Original Paper

The Ameliorative Effect of Pectin-Insulin Patch On Renal Injury in Streptozotocin-Induced Diabetic Rats

Ntethelelo Sibiya^a Phikelelani Ngubane^a Musa Mabandla^a

^aSchools of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

Key Words

Hyperglycaemia • Nephron • Albumin excretion rate • Oxidative status • Creatinine clearance

Abstract

Background/Aims: Renal damage and dysfunction is attributed to sustained hyperglycaemia in overt diabetes. Subcutaneous insulin injections are beneficial in delaying the progression of renal dysfunction and damage in diabetics. However, the current mode of administration is associated with severe undesirable effects. In this study, we evaluated the ameliorative effects of pectin-insulin dermal patches on renal dysfunction in diabetes. **Methods:** Pectin-insulin patches (20.0, 40.8 and 82.9 µg/kg) were applied on the skin of streptozotocin-induced diabetic rats, thrice daily for 5 weeks. Blood glucose concentration, blood pressure and urine output volume were recorded on week 5 after which the animals were sacrificed after which the kidneys and plasma were collected. Kidney nephrin expression and urinary nephrin concentration, albumin excretion rate (AER), creatinine clearance (CC) and albumin creatinine ratio (ACR) were assessed. **Results:** Patch application resulted in reduced blood glucose concentration and blood pressure. Furthermore, pectin-insulin patch treatment resulted in increased kidney nephrin expression and reduced urinary nephrin concentration. AER, CC ACR were also reduced post patch application. **Conclusions:** The application of pectin-insulin patch limited diabetes associated kidney damaged and improved kidney function. These observations suggest that pectin-insulin patches may ameliorate kidney dysfunction that is associated with chronic subcutaneous insulin administration.

© 2017 The Author(s)
Published by S. Karger AG, Basel

Introduction

Chronic kidney diseases are the second leading cause of mortality and morbidity in diabetes mellitus [1]. The progression of chronic kidney disease is reported to be proportional to sustained hyperglycaemia in both uncontrolled type 1 and type 2 diabetes [2]. Chronic kidney disease is associated with perturbations in essential kidney functions such as declining glomerular filtration rate (GFR), electrolyte imbalance, increased blood pressure and increased protein/albumin excretion [2]. These functional changes are mediated by morphological changes in the kidney including thickening of the glomerular basement membrane, mesangial cell expansion, extracellular matrix deposition and podocyte loss or damage [3]. In experimental diabetes, morphological changes have also been associated with oxidative stress such as increased and reduced lipid peroxidation markers and antioxidant markers, respectively. Oxidative stress is a hallmark of kidney disease in diabetes and is a result of sustained hyperglycaemia [4].

The loss or damage of podocytes in diabetes has been partly attributed to the harmful effects of sustained uncontrolled haemodynamic and glycaemic perturbations [5, 6]. Furthermore, it has been shown that podocyte function and survival is modulated directly by insulin hence absence of insulin leads to detrimental effects on kidney function [7]. The loss of podocytes compromises the glomerular filtration barrier which leads to proteinuria [8]. In overt diabetes, podocyte constituents such as nephrin, podocin and podocalyxin have been detected in urine and its concentration correlates significantly with albuminuria [9]. Therefore, the assessment of urinary nephrin and podocalyxin concentration has been shown to predict the progression of chronic kidney disease [10]. Insulin treatment delays the onset and progression of chronic kidney disease in diabetes as evidenced by decreased urine albumin concentration and normalised glomerular filtration rate and oxidative status [11]. However, despite these beneficial effects, subcutaneous insulin injections are associated with deleterious side effects due to the supraphysiological insulin concentrations administered. The elevated insulin concentration precipitates the development of oedema because of increased sodium re-absorption and increased podocyte contractility which promotes glomerular permeability and hence increased protein excretion [12].

These undesirable effects of subcutaneous insulin administration together with the inability to restore adequate renal function with this treatment precipitate the need to develop and evaluate alternative insulin delivery systems. In our laboratory, we have successfully developed a transdermal pectin-insulin patch formulation that can deliver physiologically relevant insulin concentration which can maintain normal glycaemic control and ameliorate certain diabetes associated complications in streptozotocin (STZ) induced-diabetic rats [13, 14]. Accordingly, in the study, we sought to evaluate the effects of transdermal pectin-insulin patch application on markers of kidney function such as blood pressure, creatinine clearance, albumin excretion rate, kidney nephrin expression and urinary nephrin concentration. We also investigated the effect of the patch application on oxidative stress markers such as kidney superoxide dismutase (SOD) and malondialdehyde (MDA) concentration.

Materials and Methods

Patch preparation

Pectin-insulin dermal patches of different insulin concentrations were prepared using a previously described protocol [13]. Briefly, amidated low methoxyl pectin (6 g) was dissolved in distilled water (100 mL) and mixed by agitation for 30 minutes. Step wise, DMSO (3 mL), Eucalyptus (1.5 mL), Vitamin E (1.5 mL) and purimycin (100 µL) were added and agitated for another 30 minutes. Afterwards, insulin (832, 1664 and 3328 µg) was added into separate beakers and mixed by agitation in a water bath (37°C) for 15 minutes. Thereafter, mixture aliquots (11 mL) were transferred to petri dishes (113 cm²) and a CaCl₂ (2 %) solution was added to the mixture. The mixture was left at room temperature for 10 minutes. Afterwards, all petri dishes were stored at 4°C until use.

Animals

Male Sprague-Dawley rats (250–300 g) used in this study were raised and kept in the Biomedical Research Unit of the University of KwaZulu-Natal under the normal laboratory conditions (temperature and humidity) in a 12 h day: 12 h night cycle. The animals were given contact to water *ad libitum* and standard rat chow (40 g) daily (Meadow Feeds, Pietermaritzburg, South Africa). All the animal experiments were revised and accepted by the Animal Research Ethics Committee of the University of KwaZulu-Natal (AREC/080/015D). The animals were allowed to acclimatise for 5 days to live in the metabolic cages.

Diabetes induction

Diabetes (type 1) was induced as previously described well-established protocol [15]. A week later after the streptozotocin-induction, a blood glucose concentration more than 20 mmol/L in rats was considered as stable diabetes.

Experimental groups and treatment

STZ-induced experimental animals were divided into 5 groups (n=6 per group) and were housed individually in Makrolon polycarbonate metabolic cages (Techniplast, Labotec, South Africa). Three animal groups (Groups 1-3) were individually treated with 3 concentrations (20.0, 40.8 and 82.9 µg/kg, p.o) of transdermally applied pectin-insulin patches. Group 4 rats were treated with a drug free pectin patch and served as the negative control. Group 5 rats were injected with insulin (175 µg/kg, SC) and served as the positive control. Non-diabetic animals served as the absolute control. Two days prior to the application of the pectin-insulin patches, the rats were shaved on the dorsal region of the neck [14]. The pectin-insulin patches were applied thrice daily, 8 hours apart for 5 weeks. At the end of week 5, blood glucose concentration was measured 4 hours post patch application or subcutaneous insulin injection using OneTouch select glucometer (Lifescan, Mosta, Malta, United Kingdom). Systolic (SBP) and diastolic (DBP) blood pressure, body weight, 24-hour water and food consumption and urine output volume were recorded. SBP and DSP were monitored using the non-invasive tail cuff method with photoelectric sensors (IITC Model 31 Computerised Blood Pressure Monitor, Life Sciences, Woodland Hills, California, USA) as previously described [16]. Urine samples were collected for the analysis of nephrin, albumin and creatinine concentration.

Plasma and tissue collection

All animals were anaesthetised by exposing to halothane for 3 minutes via a gas anaesthetic chamber (100 mg kg⁻¹). Blood samples were collected by cardiac puncture into individual pre-cooled heparinized containers. Afterwards, the blood was centrifuged at 10 xg in a Hermle Laborechnik GmbH centrifuge (Wehingen, Germany) for plasma collection. The kidneys were removed and weighed before freezing in liquid nitrogen. Thereafter, the kidneys and plasma were stored in an Ultra Bio Freezer (Snijers Scientific, Tilburg, Netherlands) at –80°C.

Biochemical analysis

Biochemical analysis was done in duplicates. Protein concentration was quantified using the BCA assay (Sigma Aldrich Chemical Company, Missouri, and St Louis, USA). All samples were standardized to one protein concentration (1 mg/mL). Creatinine and albumin concentration in plasma and urine samples were analysed at the Global Clinical and Viral Laboratory, Amanzimtoti, South Africa. Nephrin, SOD and plasma insulin concentration were measured using ELISA kits (Elabscience and Biotechnology, WuHan) per the manufacturer's instructions. MDA concentration was measured using an established laboratory protocol [16]. Briefly, kidney tissue (50 mg) was homogenized in phosphoric acid (500 µL, 0.2%) after which the homogenate was supplemented with phosphoric acid (400 µL, 2%). The homogenate was then separated into two glass tubes, each receiving equal volumes of the solution (200 µL). A further addition of phosphoric acid (200 µL, of 7 %) into both glass tubes was followed by the addition of butylated hydroxytoluene (400 µL) into one glass tube (sample test) and hydrochloric acid (HCl) (400 µL, 3 mM) into the second glass tube (blank). Both solutions were heated at 100° C for 15 minutes and then allowed to cool down to room temperature. Butanol (1.5 ml) was added to the cooled solutions. The sample solution was vortexed for 1 minute to ensure rigorous mixing and then allowed to settle until 2 liquid phases could be distinguished in

the tube. The butanol phase (top layer) was transferred to Eppendorf tubes and centrifuged at 13200 xg in a Hermle Labortechnik GmbH centrifuge (Wehingen, Germany) for 15 minutes. The samples were aliquoted into wells of a 96-well microtiter plate in triplicate and the absorbance was read at 532 nm (reference λ 600nm) using a Nano spectrophotometer (BMG Labtech, Ortenburg, Baden-Wurttemberg, Germany). The absorbance was used to calculate the concentration of MDA using Beer's Law.

$$\text{Concentration} = \frac{\text{Absorbance}_{\text{Final}}}{\text{Absorption coefficient (156mmol}^{-1}\text{)}}$$

Statistical analysis

Data is expressed as means $\pm$ standard error of means (SEM). Statistical analysis was conducted using GraphPad Prism Instat Software (version 5.00, GraphPad Software, San Diego, California, USA). One-way analysis of variance (ANOVA) followed by the Tukey-Kramer post-hoc test was used to analyse differences between the controls and the experimental groups. Values of $p < 0.05$ indicate statistical significance.

Results

Energy balance

Non-diabetic control (ND), diabetic control (DC), patch treatment (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) and subcutaneous insulin (SC) groups were analysed for 24-hour food and water consumption, urine output volume and blood glucose concentration at week 5 (Table 1). Food and water consumption as well as urine output volume were increased in the diabetic controls (DC) when compared to the non-diabetic (ND) animals (DC vs ND, $p < 0.05$, Table 1). Patch and subcutaneous insulin treatment attenuated this increase in food, water intake and urine output volume (Patch treatment vs DC and SC vs DC, $p < 0.05$). Glycaemia was present in the diabetic control animals at week 5 (DC vs ND, $p < 0.05$, Table 1) while the application of the dermal patches (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) or subcutaneous insulin injection attenuated blood glucose increase in the STZ-induced diabetic animals (Patch treatment vs DC and SC vs DC, $p < 0.05$, Table 1).

Table 1. Comparison in food, water intake, urine output volume and blood glucose concentration in the non-diabetic control (ND), diabetic control (DC), diabetic animals treated with pectin-insulin patches (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) and diabetic animals subcutaneously administered with insulin (SC). Data is expressed as mean $\pm$ SEM (mean of six samples per group, $n=6$) $\alpha=p < 0.05$ when comparing DC and ND $\ast=p < 0.05$ when comparing patch and DC and $\# = p < 0.05$ when comparing SC and DC

Experimental groups	Food intake (g/100g)	Water intake (ml/100g)	Urine output (ml/100g)	Blood glucose (mmol/L)
ND	7.5 $\pm$ 0.5	8.40 $\pm$ 1.20	3.92 $\pm$ 0.14	4.9 $\pm$ 0.5
DC	21.0 $\pm$ 3.0 α	76.46 $\pm$ 7.20 α	42.01 $\pm$ 4.74 α	30.9 $\pm$ 0.6 α
20.0	11.2 $\pm$ 1.9 $\ast$	47.25 $\pm$ 4.30 $\ast$	26.62 $\pm$ 2.18 $\ast$	12.3 $\pm$ 0.8 $\ast$
40.8	11.58 $\pm$ 1.9 $\ast$	41.76 $\pm$ 5.75 $\ast$	26.30 $\pm$ 2.97 $\ast$	11.2 $\pm$ 0.2 $\ast$
82.9	8.9 $\pm$ 1.0 $\ast$	35.44 $\pm$ 4.23 $\ast$	24.42 $\pm$ 3.16 $\ast$	9.4 $\pm$ 0.7 $\ast$
SC	8.4 $\pm$ 0.9 $\#$	20.88 $\pm$ 3.20 $\#$	14.65 $\pm$ 1.2 $\#$	7.4 $\pm$ 0.6 $\#$

Systolic and diastolic blood pressure

Non-diabetic control (ND), diabetic control (DC), patch treatment (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) and subcutaneous insulin (SC) groups were analysed for systolic (SBP) and diastolic (SDP) blood pressure (Figure 1). Induction of diabetes resulted in increased systolic and diastolic blood pressure (DC vs ND, $p < 0.05$, Figure 1) at week 5 of the experimental period. Patch treatment (40.8 and 82.9 $\mu\text{g/kg}$) resulted in a reduction in both systolic and diastolic blood pressure (Patch treatment vs DC, $p < 0.05$, Figure 1).

Fig. 1. Comparison in systolic (SBP) and diastolic (DBP) blood pressure in the non-diabetic control (ND), diabetic control (DC), diabetic animals treated with pectin-insulin patches (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) and diabetic animals subcutaneously administered with insulin (SC). $\alpha = p < 0.05$ when comparing DC and ND * $= p < 0.05$ when comparing patch (40.8 and 82.9 $\mu\text{g/kg}$) and DC.

Kidney to body weight ratio

Non-diabetic control (ND), diabetic control (DC), patch treatments (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) and subcutaneous insulin (SC) groups were analysed for kidney to body (K/B) weight ratio (Table 2). Induction of diabetes increased K/B weight ratio compared to the non-diabetic control at week 5 (ND vs DC, Table 2). Patch treatment (40.8 and 82.9 $\mu\text{g/kg}$) and insulin injection reduced K/B weight ratio (Patch treatment vs DC and SC vs DC, $p < 0.05$, Table 2).

Kidney function

Non-diabetic control (ND), diabetic control (DC), patch treatment (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) and subcutaneous insulin (SC) groups were analysed for creatinine clearance (CC), plasma albumin (PA), albumin excretion rate (AER) and albumin creatinine ratio (ACR) (Table 3). Induction of diabetes resulted in a significant increase in CC (DC vs ND, $p < 0.05$, Table 3). Patch treatment (40.8 and 82.9 $\mu\text{g/kg}$) and insulin injection reduced CC (Patch treatment vs DC, SC vs DC, $p < 0.05$). Induction of diabetes resulted in a reduction of plasma albumin (DC vs ND, $p < 0.05$). Patch treatments significantly increased plasma albumin with insulin injection showing similar effects (Patch treatment vs DC, and SC vs DC, $p < 0.05$). Diabetic animals presented with increased AER (DC vs ND, $p < 0.05$). Patch treatments and insulin injection

Table 2. Comparison in kidney nephrin and urinary nephrin concentration in the non-diabetic control (ND), diabetic control (DC), diabetic animals treated with pectin-insulin patches (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) and diabetic animals subcutaneously administered with insulin (SC). $\alpha = p < 0.05$ when comparing DC and ND, * $= p < 0.05$ when comparing patch treatment and DC, # $= p < 0.05$ when comparing SC and DC.

Experimental groups	Final body weight (g)	Kidney weight (g)	K/B weight ratio (%)
ND	372.2 $\pm$ 3.6	1.1 $\pm$ 0.0	0.3 $\pm$ 0.0
DC	222.2 $\pm$ 13.3 $^{\alpha}$	1.1 $\pm$ 0.1	0.5 $\pm$ 0.2 $^{\alpha}$
20.0	265.2 $\pm$ 9.5 *	1.0 $\pm$ 0.0	0.4 $\pm$ 0.2
40.8	272.5 $\pm$ 6.3*	1.0 $\pm$ 0.0	0.3 $\pm$ 0.0*
82.9	276.7 $\pm$ 6.5*	1.0 $\pm$ 0.1	0.3 $\pm$ 0.1*
SC	277.3 $\pm$ 8.0#	1.0 $\pm$ 0.0	0.3 $\pm$ 0.0#

Table 3. Comparison in creatinine clearance (CC), plasma albumin (PA), albumin excretion rate (AER), albumin creatinine ratio (ACR) in the non-diabetic control (ND), diabetic control (DC), diabetic animals treated with pectin-insulin patches (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) and diabetic animals subcutaneously administered with insulin (SC). Data is expressed as mean $\pm$ SEM (mean of six samples per group, $n=6$) $\alpha=p<0.05$ when comparing DC and ND $*=p<0.05$ when comparing patch and DC and $\#p<0.05$ when comparing SC and DC

Experimental groups	CC (ml/min/250g)	PA (g/L)	AER (mg/day)	ACR
ND	2.2 $\pm$ 0.1	18.6 $\pm$ 1.2	0.56 $\pm$ 0.1	7.4 $\pm$ 0.7
DC	5.5 $\pm$ 0.2 α	11.0 $\pm$ 0.5 α	4.0 $\pm$ 0.5 α	22.1 $\pm$ 1.2 α
20.0	4.6 $\pm$ 0.1	13.3 $\pm$ 1.2	2.7 $\pm$ 0.4	17.1 $\pm$ 1.0
40.8	3.9 $\pm$ 0.1	15.2 $\pm$ 0.6*	2.7 $\pm$ 0.2*	13.5 $\pm$ 0.35*
82.9	2.5 $\pm$ 0.12*	15.3 $\pm$ 1.5*	1.6 $\pm$ 0.1*	11.4 $\pm$ 1.5*
SC	2.4 $\pm$ 0.2 $\#$	16.0 $\pm$ 1.3 $\#$	0.8 $\pm$ 0.0 $\#$	10.7 $\pm$ 1.1 $\#$

decreased AER (Patch treatment vs DC, SC vs DC, $p<0.05$). Diabetic control animals showed elevated ACR (DC vs ND, $p<0.05$). Patch treatments decreased ACR with insulin injection showing similar effects (Patch treatment vs DC and SC vs DC, $p<0.05$).

Kidney nephrin and urinary nephrin concentration

Non-diabetic control (ND), diabetic control (DC), patch treatment (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) and subcutaneous insulin (SC) groups were analysed for kidney nephrin and urinary nephrin concentration (Figure 2). Induction of diabetes decreased kidney nephrin expression significantly in comparison to the non-diabetic control (DC vs ND, $p<0.05$). Patch treatment (40.8 and 82.9 $\mu\text{g/kg}$) and insulin injection resulted in an increase in kidney nephrin expression (Patch treatment vs DC and SC vs DC, $p<0.05$, Figure 2). On the other hand, induction of diabetes significant-

Fig. 2. Comparison in kidney nephrin and urinary nephrin concentration in the non-diabetic control (ND), diabetic control (DC), diabetic animals treated with pectin-insulin patches (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) and diabetic animals subcutaneously administered with insulin (SC). $\alpha=p<0.05$ when comparing DC and ND, $*=p<0.05$ when comparing patch treatment and DC, $\#p<0.05$ when comparing SC and DC.

Fig. 3. Comparison in kidney superoxide dismutase (SOD) and malondialdehyde (MDA) concentration in the non-diabetic control (ND), diabetic control (DC), diabetic animals treated with pectin-insulin patches (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) and diabetic animals subcutaneously administered with insulin. $\alpha=p<0.05$ when comparing DC and ND, $*=p<0.05$ when comparing patch treatment and DC, $\#p<0.05$ when comparing SC and DC.

Fig. 4. Comparison in plasma insulin in the non-diabetic control (ND), diabetic control (DC), diabetic animals treated with pectin-insulin patches (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) and diabetic animals subcutaneously administered with insulin. $\alpha = p < 0.05$ when comparing DC and ND, $* = p < 0.05$ when comparing patch treatment and DC, $\# = p < 0.05$ when comparing S.C and DC.

ly increased urinary nephrin concentration (DC vs ND, $p < 0.05$, Figure 2). Interestingly, patch treatment significantly decreased urinary nephrin concentration and similar effects were observed in diabetic animals receiving insulin injection (Patch treatment vs DC and SC vs DC, $p < 0.05$, Figure 2).

Markers for oxidative stress

Non-diabetic control (ND), diabetic control (DC), patch treatment (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) and subcutaneous insulin (SC) groups were analysed for kidney superoxide dismutase (SOD) and malondialdehyde (MDA) concentration (Figure 3). Induction of diabetes significantly reduced and increased SOD and MDA concentration, respectively (DC vs ND, $p < 0.05$, Figure 3). Patch treatment (82.9 $\mu\text{g/kg}$) and insulin injection increased SOD concentration (Patch treatment vs DC and SC vs DC, $p < 0.05$, Figure 3). Both patch treatment and subcutaneous insulin injection decreased MDA concentration (Patch treatment vs DC and SC vs DC, $p < 0.05$, Figure 3).

Plasma insulin

Non-diabetic control (ND), diabetic control (DC), patch treatments (20.0, 40.8 and 82.9 $\mu\text{g/kg}$) and subcutaneous insulin (SC) groups were analysed for plasma insulin (Figure 4). Induction of diabetes decreased in terminal plasma insulin (ND vs DC, Table 2). Patch treatment (20.0 40.8 and 82.9 $\mu\text{g/kg}$) and insulin injection resulted in an increase in terminal plasma insulin (Patch treatment vs DC and SC vs DC, $p < 0.05$, Figure 4).

Discussion

Currently, diabetic nephropathy remains irreversible, however, strict glycaemic and haemodynamic control remain significant measures that delay the progression of renal dysfunction and damage [17]. The current study investigated whether use of the pectin-insulin patch ameliorated renal dysfunction observed in streptozotocin-induced diabetic rats. Herein, we are reporting the effect of transdermal application of pectin-insulin patch on blood pressure, creatinine clearance, albumin excretion, oxidative status, nephrin concentration and kidney glomerulus morphological changes after a 5-week experimental period.

Previous studies reported that a pectin-insulin patch formulation can deliver physiological amounts of insulin and improve diabetes associated hyperglycaemia [13]. In agreement with previous reports, the daily use of the patch application in streptozotocin-induced diabetic rats significantly attenuated blood glucose concentration. This confirmed the ability of the patch to deliver insulin into the circulation. Furthermore, plasma insulin levels were augmented hence explaining the decrease in blood glucose. However, in comparison with previous study done by Hadebe et al. (2013), the current study demonstrated lower plasma insulin levels despite higher insulin doses used. In the study, a polymer component (pectin) was increased thus to allow increase insulin loading in the patch. We may speculate that this altered the speed of insulin release hence partly explaining a reduced potency in glycaemic control compared to the previous study. This may however be of advantage to minimise the times one may need to re-apply the next patch and to avoid hypoglycaemic

episodes. Diabetes mellitus is associated with polyphagia and polyuria [18]. Insulin therapy has been shown to abolish polyphagia and polyuria [19]. This effect was also present in the patch treated animals suggesting that the patch application does have an ameliorative effect in diabetes induced rodents. Maintaining normoglycaemia is critical in delaying the progression to diabetes complications including renal disturbances [20].

High blood pressure in diabetes has also been implicated in progressive renal disturbances and failure [21]. Therefore, monitoring and maintaining blood pressure within a normal range is emphasized [22]. In the study, the application of the patch restored blood pressure to normal as evidenced by systolic and diastolic blood pressure observations. We may attribute these observations to the improved electrolyte balance handling and decreased blood viscosity [23, 24]. Sustained high blood pressure and hyperglycaemia are associated with renal morphological alterations such as mesangial expansion, extracellular matrix deposition, thickening of the basement membrane and podocyte loss [24-27, 6, 28, 29]. Progressively, these structural changes are accompanied by the loss in kidney function [30]. The onset of diabetes is associated with increased glomerular filtration rate (GFR), increased sodium re-absorption and proteinuria [25, 6, 31]. The application of the pectin-insulin patch evoked a normal creatinine clearance response, which may suggest the beneficial effects of the patch in delaying the onset or progression of kidney dysfunction.

In diabetes, the presence of sustained hyperglycaemia have debilitating consequences including an increase in oxidative stress that is accompanied by an inflammatory response [32]. Hyperglycaemia evokes oxidative stress via the increased production of oxidants and suppressed expression of antioxidants [33, 10]. In this study, we investigated the presence of oxidative stress by assessing MDA and SOD concentration in kidney tissue. SOD is a potent antioxidant with an ability to neutralise superoxide anion, its expression and activity has been shown to be reduced in diabetes. MDA is an end-product of lipid peroxidation; thus, it may serve to predict the damage caused by the oxidative stress [34]. The decrease in oxidative stress following treatment with the pectin-insulin patch, may be partly due to the attainment of normoglycaemia in the diabetic rats. Hyperglycaemia activates pathways such as protein kinase C, polyol and NADPH oxidases which ensure high levels of oxidative stress with subsequent damage to crucial cellular components [34]. Oxidative stress in the kidney is associated with podocyte injury or loss which leads to the deterioration of the glomerular filtration barrier and hence proteinuria [35]. Reports have also indicated that podocyte survival is insulin dependent and in the absence of insulin, their survival is critically compromised [36, 37]. For this reason, in both type 1 and 2 diabetes, podocytes constituents including podocin, podocalyxin and nephrin have been detected in urine, suggesting podocyte injury or loss [38-40]. The presence of podocyte constituents and proteins in urine is a marker for the progression of renal damage and correlates with hyperglycaemia, increased blood pressure and albuminuria [41]. The loss of podocytes leads to proteinuria, glomerulosclerosis and adhesion of parietal cells on the glomerular basement membrane ultimately leading to diabetic nephropathy [42]. In this study, only nephrin was assessed as an indication of podocytes loss since it has been shown to correlate well with duration and severity of renal dysfunction. Insulin patch application attenuated the increase in urinary nephrin concentration suggesting a protective effect against podocyte damage and loss. This may suggest that with patch treatment, the integrity of the glomerular barrier is maintained. This was accompanied by an attenuation in albumin excretion and decrease in the albumin creatinine ratio (ACR). The decline in albuminuria was accompanied by an increase in plasma albumin concentration in patch treated diabetic animals which is critical for haemodynamic control [43]. Ultrafiltration of albumin has been shown to exacerbate renal cell proliferation, apoptosis of tubular cells and podocytes through increased expression of endothelin-1 and interleukin-8 [44]. Therefore, the degree of albuminuria can be used to predict kidney damage and failure in overt diabetes [45, 46]. In this study, the ability of the insulin patch to reduce albumin excretion may be a result of GFR normalisation and podocyte survival which perhaps may be partly mediated via restored metabolic and haemodynamic factors.

Anti-hyperglycaemic agents including insulin have been shown to attenuate albuminuria in diabetes [47]. These findings further emphasize the importance of insulin on glomerular barrier functionality. In this study, diabetic control animals presented with increased kidney to body weight ratio, suggesting the development or presence of kidney hypertrophy [48]. Kidney hypertrophy has been ascribed to the inflammation, cellular matrix accumulation, mesangium proliferation, increased expression of transforming growth factor (TGF) and thickening of the glomerular basement membrane [49-51]. Progressively, the number and size of the glomerulus is significantly reduced. Application of the pectin-insulin patch attenuated kidney hypertrophy as evidenced by the reduced kidney to body ratio. These observations correlate with the attenuation in blood pressure increase, hyperglycaemia and oxidative stress. These results suggest that the pectin-insulin dermal patch protects the kidneys from damage that occurs in the streptozotocin induced diabetic animal model.

Conclusion

The overall observations are of therapeutic relevance considering the renal complications that manifest in overt diabetes. The overall risk of renal diseases in diabetics is extremely high compared to non-diabetics and the renal disturbances in diabetes are associated with high mortality [52]. The pectin-insulin patch application offers glycaemic and hemodynamic control. Furthermore, it attenuates diabetes associated kidney damaged and improves kidney function. These observations encourage further evaluations and developments in pectin-insulin patch application.

Disclosure Statement

The authors state that they have no conflicts of interest.

Acknowledgements

The study was funded by the Technology Innovation Agency South Africa, the funders had no role in the study design, data collection and manuscript preparation.

References

- 1 Vallon V, Komers R: Pathophysiology of the diabetic kidney. *Comprehensive Physiol* 2011;1:1175-1232.
- 2 Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS: Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *Am J Kidney Dis* 2003;41:1-12.
- 3 Wolf G, Ziyadeh FN: Cellular and molecular mechanisms of proteinuria in diabetic nephropathy. *Nephron Physiol* 2007;106:26-31.
- 4 Sheetz MJ, King GL: Molecular understanding of hyperglycemia's adverse effects for diabetic complications. *JAMA* 2002;288:2579-2588.
- 5 Forbes JM, Bonnet F, Russo LM, Burns WC, Cao Z, Candido R, Kawachi H, Allen TJ, Cooper ME, Jerums G: Modulation of nephrin in the diabetic kidney: association with systemic hypertension and increasing albuminuria. *J Hyperten* 2002;20:985-992.
- 6 Susztak K, Raff AC, Schiffer M, Böttinger EP: Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. *Diabetes* 2006;55:225-233.
- 7 Van den berg J, Weerman M, Assmann K, Weening J, Florquin S: Podocyte foot process effacement is not correlated with the level of proteinuria in human glomerulopathies. *Kidney Int* 2004;66:1901-1906.

- 8 White KE, Bilous RW, Marshall SM, El Nahas M, Remuzzi G, Piras G, De Cosmo S, Viberti G: Podocyte number in normotensive type 1 diabetic patients with albuminuria. *Diabetes* 2002;51:3083-3089.
- 9 Hale LJ, Coward RJ: Insulin signalling to the kidney in health and disease. *Clin Sci* 2013; 124:351-370.
- 10 Kashiwara N, Haruna Y, K Kondeti V, S Kanwar Y: Oxidative stress in diabetic nephropathy. *Curr Med Chem* 2010;17:4256-4269.
- 11 Ruggenenti P, Schieppati A, Remuzzi G: Progression, remission, regression of chronic renal diseases. *The Lancet* 2001;357:1601-1608.
- 12 Hirshberg B, Muszkat M, Marom T, Muszkat M: Natural course of insulin edema. *J Endocrinol Invest* 2000;23:187-188.
- 13 Hadebe SI, Ngubane PS, Serumula M, Musabayane CT: Transdermal delivery of insulin by amidated pectin hydrogel matrix patch in streptozotocin-induced diabetic rats: Effects on some selected metabolic parameters. *Plos One* 2014;9:1-9.
- 14 Ngubane PS, Hadebe SI, Serumula MR, Musabayane CT: The effects of transdermal insulin treatment of streptozotocin-induced diabetic rats on kidney function and renal expression of glucose transporters. *Ren Fail* 2015;37:151-159.
- 15 Musabayane CT, Munjeri O, Bwititi P, Osim EE: Orally administered, insulin-loaded amidated pectin hydrogel beads sustain plasma concentrations of insulin in streptozotocin-diabetic rats. *J Endocrinol* 2000;164:1-6.
- 16 Madlala HP, Van Heerden FR, Mubagwa K, Musabayane CT: Changes in renal function and oxidative status associated with the hypotensive effects of oleanolic acid and related synthetic derivatives in experimental animals. *PloS one* 2015;10:e0128192.
- 17 Saleh J, Yusef H, Salawu F: Prevention of diabetic nephropathy. *Nig Med Pract* 2008;53:28-32.
- 18 Swanston-Flatt SK, Day C, Bailey CJ, Flatt PR: Evaluation of traditional plant treatments for diabetes: studies in streptozotocin diabetic mice. *Acta Diabetologia Latina* 1989;26:51-55.
- 19 Van den Berghe G, Wouters PJ, Bouillon R, Weekers F, Verwaest C, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P: Outcome benefit of intensive insulin therapy in the critically ill: insulin dose versus glycemic control. *Crit Care Med* 2003;31:359-366.
- 20 Mesotten D, Van den Berghe G: Clinical potential of insulin therapy in critically ill patients. *Drugs* 2003;63:625-636.
- 21 Jafar TH, Stark PC, Schmid CH, Landa M, Maschio G, de Jong PE, de Zeeuw D, Shahinfar S, Toto R, Levey AS: Progression of chronic kidney disease: the role of blood pressure control, proteinuria, and angiotensin-converting enzyme inhibition: a patient-level meta-analysis. *Annals Int Med* 2003;139:244-252.
- 22 Norris K, Vaughn C: The role of renin-angiotensin-aldosterone system inhibition in chronic kidney disease. *Exp Rev Cardiovasc Ther* 2003;1:51-63.
- 23 Lowe G, Lowe J, Drummond M, Reith S, Belch J, Kesson C, Wylie A, Foulds W, Forbes C, MacCuish A: Blood viscosity in young male diabetics with and without retinopathy. *Diabetologia* 1980;18:359-363.
- 24 Bwititi P, Machakaire T, Nhachi C, Musabayane C: Effects of *Opuntia megacantha* leaves extract on renal electrolyte and fluid handling in streptozotocin (STZ)-diabetic rats. *Ren fail* 2001;23:149-158.
- 25 Feldt-Rasmussen B, Mathiesen E, Deckert T, Giese J, Christensen NJ, Bent-Hansen L, Nielsen MD: Central role for sodium in the pathogenesis of blood pressure changes independent of angiotensin, aldosterone and catecholamines in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1987;30:610-617.
- 26 Nosadini R, Velussi M, Brocco E, Bruseghin M, Abaterusso C, Saller A, Dalla Vestra M, Carraro A, Bortoloso E, Sambataro M: Course of renal function in type 2 diabetic patients with abnormalities of albumin excretion rate. *Diabetes Am Diabetes Assoc* 2000;49:476-484.
- 27 Drummond K, Mauer M: The early natural history of nephropathy in type 1 diabetes II. Early renal structural changes in type 1 diabetes. *Diabetes* 2002;51:1580-1587.
- 28 Zafar M, Naqvi SN-u-H, Ahmed M, Kaimkhani ZA: Altered Kidney Morphology and Enzymes in Streptozotocin Induced Diabetic Rats. *J Intern Morphol* 2009;27.
- 29 Omran OM: Effects of thymoquinone on STZ-induced diabetic nephropathy: an immunohistochemical study. *Ultrastructural Pathol* 2014;38:26-33.
- 30 Steffes MW, Østerby R, Chavers B, Mauer SM: Mesangial expansion as a central mechanism for loss of kidney function in diabetic patients. *Diabetes* 1989;38:1077-1081.

- 31 Premaratne E, MacIsaac RJ, Finch S, Panagiotopoulos S, Ekinici E, Jerums G: Serial measurements of cystatin C are more accurate than creatinine-based methods in detecting declining renal function in type 1 diabetes. *Diabetes Care* 2008;31:971-973.
- 32 Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001;3414:813-820.
- 33 Onozato ML, Tojo A, Goto A, Fujita T, Wilcox CS: Oxidative stress and nitric oxide synthase in rat diabetic nephropathy: effects of ACEI and ARB. *Kidney Int* 2002;61:186-194.
- 34 Baynes JW: Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991;40:405-412.
- 35 Asanuma K, Mundel P: The role of podocytes in glomerular pathobiology. *J Clin Exp Nephrol* 2003;7:255-259.
- 36 Bridgewater DJ, Ho J, Sauro V, Matsell DG: Insulin-like growth factors inhibit podocyte apoptosis through the PI3 kinase pathway. *Kidney Int* 2005;67:1308-1314.
- 37 Hale LJ, Hurcombe J, Lay A, Santamaría B, Valverde AM, Saleem MA, Mathieson PW, Welsh GI, Coward R: Insulin directly stimulates VEGF-A production in the glomerular podocyte. *J Am Physiol-Renal Physiol* 2013;305:182-188.
- 38 Doublier S, Salvidio G, Lupia E, Ruotsalainen V, Verzola D, Deferrari G, Camussi G: Nephron Expression Is Reduced in Human Diabetic Nephropathy Evidence for a Distinct Role for Glycated Albumin and Angiotensin II. *Diabetes* 2003;52:1023-1030.
- 39 Pätäri A, Forsblom C, Havana M, Taipale H, Groop P-H, Holthöfer H: Nephropathy in diabetic nephropathy of type 1 diabetes. *Diabetes* 2003;52:2969-2974.
- 40 Wang G, Lai FM-M, Lai K-B, Chow K-M, Kwan BC-H, Li PK-T, Szeto C-C: Urinary messenger RNA expression of podocyte-associated molecules in patients with diabetic nephropathy treated by angiotensin-converting enzyme inhibitor and angiotensin receptor blocker. *Eur J Endocrinol* 2008;158:317-322.
- 41 Ziyadeh FN, Wolf G: Pathogenesis of the podocytopathy and proteinuria in diabetic glomerulopathy. *Curr Diabetes Rev* 2008;4:39-45.
- 42 Kriz W, Lemley KV: The role of the podocyte in glomerulosclerosis. *Curr Opin Nephrol Hypertens* 1999;8:489-497.
- 43 Bosch JP, Lew S, Glabman S, Lauer A: Renal hemodynamic changes in humans: response to protein loading in normal and diseased kidneys. *J Am Med* 1986;81:809-815.
- 44 Wolf G, Ziyadeh FN: Molecular mechanisms of diabetic renal hypertrophy. *Kidney Intern* 1999;56:393-405.
- 45 Clausen P, Jacobsen P, Rossing K, Jensen J, Parving HH, Feldt-Rasmussen B: Plasma concentrations of VCAM-1 and ICAM-1 are elevated in patients with Type 1 diabetes mellitus with microalbuminuria and overt nephropathy. *Diabetic Med* 2000;17:644-649.
- 46 Zandi-Nejad K, Eddy AA, Glassock RJ, Brenner BM: Why is proteinuria an ominous biomarker of progressive kidney disease? *Kidney Int* 2004;66:76-89.
- 47 Brakis G, Viberti G, Weston WM: Rosiglitazone reduces urinary albumin excretion in type 2 diabetes. *J Human Hypertens* 2002;106:679-684.
- 48 DiPetrillo K, Coutermarsh B, Gesek FA: Urinary tumor necrosis factor contributes to sodium retention and renal hypertrophy during diabetes. *Am J Physiol Renal Physiol* 2003;284:F113-F121.
- 49 Yu R, Bo H, Villani V, Spencer PJ, Fu P: The Inhibitory effect of rapamycin on toll like receptor 4 and interleukin 17 in the Early Stage of Rat Diabetic Nephropathy. *Kidney Blood Press Res* 2016;41:55-69.
- 50 Zhu D, Zhang L, Cheng L, Ren L, Tang J, Sun D: Pancreatic Kininogenase ameliorates renal fibrosis in streptozotocin induced-diabetic nephropathy rat. *Kidney Blood Press Res* 2016;41:9-17.
- 51 Yu QX, Zhang H, Xu WH, Hao F, Liu SL, Bai MM, Mu JW, Zhang HJ: Effect of Irbesartan on chemerin in the renal tissues of diabetic Rats. *Kidney Blood Press Res* 2015;40:467-77.
- 52 Stenvinkel P: Chronic kidney disease: a public health priority and harbinger of premature cardiovascular disease. *J Int Med* 2010;268:456-467.