cellular and molecular level when administered to obese patients. Moreover, administration of exenatide (induction of nephrotoxicity by cisplatin resulted in improvement of reduced glutathione (GSH) level in renal tissue pointing to antioxidant activity. Our results were in consistence with El-Gohary and Said (2015) who cited that exenatide has a protective role against ischemia reperfusion in diabetic rats. On the basis of the above mentioned data the present work can postulate that therapy with cisplatin, it is recommended to administrate exenatide before or concomitantly in order to protect against potential induction of nephrotoxicity by cisplatin through its anti-inflammatory and antioxidant activities. Moreover, this renoprotective effect of exenatide is not attributed to its antidiabetic action but it is a direct action regardless of presence of hyperglycemia.

5. Conclusion

From the results of the present study it can be concluded that the use of exenatide improved the biochemical and histopathological changes that occur in the renal tissue by cisplatin.

6. Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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