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The Effect of Vardenafil (a PDE Type 5 Inhibitor) on Renal Function in the Diabetic Rabbit: A Pilot Study

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Abstract. *Background: Diabetic nephropathy is a common cause of impaired renal function. We investigated the effect vardenafil, a phosphodiesterase type 5 (PDE-5) inhibitor, has on renal function in the diabetic rabbit. Materials and Methods: Blood was taken at 4 and 6 months from control and alloxan-induced diabetic animals (n=8, in each group) and biochemical variables pertaining to renal function determined. A 7-month sample was also analysed after giving control and diabetic animals (n=4 in each group) either vardenafil (3 mg/kg) or vehicle to drink for 4 weeks. Spot urine total protein/ creatinine ratio (TP/C) was determined at 4 and 6 months. At 7 months a 24 h-urine sample was collected to measure TP/C and creatinine clearance (CrCl). Results: There was a significant increase in serum creatinine concentration after 6 months diabetes, which was significantly reduced by vardenafil. TP/C from diabetic rabbit spot urine samples at 6 months were significantly elevated compared to control animals, indicating the presence of proteinuria. Vardenafil treatment caused a normalisation of TP/C. Diabetic animals receiving vardenafil showed a significant improvement in CrCl when compared with diabetic animals given vehicle. Conclusion: These findings highlight a potential role for vardenafil in the treatment of diabetic nephropathy.*

Chronic kidney disease is increasing worldwide at an annual rate of 8%, with the prevalence higher in developing countries than in the developed world (1). Diabetic nephropathy is one of the most common causes of this problem (1). In fact it is the commonest cause of end stage renal failure in many countries and is associated not only with a high morbidity rate but also an increase in mortality (2-5). It can affect 20-30% of the diabetic population and presents in its earliest stage with an increased excretion of albumin (microalbuminuria) in the urine (4). There is also evidence of an increase in systemic and vascular markers of inflammation (6), with the progressive growth of the kidney (7). Accompanying these changes are abnormalities in the blood biochemical indices of renal function, which precede renal failure (8).

The primary treatment of diabetic nephropathy has focused on the integrated targeting of glycaemic and blood pressure control to reduce microalbuminuria (3, 9, 10).

Some patients, however, progress to end stage renal disease and require renal replacement therapy. It has been estimated that this treatment can cost as much as 40,000-50,000 Euros /patient each year (5). Not surprisingly, with the increasing number of diabetics on renal replacement therapy a financial strain is placed on health care systems (2). Thus, the need to develop new treatment strategies for diabetic nephropathy is obvious.

In the present study, we investigate the effect oral vardenafil, a phosphodiesterase type 5 (PDE-5) inhibitor, has on diabetic renal function.

Materials and Methods

Induction of diabetes. Adult mature male rabbits (n=8), fed *ad libitum*, were injected intravenously with alloxan (65 mg/kg made up in 1 ml/kg, saline), while 8 control animals were injected with the saline vehicle alone (1 ml/kg), after a blood sample was taken. Diabetic animals received 3 subcutaneous injections of 10 ml of 50% glucose, 4 h apart on the first day of alloxan treatment. A final glucose injection (10 ml of a 50% glucose solution) was administered on the morning (7. 30 am) of the second day. This procedure was carried out to counteract the hypoglycaemia caused by insulin release from necrosed pancreatic beta cells due to the acute action of alloxan.

Within 1 week of the alloxan injection, blood samples not exceeding more than 10% of the total blood volume were taken to confirm diabetes. Thereafter, blood samples not exceeding 15% of the total blood volume were taken at 4 and 6 months to monitor serum biochemical variables that directly relate to renal function

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(urea, sodium, and creatinine), as well as, glucose and bicarbonate. Spot urine samples were also collected at 4 and 6 months from control and diabetic animals for the measurement of total protein and creatinine concentrations.

Experimental animal groups. After 6 months the control and diabetic animals were divided into 4 groups.

Group 1 (4 control rabbits) and Group 2 (4 diabetic rabbits) were given vardenafil (3 mg/kg, Bayer Healthcare AG, Germany) made up in 120 ml HCl acid water, pH 4.5, to drink each morning for 4 weeks, this was followed by HCl acid water, pH 4.5, given *ad libitum*. Animals in Group 3 (4 control rabbits) and Group 4 (4 diabetic rabbits) received HCl acid water, pH 4.5, to drink *ad libitum* for 4 weeks. The vardenafil dose used has been reported to be the minimum necessary to elicit urological changes following partial bladder outlet obstruction in the rat (11).

Rabbits in all four experimental groups readily drank the vardenafil solution or vehicle.

The final 7-month blood sample was taken after 4 weeks vardenafil or vehicle treatment.

All animals were placed in metabolic cages at 7 months to collect 24 h urine samples to measure total protein and creatinine concentrations, as well as to determine creatinine clearance (CrCl).

Kidney sections were also collected from control and diabetic vehicle-treated rabbits for transmission electron microscopy (TEM). Animals were weighed at the start and the end of the study.

Statistical analysis. For parametric analysis the results are expressed as mean±SD using a Student's unpaired or paired *t*-test, with statistical significance accepted at $p<0.05$. For nonparametric analysis, the results are expressed as median with range using the Mann Whitney unpaired test and the Wilcoxon paired test. Values from both tests were considered significant at $p<0.05$

Results

The starting weights of control rabbits were less than the diabetic rabbits [control: 3.0 (2.8 -3.2kg); diabetic: 3.3 (3.2-3.4 kg; $p=0.002$) Mann Whitney test, n=8]. At the end of 7 months, the control animals were significantly ($p=0.008$; Wilcoxon test) heavier than their starting weights, while the diabetic animals showed no significant change from their starting weights, [control: 3.9 (3.6 - 4.3kg); diabetic: 4.0 (2.2-4.8 kg) n=8]. The final weight of the control animals was not significantly different from the final weight of the diabetic animals.

Serum glucose concentration was significantly elevated 1 week after the alloxan injection compared to vehicle-treated control animals (similar to serum glucose concentration in Table I). Acid water treatment did not induce metabolic acidosis, since there were no significant difference in serum bicarbonate concentration in control and diabetic animals before and after acid water or vardenafil treatment. For example, 6 month diabetic serum bicarbonate before vardenafil treatment was 26 mmol/l (20-28 mmol/l, n=4); 7 month diabetic serum bicarbonate after vardenafil treatment was 26 mmol/l (22-27 mmol/l, n=4).

Table I. *Serum biochemical variables from 6-month control and diabetic rabbits: evidence of renal impairment.*

Biochemical variable	Control (n=8) mean±SD	Diabetes (n=8) mean±SD	*P*-value (unpaired Student's *t*-test)
Glucose (mmol/l)	7.5±0.4	26.2±4.7	<0.0001
Sodium (mmol/l)	143±1	131±4	0.0001
Urea (mmol/l)	5.2±0.3	9.7±2.0	<0.0001
Creatinine (μmol/l)	79.0±10	95.0±9	0.0045

Blood samples analysed from 4-month (results not shown) and 6-month diabetic animals (Table I) revealed impaired renal function as there was a significant increase in serum creatinine and urea concentrations. There was also a significant increase in serum glucose concentration, with a significant fall in serum sodium concentration.

Serum creatinine (μmol/l) concentration from diabetic rabbits before and after oral vardenafil treatment. The diabetes-induced increase in serum creatinine concentration was significantly reduced by vardenafil (6-month diabetic creatinine: 97±13 μmol/l; n=4 *vs.* 7-month diabetic creatinine following vardenafil: 87±12 μmol/l; n=4, $p=0.015$; paired Student's *t*-test.

Urinary total protein (g/l) / creatinine (mmol/l) ratio (TP/C) from control and diabetic rabbits with and without oral vardenafil treatment. TP/C from diabetic rabbit urine samples at 6 months but not at 4 months (results not shown) was significantly elevated compared with controls, indicating the presence of proteinuria. Control TP/C: n=8, 0.0116 (0.0091-0.0191) *vs.* diabetic TP/C: n=8, 0.0227 (0.0096-0.1632), $p<0.038$ (Mann Whitney, unpaired test). Vardenafil treatment caused a normalisation of TP/C from diabetic animals (n=3) at 0.0143 (0.0118-0.0176).

Creatinine clearance (CrCl) following 4 weeks' oral vardenafil or vehicle treatment from 7-month control and diabetic rabbits. Control rabbits receiving vehicle had a CrCl of 9.3±3.3 ml/min, a value not statistically different from control animals receiving oral vardenafil (8.0±0.5 ml/min).

Diabetic vehicle-treated rabbits had a fall in CrCl compared with vehicle-treated controls, however, the difference was not statistically significant.

In contrast, diabetic animals receiving vardenafil showed a significant improvement in CrCl compared with diabetic animals given vehicle: diabetic vehicle-treated, 6.1±3.7 ml/min, n=4 *vs.* diabetic vardenafil-treated, 11.3±1.0 ml/min, n=4, $p=0.035$ (unpaired Student's *t*-test). This improvement in CrCl was not statistically different from

control animals receiving vehicle. Similar results were found even when CrCl was expressed per kg (results not shown).

Transmission electron microscopy (TEM) comparison of kidney sections taken from vehicle-treated control and diabetic rabbits. TEM revealed no significant evidence of morphological changes between control and diabetic rabbit kidney sections following vehicle treatment.

Discussion

The serum biochemical data presented in this study demonstrate that significant renal impairment is evident 6 months following the induction of diabetes (a rise in serum creatinine and urea and a fall in sodium concentrations). We have previously reported similar findings. In addition, we found renal impairment starts much earlier than we report here (3 months after the induction of diabetes) (12). The effect of vardenafil (a PDE-5 inhibitor) on the erectile process and in particular its beneficial actions on patients with erectile dysfunction are well-documented (13,14).

Here, for the first time, we report that 4-week treatment with oral vardenafil significantly reduced the diabetic-induced increase in serum creatinine concentration. We also report that the diabetic rabbits had an elevated urinary TP/C [a test for proteinuria (15)], which was normalised by vardenafil. Taken together, these findings imply that vardenafil can reduce proteinuria and improve the renal status in diabetic nephropathy. It appears that this property is only evident when kidney function is impaired, since vardenafil had no effect on the renal function of control rabbits.

The early stages of diabetic nephropathy are characterised by an increase in glomerular hyperfiltration, which increases the glomerular filtration rate (GFR) and is believed to contribute to the progression of renal impairment (7, 16). As the nephropathy progresses, renal function deteriorates and a reduction in GFR becomes evident (17, 18).

In our study, we measured CrCl, an index of GFR (19), and found that control animals had similar CrCl values to the GFR values previously reported for control rabbits (20). We also found that diabetic vehicle-treated animals did not show evidence of glomerular hyperfiltration (elevated CrCl), when compared with vehicle-treated control animals. In fact, our data suggest that diabetic animals were moving into the phase when GFR starts to fall and before significant morphological changes become evident.

Interestingly, diabetic rabbits that received vardenafil showed a significant increase in CrCl, compared with diabetic vehicle-treated animals, providing further evidence of drug-induced improvement in renal function. Importantly, this increase did not induce glomerular hyperfiltration, since CrCl was not significantly greater than that obtained from control vehicle-treated animals. Nor was the increase related to differences in the final body weight of the animals in each group, since results were similar when CrCl was expressed per kg.

A possible mechanism for the action of vardenafil on diabetes-induced impaired renal function can be inferred from previous studies. It has been proposed that glomerular hyperfiltration is significantly dependent upon an increase in nitric oxide (NO) activity in the early phase of diabetic nephropathy (21). However, in the later phase when the GFR starts to fall a concomitant reduction in NO activity seems to occur. The diabetes-induced reduction in NO activity could be due to a defect in synthesis or quenching through the production of superoxide radicals and advanced glycosylation end products (22, 23). In the context of renal function an increase in NO/cGMP activity would cause renal vasodilation. Thus, vardenafil, a potent and highly selective PDE-5 inhibitor may be restoring GFR, reducing serum creatinine and urinary TP/C by enhancing NO-induced cGMP formation/accumulation, as with cavernosal tissue (24).

Cyclosporin A, a potent immunosuppressive agent, causes nephrotoxicity characterized by similar renal changes to those reported here, *i.e.* elevated serum creatinine levels and a decrease in CrCl (25, 26). FR226807 (Fujisawa Pharmaceutical, Japan) another PDE-5 inhibitor was found to improve cyclosporin A-induced nephrotoxicity in spontaneous hypertensive rats, as did sildenafil (25). This finding suggests that PDE-5 inhibitors have a beneficial effect on impaired renal function. These authors also suggested that the effect of FR226807 was probably due to an increase in cGMP content in the kidney, rather than *via* reducing blood pressure.

An important finding from that study was that cyclosporin A-induced pathological changes in renal morphological were improved by FR226807. Further work is required to determine whether vardenafil treatment can arrest or delay the known renal morphological changes that are associated with diabetic nephropathy.

Finally, the present study suggests a possible role for vardenafil in the treatment of diabetic nephropathy.

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