EFFECTS OF TRANSDERMALLY DELIVERED INSULIN ON SOME SELECTED METABOLIC PARAMETERS OF STREPTOZOTOCIN-INDUCED DIABETIC MALE SPRAGUE-DAWLEY RATS

By Silindile I. Hadebe, Phikelelani S. Ngubane, Metse Serumula & Cephas T Musabayane
School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa.

INTRODUCTION

The tight glycaemic control in type 1 diabetes which requires uncomfortable multiple insulin injections is associated with patients' non-compliance. Therefore, methods which can deliver sustained therapeutic insulin concentrations into the blood may be beneficial with glycaemic control. Studies in our laboratory are concerned with methods that sustain controlled insulin release into the bloodstream based on the topically applied pectin hydrogel insulin (PI) matrix patch. In the present study, we were mainly interested in determining whether transdermally delivered insulin can minimize risk of diabetic complications.

OBJECTIVES

The objectives of this study were to investigate whether topically applied pectin insulin (PI) amidated matrix patch can:

i. sustain controlled insulin release into the bloodstream
ii. control some selected deranged metabolic parameters in STZ-induced diabetic rats.
iii. influence the expression of insulin-stimulated enzymes and facilitative glucose transporters in STZ-induced diabetic rats.

MATERIALS AND METHODS

Patch preparation
The amidated pectin hydrogel matrix patch was prepared using a previously described protocol described by Musabayane *et al*. 2003 with slight modifications[1]. Amidated PI patches with specified pectin/insulin concentrations were prepared by adding 4g of pectin to 100ml of deionised water in a petri dish with subsequent solidification with 2% CaCl2 to give various amounts (11.01, 17.81, 42.64 and 74.98 µg). Patches with measured widths containing 0.74, 1.20, 2.87 and 5.04 µg of insulin translating to a dosage of 2.47, 3.99, 9.57 and 16.80 µg/kg, respectively were cut out and placed on hydrofilm that served as backing material

Dissolution studies
Pectin insulin (PI) hydrogel matrix patch formulations containing various amounts of insulin (11.01, 17.81, 42.64 and 74.98 µg) were dissolved in Sorenson's phosphate buffer at a pH of 7.2 to determine the amount of insulin that was incorporated into each patch.

Study design
The study was designed to establish the effects of PI hydrogel matrix patch formulation on selected metabolic parameters in experimental diabetes.

Acute studies
Oral glucose tolerance (OGT) responses
OGT responses were evaluated in separate groups of non-diabetic and STZ-induced diabetic groups of rats following topical application of PI matrix patch on the back of the neck. The animals were fasted overnight (18 h), followed by measuring blood glucose (time 0). Subsequently, OGT responses to topically applied insulin pectin (PI) hydrogel patches at various doses of insulin (2.47, 3.99, 9.57 and 16.80 µg/kg) were monitored. Rats sham treated with drug free pectin hydrogel matrix patches and insulin (175 µg/kg, s.c.) served as control animals and positive control animals, respectively. Blood glucose was measured before glucose loading and at 15 minutes intervals for the first hour and then hourly for the subsequent 5 hours after glucose-loading.

Pharmacokinetic studies
To investigate whether PI matrix patches applied topically onto the skin delivered insulin into the bloodstream, plasma insulin concentrations were measured in separate parallel groups of STZ-induced diabetic rats as prepared for OGT responses.

Short-term effects
Short-term (5 weeks) effects were assessed in animals applied thrice daily 8 hours apart with topical PI patches containing various doses of insulin (3.99, 9.57 and 16.80 µg/kg). Animals treated with drug-free pectin and insulin (175µg/kg, s.c.) acted as untreated and treated positive controls, respectively. Blood samples and tissue samples were collected for the measurement of selected biochemical parameters and effects on the expression of insulin-stimulated enzymes and facilitative glucose transporters.

Statistical analysis
All data were expressed as means ± standard error of means (S.E.M.). The $AUC_{0\text{-}360min}$ values were calculated using blood glucose concentrations following topical application of PI matrix patch. Statistical comparison of the differences between the control means and experimental groups was performed with GraphPad InStat Software (version 5.00, GraphPad Software, San Diego, California, USA), using one-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparison test. A value of $p<0.05$ was considered significant.

RESULTS

Dissolution studies
Table 1 shows the amount of insulin in insulin-pectin hydrogel patches. The theoretical amount of insulin in each patch was calculated from the known amount of insulin added to petri dishes during patch preparation and the area of the patches cut out of the petri dishes. The insulin incorporation into each patch ranged from 76 % to 94%.

Table 1: Insulin-loading in pectin hydrogel matrices and mean loading-efficiencies: Each value represents the mean value of six different samples.

Theoretical insulin in petri dish (µg)	Actual insulin in petri dish (µg)	Actual insulin in patch (µg)	Dosage $\mu g.kg^{-1}$	% insulin incorporation
11.72	11.01 ± 0.97	0.74 ± 0.05	2.47	94
23.43	17.81 ± 0.07	1.20 ± 0.01	3.99	76
46.86	42.64 ± 0.88	2.87 ± 0.25	9.57	91
93.70	74.98 ± 0.58	5.04 ± 0.01	16.80	80

OGT responses (Figure1)
OGT responses of groups and the area under the glucose curve (AUC) of STZ-induced diabetic rats topically applied PI hydrogel patches on the skin at various doses of insulin are shown in Figure 1.

As can be seen by Figure 1, PI patch treated diabetic rats resulted in a statistically significant decrease in blood glucose at all-time points.

In addition, the blood glucose AUC was smaller in PI hydrogel treated animals compared with respective control diabetic rats.

A dose-dependent effect on the magnitude of PI-induced blood glucose lowering was not statistically significant.

Administration of s.c. insulin demonstrated blood glucose-lowering effects in STZ-induced diabetic rats. In summary, the OGT responses and $AUC_{glucose}$ were not significantly different from those observed with s.c. insulin.

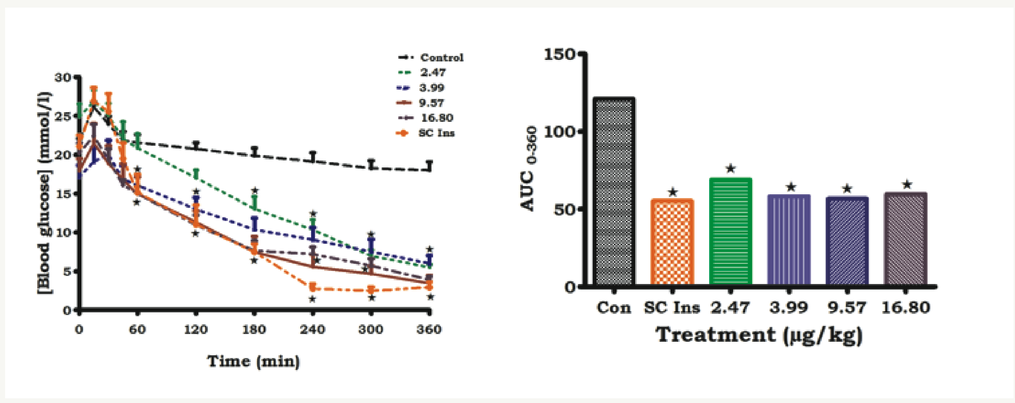

Figure 1: OGT responses **(A)** and $AUC_{glucose}$ **(B)** of STZ-induced diabetic rats to various doses of rats treated with various doses of PI hydrogel patch with control animals. (n=6 in each group).★p<0.05 by comparison with control animals

Insulin pharmacokinetics

The plasma insulin concentration remained very low in the STZ-induced diabetic group (Figure 2).

Conversely, the plasma insulin concentrations were elevated in the non-diabetic and transdermally treated groups for the duration of the experiment.

The plasma insulin concentrations in the animals treated with the high insulin doses (9.57, 16.80 µg/kg) were significantly higher ($p < 0.05$) than those found in all the other groups

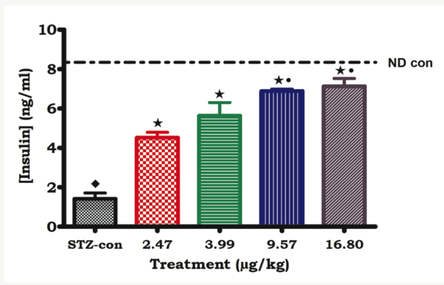

Figure 2: Comparison of plasma insulin concentrations of STZ-induced diabetic rats. (n=6 in each group).
★p<0.05 by comparison to STZ-induced diabetic control
◆p<0.05 by comparison Non-diabetic control
● p<0.05 by comparison to the lowest dose

Short-term studies
Metabolic parameters

Untreated STZ-induced diabetic rats exhibited extensive hyperglycaemia, depletion of liver and muscle glycogen concentrations by the end of the 5-week experimental period (Figure 3 and Table 2).
The reduction in glycogen production was associated with decreased expressions of the insulin-stimulated glycogen synthase (GS) and facilitative glucose transporter (GLUT4) in hepatic and skeletal muscle tissues, respectively.
Treatment with s.c. insulin (175 µg/ kg) and various doses of topically applied PI hydrogel matrix patch for 5 weeks restored the expression of GLUT-4 and GS to levels that were comparable to the non-diabetic control animals (Figure 4).

The PI treated groups showed no dose-dependent effects, however, the effects of PI were comparable to those of the s.c. treated animals.

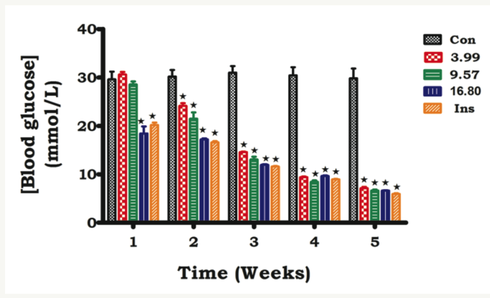

Figure 3: Effects on blood glucose of STZ-induced diabetic rats topically applied amidated PI hydrogel matrix patches on the skin. (n=6 in each group). ★p<0.05 by comparison with control animals.

Table 2: Comparison of hepatic and muscle glycogen concentrations of STZ-induced diabetic rats treated with amidated PI hydrogel patches applied onto the skin with control animals. Values are presented as means, and vertical bars indicate SEM of means (n=6 in each group).

	Glucose mmol/L	Glycogen	µg/100g/tissue
		Hepatic	Skeletal muscle
Non-diabetic control	4.51 ± 0.01	28.42 ± 0.41	2.62 ± 0.32
STZ-control	29.83 ± 2.01◆	12.36 ± 0.72◆	1.02 ± 0.21◆
STZ-TD 3.99	7.13 ± 0.28*	20.08 ± 0.56*	2.02 ± 0.09*
STZ-TD 9.57	6.65 ± 0.18*	21.26 ± 0.64*	2.34 ± 0.20*
STZ-TD 16.80	6.63 ± 0.07*	22.02 ± 1.33*	2.52 ± 0.38*
STZ-SC Ins	5.95 ± 0.11*	21.28 ± 0.94*	2.36 ± 0.21*

* p<0.05 by comparison with respective control animals
◆p<0.05 by comparison with respective non-diabetic animals

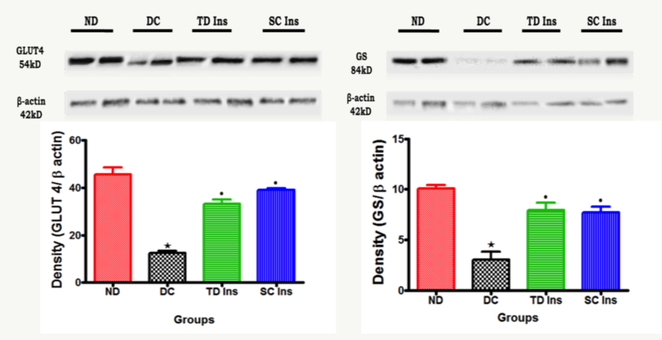

Figure 4: Comparison of the effects of topically applied PI hydrogel matrix patch and s.c. insulin insulin-stimulated enzymes and facilitative glucose transporters in STZ-induced diabetic rats
Values were obtained from Western blots for six preparations.
★p<0.05 by comparison with non-diabetic animals
● p<0.05 by comparison with respective control animals

DISCUSSION

The aim of this study was to develop a transdermal delivery formulation for controlled sustained insulin release into the bloodstream with good control of hyperglycaemia and consequent impact on alleviating complications in diabetes. A carrier for the transdermal delivery of insulin made out of a cocktail comprising (a) low methoxy pectin gelled with calcium ions (b) insulin (c) a transdermal transfer enhancing agent and (d) an antioxidant which sustained controlled release of insulin into the bloodstream of streptozotocin (STZ)-induced diabetic rats was developed. It thus appears that these patches lower blood glucose concentration and a dose-independent effect is seen.

The high insulin dose produced a significantly higher plasma concentration of insulin as compared with all the other groups. We believe that all the pancreatic ß cells were destroyed by the dose of streptozotocin used. Thus, the only explanation for this difference can be ascribed to the differences in the doses of insulin used in the patches.

CONCLUSION

Pectin hydrogel insulin patches lower blood glucose concentration in diabetic rats with concomitant amelioration of some metabolic parameters and insulin is transported through the skin using the pectin patches. We suggest that the formulation may free diabetic patients from multiple insulin injections thereby improving patient compliance.

REFERENCES

1. Musabayane CT, Munjeri O and Matavire TP. (2003). Transdermal Delivery of Chloroquine by Amidated Pectin Hydrogel Matrix Patch in the Rat, Renal Failure 25 (4): 525-534.