The efficiency of transdermal insulin deliveryby using different concentrations of insulin ointment in hyperglycemic rats

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Abstract

Background & Aims: Diabetes mellitus is one of the major issues of the healthcare systems of all societies. Patients with diabetes mellitus type 1 (insulin-dependent diabetes) have to receive daily injections of insulin in order to maintain a constant normoglycemic condition. Since this method of insulin delivery is so invasive and may end in a variety of mental and physical problems after its long-term usage, many scientists have been trying to find less invasive ways of insulin delivery to these patients. At the present study, the efficiency of transdermal insulin delivery using topical insulin ointment at different concentrations was investigated in hyperglycemic rats.

Materials and Methods: For this purpose, 36 male rats were divided into six groups. The control group was delivered a combination of Ketamin and Xylasine, and the rest were treated with different concentrations of insulin ointment following a Xylazine-induced hyperglycemia, then the effect of the ointment on blood glucose levels was investigated.

Results: Our data showed that high concentration (1.73%) of insulin ointment can be as effective as injectable insulin in decreasing blood glucose levels of hyperglycemic rats.

Conclusion: It is concluded that this way of insulin delivery can be a potential alternative to the conventional injectable method. However, more studies are needed in order to confirm its quality and usage in human models.

Keywords: Diabetes, Insulin ointment, Rat, Transdermal delivery

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Introduction

Application of medicines to the skin has been a method of drug delivery for a long time in order to elicit local effects. However, it has been also used during recent years as a route for systemic administration of some drugs such as nicotine for the cessation of smoking in addicts (1). A variety of advantages including: Non-invasiveness, overcoming the first pass effect, decreasing gastric irritation, preventing drug degradation among many others has made this method to some extent superior to other methods of drug administration (2).

The main barrier against transdermal drug delivery is the low permeability of the skin to exogenous compounds mainly due to the Stratum Corneum, a morphologically unique bio-membrane present in the skin (3). Although many chemical and physical penetration enhancers such as surfactants and fatty (4), ionophoresis (5), sonication (6), Ultrasound (7, 8), microneedles (3) have been used thus far to overcome this problem (9). Many attempts have remained unsuccessful in increasing the permeability of the skin to a promising extent especially in drugs with large molecular structures (10).

Only ten drugs had been confirmed as transdermal patches in the world market by 2001 including: Scopolamine (hyoscine), nitroglycerine, clonidine, estradiol (with and without norethisterone or levonorgestrel), testosterone, fentanyl and nicotine, and

lidocaine (11). Many efforts have been made by different researchers worldwide to add to the number of these drugs with especial qualities suitable to be delivered as transdermal medications. Insulin is a drug which is used by increasingly a large number of people suffering from diabetes mellitus type 1 (insulin-dependent diabetes) (12). Diabetic patients have to receive daily injections of insulin in order to maintain a nearly normoglycemic condition.

At the present, subcutaneous injection of insulin is the most conventional way of insulin delivery to diabetic patients which may lead to a variety of physical and mental problems after its long-term invasive usage (13). This fact has drawn the attention of many researchers worldwide to find less invasive ways of insulin delivery to these patients. There have been few studies conducted so far regarding transdermal insulin delivery using insulin as a topical ointment. All of these studies lack the information regarding the different concentrations of insulin ointment, resulting in inadequate data on its efficiency in decreasing blood glucose (14, 15). This study tries to investigate the possible hypoglycemic effect of topical insulin ointment at different concentrations in hyperglycemic rats.

Materials and Methods

Ointment preparation:

The insulin ointment was prepared using pure human insulin (Humulin® R, rDNA U-100, Indianapolis, IN) and eucerin. Eucerin is commonly used for therapeutic and cosmetic purposes (16). It is among one of five major types of substances used in preparation of ointments (17). Desirable pharmacological properties of eucerin, such as good drug release potential, and being hydrophilic (17), has made it a good choice to be used in the preparation of oil-based ointments. 4, 8, 9.4 and 10.2 ml of regular insulin were calculated to be mixed in 2 g of eucerin in order to reach concentrations of 0.69%, 1.36%, 1.6%, and 1.73% of insulin ointments, respectively. The mentioned concentrations were calculated as weight/weight percentages (18).

To prepare the ointment for the treatment of each group, specific amounts of insulin had been gradually added to 2 g of eucerin while the mixture was being stirred. Once the mixture lost its consistency, the addition of insulin was seized for a while, and the mixture was stirred again so that it could regain its consistency then the remaining amount of insulin was added. Under these conditions, insulin could be readily mixed into eucerin. Finally, the ointment was placed in 4 °C until commencement of the experiment.

Animal experiments:

36 male Wistar rats (250-360 g) were divided into 6 groups of six including one control (C) group and five treatment (T) groups. All the animals were fed on standard chow diet, and they were kept in a stress-free condition two weeks before the experiment. A combination of ketamin hydrochloride (60 mg/kg) and xylazine hydrochloride (10 mg/kg) was intra-peritoneally injected in all animals to anesthetize the animals, and to reach a temporary (12 hours) but constant hyperglycemia. 25 cm^2 of the abdominal surface of each animal was carefully shaved in the second (T_2), third (T_3), forth (T_3), and the fifth (T_5) treatment group, and the skin was cleaned up in order to remove any remaining hair (Figure 1).By the initiation of the experiment, the animals in T_2, T_3, T_4 and T_5 groups were treated with the concentrations of0.69%, 1.36%, 1.6%, and 1.73%of the insulin ointment on their shaved abdominal skin, respectively. Each of the animals in the first treatment group (T_1) received a subcutaneous injection of insulin.

Since it had been shown in previous studies that a dose of 0.25 U/Kg of insulin is the most effective one in decreasing the blood glucose levels of hyperglycemic rats, the same dose of insulin was used to be injected for the first treatment group (14). Blood samples were collected from the tail vein of the animals once before anesthesia to confirm the uniformity of the glucose levels in all animals, and for the second time after anesthesia and prior to the administration of insulin to obtain a baseline glucose level. Additional blood

samples were taken every 15 minutes postanesthesia for a period of 90 minutes. The blood glucose levels were measured and recorded using an ACCU-CHEK™ glucometer (Roche Diagnostics Co., Indianapolis). Each sample was tested at least twice to confirm the accuracy of the readings. The blood glucose levels of all animals were recorded as the mean ± standard deviation of each group in every 15 minutes of the whole 90 minutes experimental period. Statistical analysis was performed using SPSS 16. One-way ANOVA statistical test was used to confirm the uniformity of the blood glucose levels and the weights in all animals prior to anesthesia.

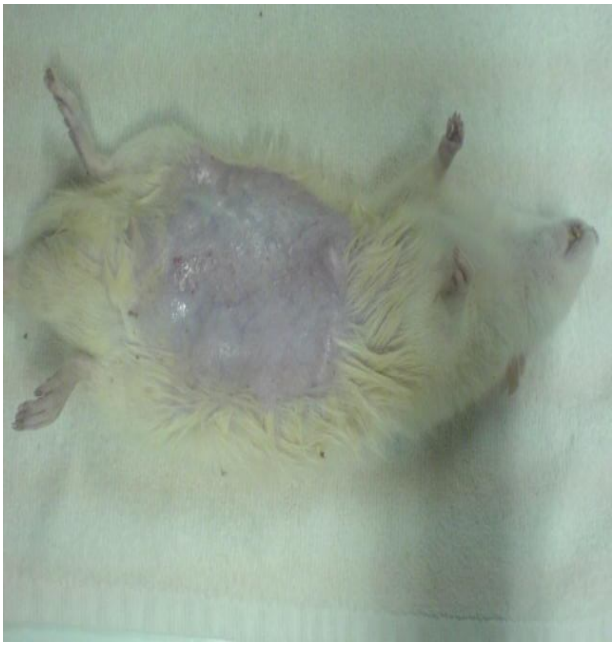

Figure 1: A rat placed in a dorsal decubitus position for ointment insulin administration.

Results

The blood glucose levels before anesthesia was within a range of 93 to 118 mg/dl. The mean and standard deviation weight of the rats in the study groups were measured (298±30.28). One-way ANOVA statistical test between six studied groups showed that there was no significant difference between the weight of the rats (p=0.186) and their blood glucose concentration before anesthesia (p=0.423).

The average and standard deviation of initial blood glucose level at the beginning of the experiment (the average baseline glucose level) was 204 ± 18 mg/dl for the 36 experiments. The mean and standard deviation of blood glucose level changes were obtained in each group during the experimental period. The results were graphed in Figure 2.

The non-parametric multiple comparisons statistical test(Dunnett) showed that there was no significant difference between the mean glucose levels in the treatment groups in comparison with the control group immediately after anesthesia (P>0.05).Multiple comparisons (Dunnett) test was also performed on the mean blood glucose levels between the control group and all treatment groups at different times of the experiment (Table 1). There was a significant difference between the blood glucose levels of the animals60 minutes after anesthesia and their baseline values (immediately after anesthesia) in all treatment groups comparing to the control group (p<0.05).

Little modifications were observed in the blood glucose levels of the animals in T_2, T_3 and T_4 insulin ointment groupsin45 minutes post anesthesia with the ranking order of 1.6%>1.36%>0.69%(p>0.05). However, the change of blood glucose levels in T_5 (1.73% ointment) and T_1 (insulin injection) groups significantly differed from that of the control group (p<0.05) during the whole experiment period of 90 minutes. The blood glucose lowering activity of the 1.73%insulin ointment was almost equal to that of the injectable insulin. It may indicate that transdermal penetration of insulin can be increased by higher ointment concentrations.

Table 1: The results of multiple comparisons (Dunnett) test between the mean blood glucose levels in the studied groups of animals at different times of the experiment

Time (min)	Multiple Comparisons (Dunnett)	P-Value
15	T1 - C	0.025
	T2 - C	0.085
	T3 - C	0.924
	T4 - C	1.00
	T5 - C	0.019
30	T1 - C	0.001
	T2 - C	0.055
	T3 - C	1.00
	T4 - C	0.435
	T5 - C	0.001
45	T1 - C	0.001
	T2 - C	0.005
	T3 - C	0.247
	T4 - C	0.004
	T5 - C	0.001
60	T1 - C	0.001
	T2 - C	0.011
	T3 - C	0.018
	T4 - C	0.004
	T5 - C	0.001
75	T1 - C	0.001
	T2 - C	0.001
	T3 - C	0.001
	T4 - C	0.001
	T5 - C	0.001
90	T1 - C	0.002
	T2 - C	0.001
	T3 - C	0.001
	T4 - C	0.001
	T5 - C	0.001

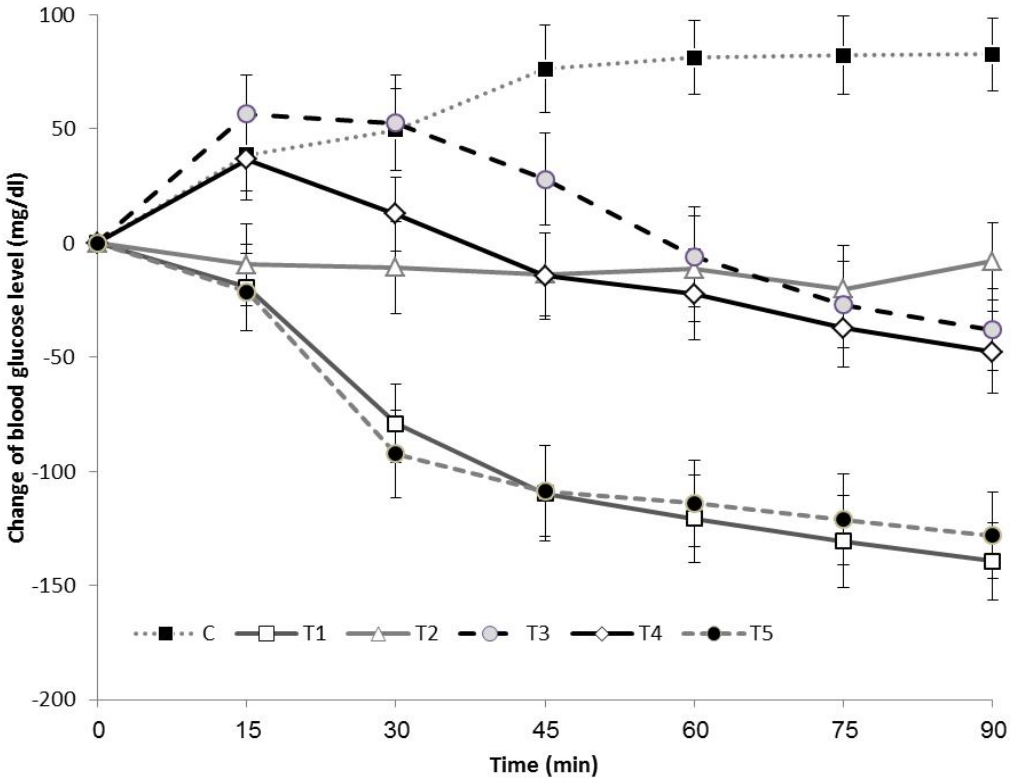

Figure2: Themean and standard deviation of blood glucose level changes in each group during experimental period

Discussion

Transdermal delivery of insulin can be a potential and desirable alternative for its injectable form, if it is proven to be at least partially as efficient as its subcutaneous route. In a study, insulin was successfully delivered to hairless rats through the skin using a preparation of insulin solution at a concentration of 100 U/ml exposed to ultrasound (19). In two other similar studies which was conducted by Park *et al.* the same preparation of insulin was administered to rat and pig models along with the application of ultrasound as a method to enhance the transdermal permeation of insulin molecules (14, 15). What all of the mentioned studies lack, is probably the application of different concentrations of insulin. In the present study, we attempted to investigate the efficiency of different concentrations of insulin as an ointment, and to define the most efficient concentration at which insulin can be delivered through the skin. According to the best of our knowledge, this is the first study in which ointment of insulin has been applied to the skin at different concentrations. Our data showed that the transdermal permeation of insulin will be increased by the increasing concentrations of insulin ointment. Moreover, 1.73% ointment was shown to be almost as effective as the injectable insulin in decreasing blood glucose levels of xylasine-induced hyperglycemic rats which indicates that application of insulin ointment can be a promising non-invasive alternative to its injectable form.

However, an effective permeation enhancer that may reduce the effective concentration of insulin by increasing the skin permeability remains to be discovered. The principal resistant layer against transdermal drug delivery is stratum corneum layer of the skin (20). There have been many studies conducted so far to find ways of enhancing skin permeability to different drugs by overcoming this layer. Exposure to ultrasound (21) and the usage of micro-scale devices (22) among many other ways have been used to solve this problem; however, applying these factors along with

the application of insulin ointment may result in the invention of new approaches.

It is evident that the body`s glucose response to injectable insulin in humans is variable depending on factors such as exercise, body weight and the most recent meal (23). The required insulin dose for a diabetic adult to maintain a normoglycemic condition is 0.5-1 U/Kg/Day (24). It has been also shown that the dosage of insulin that is efficient in decreasing blood glucose levels varies among species (25). Other *in vitro* and clinical experiments are necessary in order to investigate the efficiency of insulin ointment in humans, and to specify the quality and the exact concentration of the ointment to be used as an alternative to injectable form. Finally based on this information, transdermal delivery of insulin ointment may pave the way to a promising future in the treatment of diabetes after its approval by adequate animal and clinical studies.

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Conflicts of Interest:

The authors declare no conflict of interest.

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