Ameliorating effect of cobalt chloride on renal failure and glucose lowering effect in diabetic nephropathy induced in uninephrectomized diabetic rat

Deshmukh Aaishwarya,[1,2] Patel Jayvadan,[3] Prajapati Ashish[4]

[1]Department of Pharmacology, Shankersinh Vaghela Bapu Institute of Pharmacy, Unava- Gujarat; [2]Bhagwant University, Ajmer, Rajasthan; [3]Department of Pharmaceutics, Nootan Pharmacy College, Visnagar-Gujarat; [4]Department of Quality Assurance, Shree Sarvajanik Pharmacy College, Mehsana-Gujarat, India

Abstract

Diabetic nephropathy, a progressive development of renal insufficiency in the setting of hyperglycemia, is the major single cause of chronic renal failure (CRF) in which hypoxia plays a critical role. This study evaluated the efficacy of cobalt chloride, a Prolyl 4-hydroxylase (PHD) inhibtor, in amelioration of renal injury, as well as its effect on hyperglycemia in uninephrectomized diabetic rat. The effect of cobalt chloride ($CoCl_2$,10 mg/kg, i.p. OD) treatment on plasma urea, creatinine, uric acid, electrolytes like sodium, potassium, chloride, as well as blood glucose levels were checked along with measurement of the dry weight of contralateral kidney in different groups. A significant rise in plasma urea, creatinine and uric acid levels was observed in uninephrectomized diabetic rat. Cobalt chloride (10 mg/kg, i.p. OD) treatment for seven continuous days, followed by intermittent dosing for 30 days, showed improvement in renal injury by a significant fall in the plasma urea, creatinine and uric acid levels with restoration to partially normal values as compared to an uninephrectomized uninephrectomized diabetic group. A significant change in plasma electrolyte levels was observed which was partially normalized in the cobalt chloride group along with a reduction in the dry weight of kidney. A significant decrease in the blood glucose level was observed in the $CoCl_2$ treated group as compared to the uninephrectomized diabetic group. Our study shows the effect of $CoCl_2$ in amelioration of renal failure and antihyperglycemic effect.

Introduction

Diabetic nephropathy is the most recurrent cause of end-stage renal disease (ESRD) in developed countries.[1] Hypoxia plays a critical role in the pathogenesis and progression of chronic renal failure.[2] Regulatory mechanisms are exerted by hypoxia by influencing gene expression through a family of transcription factors known as hypoxia inducible factors (HIFs).[3] HIFs are heterodimers composed of two different oxygen-dependent α-subunits and a constitutive β-subunit, regulation of which is exerted by oxygen-dependent proteolysis of the a-subunit. HIFs govern transcriptional activity of a host of genes which are cell/tissue protective.[4-7] Irrespective of the underlying cause, there is growing evidence to suggest involvement of regional renal hypoxia in the pathophysiology of acute kidney injury (AKI).[8,9] Furthermore, chronic hypoxia appears to play an important role in the progression of chronic renal failure.[10] Diabetes is certainly a chief risk factor and a leading cause of end-stage kidney disease in developed countries.[11]

Compelling evidence points to induction of an early hypoxia in diabetic kidneys. A study utilizing blood oxygen level dependent (BOLD)-MRI has shown that kidneys of streptozotocin-induced diabetic rats are hypoxic even at an early stage.[12] In a hypertensive type 2 diabetic nephropathy rat model (SHR/NDmcr-cp), it has been documented that the accumulation of pimonidazole, a compound incorporated into hypoxic cells leads, to renal hypoxia.[13] Furthermore, a study of intrarenal haemodynamics in human type 2 diabetic patients showed correlation between a decreased peritubular capillary flow and tubular dysfunction, thus supporting pathogenic role of chronic hypoxia in diabetic kidney.[14] Under hypoxic conditions, HIF instead of being hydroxylated, transactivates in the nucleus a host of genes involved in the adaptation to hypoxia.[15] Interestingly, cobalt inhibits HIF degradation by PHDs, thus enhancing HIF activity.[16]

This study was designed to explore the role of cobalt chloride induced augmentation on HIF activity. While our ultimate goal was to treat chronic hypoxia in diabetic nephropathy induced chronic renal failure, in this study we used a model of unilateral nephrectomy followed by STZ treatment to investigate the effects of our approach in ameliorating renal failure. We tested a hypothesis that administration of cobalt chloride retards the progression of renal failure and improves renal function with a significant decrement in the plasma glucose levels to which we obtained consistent results.

Correspondence: Aaishwarya B. Deshmukh, Department of Pharmacology, Shankersinh Vaghela Bapu Institute of Pharmacy, Unava-Gujarat, India.
Tel. +91.79.2397.6171 - Fax: +91.79.2397.6172.
E-mail: aaishwarya.22@gmail.com

Key words: chronic hypoxia, hypoxia inducible factor-1α, cobalt chloride, diabetic nephropathy.

Received for publication: 29 September 2011.
Revision received: 6 December 2011.
Accepted for publication: 13 December 2011.

Drugs and Therapy Studies 2012; 2:e5
doi:10.4081/dts.2012.e5

Materials and Methods

Animals

Eight-week old Sprague-Dawley (SD) rats from Torrent Research Centre, Bhat, Gujarat, India, were maintained in well controlled supplied air, humidity (<70%) and temperature (<30° C) with a 12 h day and night cycle at the Central Animal Facility, Nootan Pharmacy College, Visnagar, India (CPCSEA n. 1244/ac/08/CPCSEA). Each rat was individually housed in a plastic box cage and had free access to untreated tap water and standard rat chow (Pranav Agro Ltd., Ahmedabad, India) according to the norms of IAEC (Institutional Animal Ethics Committee) and CPCSEA. After surgery, animals were inspected daily for level of activity and healing of surgical wounds.

Induction of diabetic nephropathy by unilateral nephrectomy followed by STZ administration

All rats were initially subjected to removal of the right kidney to accelerate the development of diabetic nephropathy, as described previously.[17] Male SD rats were anesthetized by intraperitoneal injection of a mixture of ketamine (60 mg/kg ip) and xylazine (6.5 mg/kg ip). The anesthetized rats were placed on their ventral surface on a homeothermic heating pad; a dorsoventral incision parallel to spinal cord was made in the skin and muscle layer. The right kidney was freed gently of connective tissue and pulled out by grasping the perirenal fat. A silk thread was passed from just above between the renal artery and ureter, and a knot was tightened, the right kidney was removed giving

a cut before the knots at the artery and ureter, respectively, and the cavity closed by double sutures of muscle and skin, allowing animals to recover. Post-operative care included administration of normal saline (2 mL/animal), application of povidone, buprenorphine HCl (0.03 mg/kg, s.c. o.d./3 dyas) and benzyl penicillin (20,000 IU/kg, im, bid/3 days).

One week post surgery, a single intraperitoneal injection of streptozotocin (Future Delhi, India) (40 mg/mL in 0.1 moL/1 phosphate/0.4 moL/L citrate buffer, pH 6.5) at a dose of 45 mg/kg body weight was injected in the uninephrectomized animals. The animals had free access to water at all times and feed was available *ad libitum*. Diagnosis of diabetes was established 48 h after the streptozotocin injection by determination of the tail vein blood glucose concentration by using a glucometer.[18] Any streptozotocin-treated animal which at this time had a 4-6 h fasting blood glucose concentration of less than 200 mg/dL was eliminated from the study.

Sham surgery

Animals in the Sham group underwent the same surgical procedure as above but the kidney was neither cut or removed, only fatty material was removed and the kidneys were touched with forceps and threads. Similar post-operative care procedures were followed. After surgery, rats were placed individually in cages with free access to food and water.

Grouping of animals

Animals were randomized according to body weight and divided into four groups as follows before surgery: i) control animals (n=06); ii) animals with sham surgery (n=06); iii) animals with unilateral nephrectomy followed by STZ administration (n=06) without treatment (uninephrectomized diabetic); iv) animals with unilateral nephrectomy followed by STZ administration (n=06) with $CoCl_2$ treatment (treatment group).

Estimation of parameters

Biochemical parameters

Blood samples were collected at basal and after 1, 3 and 5 weeks of study from a sublingual vein. The plasma was separated by centrifugation at 4000 rpm for 10 min at 4°C and was used to estimate creatinine (Jaffé method), uric acid and urea (Kinetic UV test) by semi-autoanalyzer (Erba Mannheim's) and methods described previously.[19]

Plasma electrolytes

Plasma sodium (Na) and potassium (K) concentrations were determined by standard flame photometry and chloride (Cl) by the method of Schales and Schales.[20]

Estimation of plasma glucose (mg/dL)

Blood was withdrawn from tail vein and glucose level was estimated at basal, after 48 h and at the fifth week by commercially available glucose kits (Horizon®, OneTouch – Johnson & Johnson, India) based on a glucose oxidase method.[21]

Change in the dry contralateral kidney weight

Changes in dry weight of contralateral kidney were estimated by comparing the weight of the kidney with that from an animal which had undergone a sham nephrectomy. Change in the weight of $CoCl_2$ treatment group was estimated and compared with that of the uninephrectomized diabetic group.[22]

Cobalt chloride preparation and treatment

Cobalt chloride hexahydrate ($CoCl_2.6H_2O$) was obtained from SD Fine Chemicals, Mumbai, India. Cobalt chloride hexahydrate is magenta colored, in a crystalline powdered form, with great solubility in normal saline. Based on a study and its LD_{50} value in rat by intraperitoneal route, a 10 mg/kg dose was selected for use in this study.[23] Cobalt chloride hexahydrate solution was freshly prepared in normal saline by weighing 200 mg quantity of cobalt chloride hexahydrate in 20 mL normal saline to make a $CoCl_2$ solution with a concentration 10 mg/mL. Animals were treated with cobalt chloride at the dose of 10 mg/kg, ip, OD for 30 days with continued dosing for one week, followed by intermittent dosing at days 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 and 30. The animals in groups I and II were administerd the equivalent amount of citrate buffer intravenously as well as normal saline (1 ml/kg) intraperitoneally, and group III received normal saline (1 ml/kg, ip).

Statistical analysis

Results were expressed as mean±SEM. Results from each group at each period were compared with the respective value of that period of the control group. All comparisons were carried out on software Graphpad, PRISM®, version 5 using one-way analysis of variance (ANOVA) followed by a Tukey test and unpaired t-test, depending on the type of competition. $P \leq 0.05$ was considered statistically significant and $P<0.001$ highly significant.

Result

Effect of cobalt chloride treatment on general features of animals during the study

The unilateral nephrectomy followed by STZ administration induced moderate to severe CRF in rats. The biochemical parameters for their confirmation were measured as basal and after 48 h of STZ treatment and subsequently after 1, 3 and 5 weeks. CRF were significantly induced in the diabetic nephropathy model, while control animals, as well as sham operated animals, remained normal throughout the study period. A gradual decrease in the body weight of diabetic animals was observed with a significant effect observed at the fifth week (*data not shown*).

Effect of cobalt chloride treatment on plasma creatinine, urea and uric acid

Plasma creatinine, urea and uric acid were estimated in the control, uninephrectomized diabetic and $CoCl_2$ groups. Animals in the uninephrectomized diabetic group showed a significant and stable rise in their plasma urea, creatinine and uric acid. A significant rise was observed in uninephrectomized diabetic group as compared to control after 48 h of STZ administration till the end of the 5th week at which a maximum difference was observed between these two groups. The animals which died during the study from the diabetic group also had a great increase in levels of renal biochemical parameters before their death (Figure 1).

Effect of cobalt chloride treatment on electrolyte levels

Plasma levels of sodium, potassium and chloride were measured. We observed a significant change in the electrolyte levels in group III. A significant decline in sodium level (135.55±6.36) was observed after 48 h of STZ administration which was normalized over the following week, after which a gradual decline in the sodium level was observed which was significant at the end of the 5th week. A rise in plasma potassium levels (6.60±0.35) was observed which was at its maximum at the end of the 3rd week and was differed significantly from the control group. There was a consistent fall in the plasma chloride levels in the uninephrectomized diabetic group (82.73±3.74) that was significantly different

from the control group (94.37±3.79). Treatment with cobalt chloride at the dose of 10 mg/kg, ip reduced the renal parameters, and partially normalized values were observed at the end of the study. A decline in animal mortality was also observed which was found in the diabetic group due to a worsening of renal function (Table 1).

Each value is represented as mean±S.E.M. (n=06). *P<0.05 *vs* control, °P<0.01 *vs* control, #P<0.001 *vs* control, §P<0.01 *vs* uninephrectomized diabetic.

Blood glucose level

Blood glucose level was estimated in plasma from control, uninephrectomized diabetic and $CoCl_2$ treatment groups at the start of the study, 48 h after STZ administration and at the end of the 5th week. No significant difference in basal values for glucose was observed in control or in uninephrectomized diabetic groups. But a significant rise was observed in the diabetic group after 48 h of STZ administration, with a persistent rise following thereafter; elevated plasma glucose levels were found in uninephrectomized diabetic group throughout the study. Treatment with $CoCl_2$ (10 mg/kg i.p.) decreased the plasma glucose level, although normalization of glucose was not observed in the treatment group. However, a significant difference was observed in plasma glucose levels in both groups (Figure 2).

Change in the dry contralateral kidney weight

Changes in dry weight of kidney of uninephrectomized diabetic and $CoCl_2$ group were estimated by comparing the weight of the kidney with that from an animal which had undergone a sham nephrectomy. A significant rise in the kidney weight was observed in diabetic group as compared to the sham group and a decrease in kidney weight was observed in $CoCl_2$ group (Figure 3).

Discussion

Although conventional treatments such as insulin and other antidiabetic drugs are used to reduce blood glucose levels and complications of diabetes, there is still a therapeutic need for effective drugs.[24] Diabetic nephropathy is one of the complications of diabetes and involves progressive development of renal insufficiency in the setting of hyperglycemia. Diabetic nephropathy is now a major single cause of end-stage renal failure in many countries. Reliable animal models of diabetic renal injury may be a valuable tool for identifying the molecular mechanisms and for the pre-clinical development of new therapeutic strategies.[25]

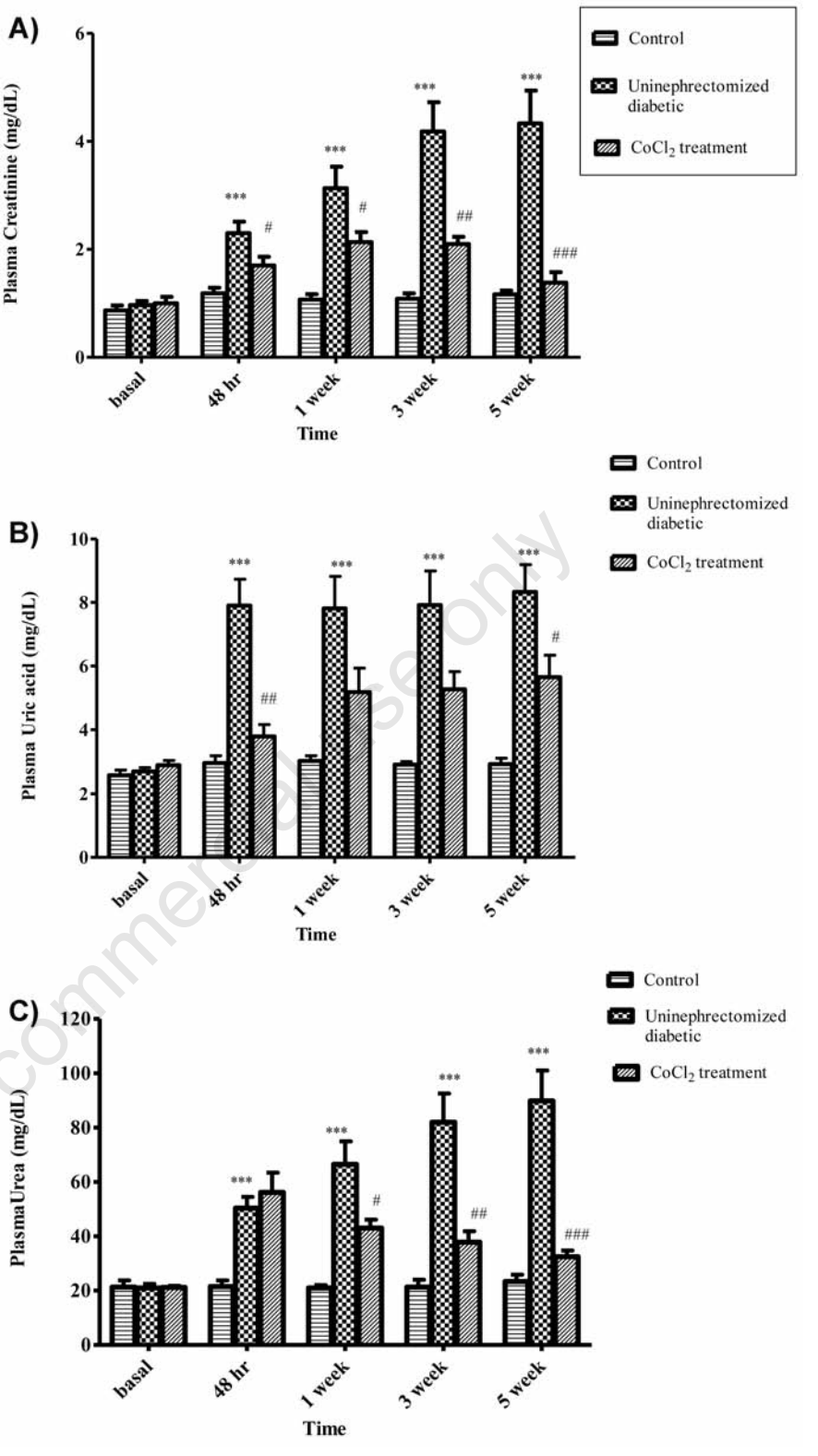

Figure 1. Effect of cobalt chloride (10mg/kg i.p.) treatment for 30 days on biochemical parameters in control, uninephrectomized diabetic and $CoCl_2$ treatment group. (A) Plasma creatinine (mg/dL). (B) Plasma Uric acid (mg/dL) and (C) plasma uric acid (mg/dL). Each value is expressed as mean±S.E.M. (n=06). ***P<0.001 *vs* control, #P<0.05 *vs* uninephrectomized diabetic, ##P<0.01 *vs* uninephrectomized diabetic, ###P<0.001 *vs* uninephrectomized diabetic.

Unilateral nephrectomy followed by streptozotocin (STZ) administration induced diabetes in rat and is a well documented model of experimental diabetes. Previous data show that the type of diabetes and characteristics differ with the dose of STZ employed and the animal species used. STZ is a pancreatic β cell toxin that induces rapid and irreversible necrosis of β cells of langerhans.[26] Moreover, STZ-induced diabetes in rodents results in development of nephropathy similar to early stage clinical diabetic nephropathy, which accelerates the progression of renal injury.[27,28] Uninephrectomy results in enlargement of the remaining kidney, further increased by the development of diabetes. A study demonstrated that uninephrectomy increases glomerular capillary pressure in SHR rats which promotes diabetic glomerular injury.[29] In a study by Utimura *et al.*, uninephrectomized (right nephrectomy) male wistar rats were made diabetic by a single intravenous injection of STZ (65 mg/kg) and blood glucose assessed two days later.[30] The blood glucose was then maintained between 300-400 mg/dL for the next eight months with insulin treatment. We employed this model to induce diabetic nephropathy which eventually leads to chronic renal failure. An imbalance between oxygen supply and consumption disturbs local metabolism and leads to tissue hypoxia. There is ompelling evidence to show that chronic hypoxia in the kidneys is the end result of multiple processes and mechanisms in patients with chronic renal disease.[31] In spite of the fact that blood flow to the kidney is relatively high, the presence of oxygen shunt diffusion between arterial and venous vessels that run in close parallel approximation keeps renal tissue oxygen tension relatively low, suggesting hypoxia as one such determinant in the sensitivity of the kidney to changes in oxygen delivery.[32,33] The hypoxia-inducible factor (HIF) is a heterodimeric nuclear factor, which is a crucial intermediate in the defense mechanisms against hypoxia, and its activation might offer a promising approach to the protection of hypoxic tissues by inducing a broad and coordinated downstream reactions. HIF is composed of two subunits, an oxygen-sensitive HIF-alpha subunit and a constitutively expressed HIF-beta subunit (also called ARNT, the aryl hydrocarbon receptor nuclear translocator). HIF stability is radically reduced by prolyl hydroxylases (PHDs) that induces oxygen-dependent hydroxylation of proline residus within the HIF proteins. The von Hippel Lindau tumor suppressor protein (pVHL) is then recruited by hydroxylated HIF, which in turn tags HIF with ubiquitin groups and targets it for degradation within the proteasome. However, under hypoxic conditions, HIF is not hydroxylated but is transactivated in the nucleus, activating a host of genes involved in the adaptation to hypoxia.[15] Furthermore, HIF activation is suboptimal in renal disease and this strategic route is consequently wide open to discussion with a wide range of evidence from a variety of studies.[16,34] Interestingly, cobalt inhibits HIF degradation by PHDs, thus enhancing HIF activity.[35] Data show that cobalt ameliorated renal failure in an obese, hypertensive type 2 diabetes rat model independent of metabolic status and blood pressure. The effect of $CoCl_2$ was attributed to the upregulation of HIF and HIF-regulated genes, and to a mitigated advanced glycation and oxidative stress.[36] A similar effect of cobalt has been previously reported in other renal injury animal models.[37-39] However, renoprotective mechanisms of cobalt remain elusive.[16] Conversely, a study demonstrated that treatment of STZ-induced diabetic rats with $CoCl_2$ results in a signifi-

Table 1. Effect of cobalt chloride (10mg/kg, i.p.) treatment for 30 days on electrolyte levels.

Parameter	Time period	Control group	Uninephrectomized diabetic group	$CoCl_2$ treatment group
Plasma Sodium (mMol/L)	basal	140.42±1.91	140.80±3.42	140.25±2.17
	48 aftre STZ	142.37±2.26	135.55±6.36*	139.51±4.74
	1 week post treatment	140.78±2.45	141.22±2.44	140.86±2.41
	3 week post treatment	140.67±3.73	138.87±4.12	142.43±4.49
	5 week post treatment	140.13±3.99	136.92±3.17	139.43±2.36
Plasma Potassium (mMol/L)	basal	4.79±0.80	4.74±0.76	4.84±1.02
	48 hrs aftre stz	4.66±0.79	6.13±0.50°	5.72±0.53
	1 week post treatment	5.12±0.65	6.00±0.63*	5.82±0.59
	3 week post treatment	4.93±0.53	6.60±0.35°	5.85±0.95
	5 week post treatment	5.57±0.57	6.20±0.79	5.89±0.43
Plasma Chloride (mMol/L)	basal	95.57±4.01	93.95±4.58	94.60±5.00
	48 hrs aftre stz	93.96±4.30	92.05±3.45	91.08±4.43
	1 week post treatment	94.22±4.03	87.39±5.09*	86.95±9.65
	3 week post treatment	93.12±5.63	89.14±7.16	91.32±7.43
	5 week post treatment	94.37±3.79	82.73±3.74#	93.90±5.77§

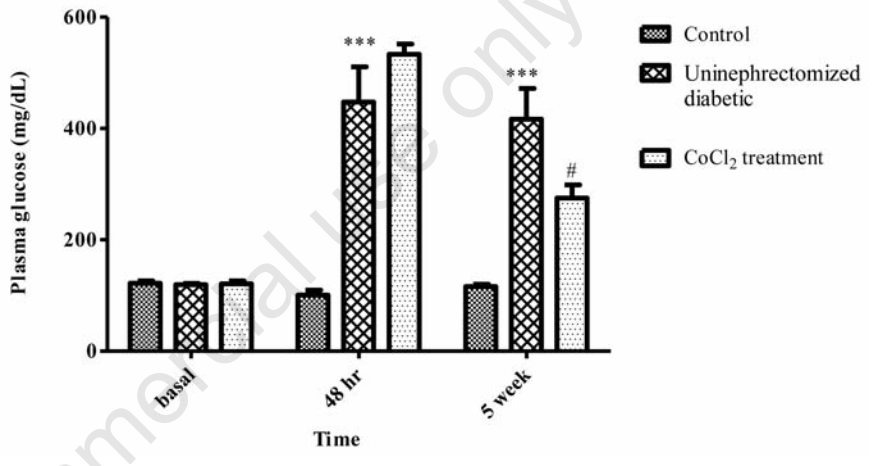

Figure 2. Effect of cobalt chloride (10mg/kg i.p.) treatment for 30 days on blood glucose in control, uninephrectomized diabetic and $CoCl_2$ treatment group. Each value is expressed as mean±S.E.M. (n=06). #P <0.05 *vs* uninephrectomized diabetic, ***P<0.001 *vs* control.

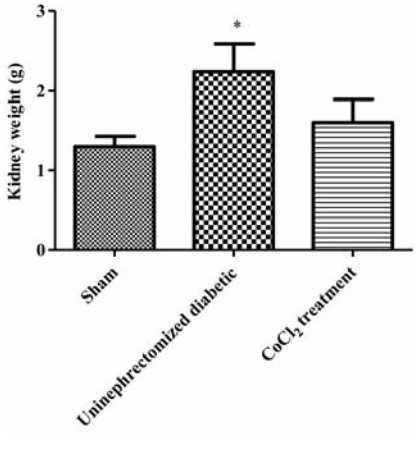

Figure 3. Effect of cobalt chloride (10 mg/kg, i.p.) treatment for 30 days on contralateral kidney weight in sham, uninephrectomized diabetic and $CoCl_2$ treatment group. Each value is expressed as mean±S.E.M. (n=06) *P <0.05 *vs* control.

cant reduction in the serum glucose concentration.[40] Thus cobalt chloride treatment has immense possibilities for renal protection and reducing hyperglycemia in rats which may establish HIF activation, as well as PHD inhibitors, as a newer therapeutic strategy for diabetic nephropathy. The present study was prompted by the earlier observations, and we aimed to study the renal protective as well as anti-hyperglycemic effect of $CoCl_2$ treatment in uninephrectomized rat.

The results of this study demonstrated that uninephrectomized STZ diabetic nephropathy leads to chronic renal failure as was found from a significant rise in plasma creatinine, uric acid and urea compared to the control group. Based on the results from animal experiments of previous studies, we hypothesized that suboptimal HIF activation as a consequence of chronic hypoxia might be increased by an agent inhibiting PHD and thus stabilizing HIF. Cobalt chloride, being a non-specific inhibitor of PHD, was employed in the study to show the effect on reducing renal failure; a significant improvement in the renal functioning was achieved based on the reduction in levels of biochemical parameters such as plasma urea, creatinine and uric acid. $CoCl_2$ treatment reduced the kidney weight in animals showing that the inflammation-induced hypertrophy is decreased with the treatment. Plasma electrolytes are essential for assessing normal renal functions. A significant change in potassium and chloride levels was observed in the uninephrectomized diabetic group that was restored in the $CoCl_2$ group. Furthermore, significantly decreased blood glucose from $CoCl_2$ treatment supported the proposition that $CoCl_2$ may mitigate hyperglycemia in diabetic animals. This is, however, a preliminary study emphasizing the need for further detailed research. The present data demonstrate that cobalt chloride, an HIF activator through the inhibition of PHDs, exhibits unique properties of improvement of chronic hypoxia and thereby mitigates the development of diabetic nephropathy as well as hyperglycemia, as shown by improved pathological changes and decreased blood glucose.

Regrettably, the beneficial effects of cobalt in diabetic patients with nephropathy are limited because of its toxicity, but accessibility of non-toxic PHD inhibitors or HIF activators might open new therapeutic opportunities.[41] Taken together, our data provide indications that HIF is an attractive target for protection against diabetic nephropathy, along with an anti-hyperglycemic effect requiring investigation also into its role in non-hypoxic kidney injuries. In conclusion, therapeutic strategies against hypoxia could be effective in diabetic conditions.

References

1. O'Connor AS, Schelling JR. Diabetes and the kidney. Am J Kidney Dis 2005;46:766-73.
2. Griffin KA, Picken M, Bidani AK. Methods of renal mass reduction is a critical modulator of subsequent hypertension and glomerular injury. J.Am.Soc. Nephrol 1994;4:2023-34.
3. Rosenberger C, Mandriota S, Jurgensen JS, et al. Expression of hypoxia-inducible factor-1 alpha and -2alpha in hypoxic and ischemic rat kidneys. J Am Soc Nephrol 2002;13:1721-32.
4. Maxwell PH. HIF-1's relationship to oxygen: simple yet sophisticated. Cell Cycle 2004;3:156-9.
5. Metzen E, Ratcliffe PJ. HIF hydroxylation and cellular oxygen sensing. Biol Chem 2004;385:223-30.
6. Semenza GL. Hydroxylation of HIF-1: oxygen sensing at the molecular level. Physiology 2004;19:176-82.
7. Rosenberger C, Rosen S, Heyman S. Current understanding of HIF in renal disease. Kidney Blood Press Res 2005;28:325-40.
8. Eckardt KU, Bernhardt WM, Weidemann A, et al. Role of hypoxia in the pathogenesis of renal disease. Kidney Int Suppl 2005;99:S46-S51.
9. Rosenberger C, Rosen S, Heyman SN. Intrarenal oxygenation in acute renal failure. Clin Exp Pharmacol Physiol 2006;33:980-88.
10. Nangaku M. Chronic hypoxia and tubulointerstitial injury: a final common pathway to end stage renal failure. J Am Soc Nephrol 2006;17:17-25.
11. Jones CA, Krolewski AS, Rogus J, et al. Epidemic of end-stage renal disease in people with diabetes in the United States population: do we know the cause? Kidney Int 2005;67:1684-91.
12. Ries M, Basseau F, Tyndal B, et al. Renal diffusion and BOLD MRI in experimental diabetic nephropathy. Blood oxygen level-dependent. J Magn Reson Imaging 2003;17:104-13
13. Izuhara Y, Nangaku M, Inagi R, et al. Renoprotective properties of angiotensin receptor blockers beyond blood pressure lowering. J Am Soc Nephrol 2005;16:631-41.
14. Futrakul N, Vongthavarawat V, Sirisalipotch S, et al. Tubular dysfunction and hemodynamic alteration in normoalbuminuric type 2 diabetes. Clin Hemorheol Microcirc 2005;32:59-65.
15. Semenza GL. HIF-1, O(2), and the 3 PHDs: how animal cells signal hypoxia to the nucleus. Cell 2001;107:1-3.
16. Salnikow K, Donald SP, Bruick RK, et al. Depletion of intracellular ascorbate by the carcinogenic metals nickel and cobalt results in the induction of hypoxic stress. J Biol Chem 2004;279:40337-44.
17. Anderson S, Rennke HG, Brenner BM. Nifedipine versus fosinopril in uninephrectomized diabetic rats. Kidney Int 1992;41:891-7.
18. Huggett AStG, Nixon DA. Enzymic determination of blood glucose. Biochem J 1957;66:12P.
19. Bartels H, Bohmer M, Heieri C. Serum creatinine determination without protein precipitation. Clin Chem Acta 1972;37:193-6.
20. Schales P, Sehales SS. A simple and accurate method for the determination of chloride in biological fluids. J. Biol. Chem 1941;140:879-84.
21. Trinder, P. Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. Ann. Clin. Biochem 1969;6;24-25.
22. Ujihara M, Nomura K, Toraya S, Demura H. Uninephrectomy inducing quantitative and qualitative changes in LH isoforms in the male rat. Acta Endocrinologica 1990;123:365-70.
23. Pawlak D, Koda M, Pawlak S, et al. Contribution of quinolinic acid in the development of anemia in renal insufficiency. Am J Physiol Renal Physiol 2003;284:693-700.
24. Vasudevan H and McNeill JH. Chronic Cobalt Treatment Decreases Hyperglycemia in Streptozotocin-Diabetic Rats. Bio Metals 2007;20:129-34.
25. Tesch GH, Allen TJ. Rodent models of streptozotocin-induced diabetic nephropathy. Nephrology 2007;12:261–6.
26. Arora S, Ojha SK, Vohora D. Characterisation of streptozotocin induced diabetes mellitus in swiss albino mice. Global Journal of Pharmacology 2009;3:81-4.
27. Rasch R, Mogensen CI.. Urinary excretion of albumin and total protein in normal and streptozotocin diabetic rats. Acta Endocrinol 1980;95:376-81.
28. Sassy-Prigent C, Heudes D, Jouquey S, et al. Morphometric detection of incipient glomerular lesions in diabetic nephropathy in rats. Protective effects of ACE inhibition. Lab Invest 1995;73:64-71.
29. Kang MJ, Ingram A, Ly H, Thai K, Scholey JW. Effects of diabetes and hypertension on glomerular transforming growth factor-beta receptor expression. Kidney Int 2000;58:1677-85.
30. Utimura R, Fujihara CK, Mattar AL, et al. Mycophenolate mofetil prevents the development of glomerular injury in experimental diabetes. Kidney Int 2003;63:209- 16.
31. Nangaku M, Inagi R, Miyata T, Fujita T.

Hypoxia and Hypoxia-Inducible Factor in Renal Disease Nephron Exp. Nephrol 2008; 110:e1-e7.
32. Fine LG, Orphanides C, Norman JT. Progressive renal disease: the chronic hypoxia hypothesis. Kidney Int Suppl 1998;65:S74-S78.
33. Nangaku M. Chronic hypoxia and tubulointerstitial injury: a final common pathway to end-stage renal failure. J Am Soc Nephrol 2006;17:17-25.
34. Katavetin P, Miyata T, Inagi R, et al. High glucose blunts vascular endothelial growth factor response to hypoxia via the oxidative stress-regulated hypoxia-inducible factor/hypoxia-responsible element pathway. J Am Soc Nephrol 2006;17:1405-13.
35. Rosenberger C, Khamaisi M, Abassi Z, et al. Adaptation to hypoxia in the diabetic rat kidney. Kidney Int 2008;73:34-42.
36. Ohtomo S, Nangaku M, Izuhara Y, et al. Cobalt ameliorates renal injury in an obese, hypertensive type 2 diabetes rat model. Nephrol Dial Transplant 2008;23: 1166-72.
37. Tanaka T, Kojima I, Ohse T, et al. Cobalt promotes angiogenesis via hypoxia-inducible factor and protects tubulointerstitium in the remnant kidney model. Lab Invest 2005;85:1292-307.
38. Tanaka T, Kojima I, Ohse T, et al. Hypoxia-inducible factor modulates tubular cell survival in cisplatin nephrotoxicity. Am J Physiol Renal Physiol 2005;289:F1123-33.
39. Matsumoto M, Makino Y, Tanaka T, et al. Induction of renoprotective gene expression by cobalt ameliorates ischemic injury of the kidney in rats. J Am Soc Nephrol 2003;14:1825-32.
40. Ybarra JA, Behrooz A, Gabriel MH, et al. Glycemia-lowering effect of cobalt chloride in the diabetic rat. Mol. Cell. Endocrinol 1997;133:151-60.
41. De Boeck M, Kirsch-Volders M, Lison D. Cobalt and antimony: genotoxicity and carcinogenicity. Mutat Res 2003;533:135-52.