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Blood glucose control in diabetic rats by transdermal iontophoretic delivery of insulin

Jue-Chen Liu [1], Ying Sun [1], Ovais Siddiqui [1], Yie W. Chien [1], Wei-min Shi [2] and John Li [2]

[1] *Controlled Drug-Delivery Research Center, College of Pharmacy and* [2] *Department of Biomedical Engineering, College of Engineering, Rutgers – The State of University of New Jersey, Piscataway, NJ 08855-0789 (U.S.A.)*

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Summary

Iontophoresis of direct current (DC) with various pulse waveform modes was used to facilitate and regulate the transdermal delivery of insulin in order to control blood glucose levels in diabetic rats. It was found that a considerable reduction in blood glucose levels can be achieved by a lower current intensity and shorter application time, using a pulse current instead of simple direct current. The effect of DC pulse waveform parameters on the blood glucose control in diabetic rats was also investigated. Frequency and current intensity were found to be the two important parameters. Blood glucose levels were observed to be better controlled when higher frequency with an on/off ratio of 1 : 1 was used.

Introduction

Transdermal delivery of drugs has gained an increasing recognition in recent years since this route of administration bypasses gastrointestinal (GI) degradation and hepatic metabolism and may also improve patient compliance. The possible use of a transdermal route for the delivery of peptides and/or proteins has met with limited success in the past (Siddiqui and Chien, 1987). In order to deliver a peptide/protein molecule through an intact skin and successfully attain a systemic effect, chemical and/or physical method(s) are required to enhance the rate of penetration of peptides/proteins through the main diffusion barrier – the stratum corneum.

Correspondence: J.-C. Liu, Controlled Drug Delivery Research Center, College of Pharmacy, Busch Campus, P.O. Box 789, Piscataway, NJ 08854, U.S.A.

Iontophoresis, a process which causes an increase in the migration of ionic species into the skin or tissues under the gradient of electrical potential (Harris, 1967), has been used in the past to enhance the percutaneous penetration of charged molecules. The degree of enhancement was generally found to depend on the ionic characteristics of the drug (Echols et al., 1975; Comeau et al., 1983; Siddiqui et al., 1985, 1987; Burnette et al., 1986, Okabe et al., 1986; Sun et al., 1986).

Iontophoresis, using simple direct current (DC), has been demonstrated to facilitate the transport of charged insulin molecules through the rabbit skin when the stratum corneum was disrupted (Kari, 1986). Recently, it was found in our laboratory that it was possible to reduce the blood glucose levels in diabetic hairless rats by administration of insulin through the intact skin using simple DC with a current density of 0.67 mA/cm^2 and an application time of 80 min (Siddiqui et al.,

1987). The possible treatment-induced discomfort and/or skin damage as well as the physiological response to current stimulation at a current density over 0.5 mA/cm^2 with the long treatment duration would, however, be one of the major concerns. It is highly desirable therefore, to improve this iontophoretic delivery technique to achieve a more efficient transdermal delivery of insulin or other peptides/proteins using a lower current intensity with a shorter treatment time.

In this report, we have investigated the efficiency of a pulse waveform in the iontophoretic delivery of insulin to achieve blood glucose control and compared the results with a simple DC iontophoretic delivery system. Studies were carried out on diabetic hairless rats and the pharmacodynamic response was monitored by measuring glucose levels in the blood.

Theoretical considerations

Electrical properties of skin

Skin manifests large impedance to charged molecules which are driven through the skin under an applied electrical field. The electrical properties of the skin are dominated by the stratum corneum which is considered to be the least conductive layer of the skin. Stratum corneum consists of multilayers of cornified cells. These electrically insulated horny cells are continuously replenished by the slow upward migration of cells from the basal cell layer of the stratum germinativum. Skin is breached by hair follicles, sweat ducts, and other glands which could provide a potential pathway for diffusion across the skin. This is referred as "shunt" pathway and is considered to be important for the percutaneous penetration of ionic species, which show an extremely poor permeation through the transcellular or intracellular route (Chien, 1982). Under the application of an electrical field, ionic species may penetrate the skin via the shunts and/or intercellular route (Siddiqui et al., 1985, 1987).

Stratum corneum shows two important electrical features. First, its impedance changes with the pulse frequency especially in man (Tregear, 1966). Secondly, it will be polarized by a direct electrical

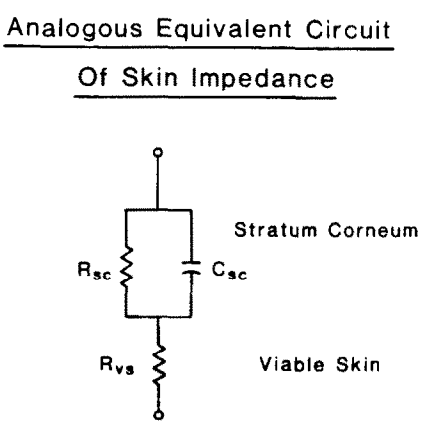

Fig. 1. Analogous equivalent circuit of skin impedance, where R_{vs} and R_{sc} are the resistors, respectively, for viable skin and stratum corneum; C_{sc} is the capacitor for the stratum corneum.

field (Yamamoto and Yamamoto, 1976). These properties may be represented by an electrical equivalent circuit shown in Fig. 1. In this equivalent circuit, R_{vs} is the pure resistance, which originates from the viable skin. This resistance (R_{vs}) to charged molecular current does not change with pulse frequency. The parallel combination of R_{sc} and C_{sc} represents the resistive and capacitive components for the impedance of the stratum corneum and is a function of pulse frequency. It has been found that the impedance of human skin decreases with the increase in frequency (Yamamoto and Yamamoto, 1976).

When a direct electrical field (i.e. simple DC mode) is used to facilitate the penetration of ionic molecules, an electrochemical polarization occurs in the skin. This polarization operates against the applied electrical field and greatly reduces the magnitude of the input current. Polarization of the stratum corneum is analogous to the charging of the capacitor C_{sc} in Fig. 1 with an input current, i. This input current decays exponentially across the skin when constant DC voltage is applied. Consequently, the efficiency of current-dependent penetration of ionic species through the skin is expected to be reduced.

To avoid the polarization of the stratum corneum, a pulse DC can be used. The pulse mode is a DC voltage which periodically alternates with the "on" and "off" of the applied voltage (Fig. 2). In the state of "on", charged molecules are forced

into the skin and the stratum corneum soon becomes polarized; while in the state of "off", no external stimulation is present and the stratum corneum becomes depolarized. The on/off ratio controls the time proportion for polarization and depolarization process in each cycle. The number of on/off cycles in each second is frequency. The duration of each cycle is the reciprocal of frequency. For instance, frequency of 2000 Hz with the on/off ratio of 1 : 1 means there are 2000 cycles in each second and each cycle lasts 1/2000 s or 0.5 ms, where the ratio of polarization-to-depolarization period is 1 : 1 (0.25 ms/0.25 ms). Similarly, when the on/off ratio is 4/1 or 8/1 at 2000 Hz, the time proportion for polarization and depolarization are 0.4 ms/0.1 ms and 0.44 ms/0.056 ms, respectively. The optimum value of on/off ratio varies with the frequency applied. When an ideal on/off ratio is selected, every new cycle starts with no residue polarization left in the skin from the previous cycle, i.e. the effect of polarization is eliminated.

The energy (E) required to overcome the penetration barrier, stratum corneum, can be expressed by:

$$E = \int [V(t)i(t)]\, dt = \int \left[i(t)^2 R_t(t)\right] dt \qquad (1)$$

where $V(t)$ and $i(t)$ are the voltage and current applied respectively and R_t is the impedance of the skin. As can be seen from Eqn. 1, less energy will be required to overcome the barrier when the skin impedance is reduced. This may be achieved by applying the current with proper frequency and on/off ratio. Therefore, it is essential to select optimum pulse mode parameters to attain the best facilitating effect of iontophoresis for a particular drug or a dosage form. The sensitivity and the tolerance of the skin in response to the frequency used should also be considered.

Instant momentum

As the iontophoretic device is switched on, current is carried by both charged drug molecules and buffer ions in the drug reservoir compartment. The effectiveness of pulse current in the delivery of insulin may also be attributed to the change in the instant momentum and entropy (Sun et al., 1986). This effect is expected to be more significant for molecules with large dimension, such as insulin, since a higher counter flow will be developed as the macromolecules are delivered through the "shunts" and "intercellular" spacings of the skin (Idol and Anderson, 1986). As a result, a pulse waveform is expected to be more effective than simple DC mode in pushing the macromolecules through the skin.

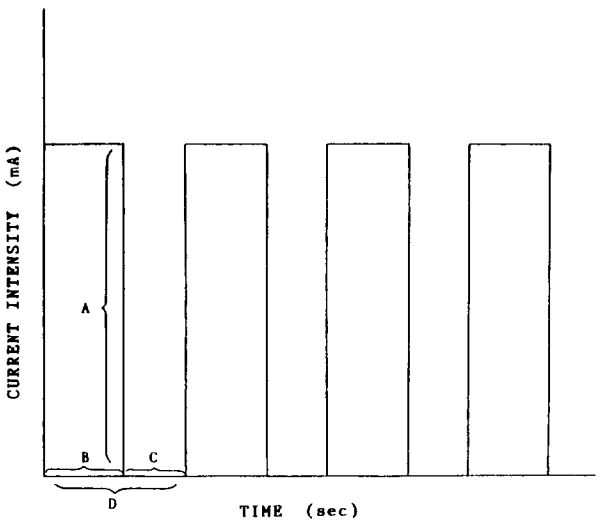

Fig. 2. Diagram of a pulse waveform profile, where A is the amplitude of a current intensity (mA), B/C are the on/off ratio, D is the duration (s) of a complete cycle and so 1/D is the frequency.

Experimental

Materials and equipments

Hairless rats (350–500 g) were obtained from the Institute of Pathology, Walter Reed Army Medical Center, Washington, DC. Aqueous solution of insulin (Iletin II/Eli Lilly, Purified Pork insulin injection, 500 IU/ml) and streptozotocin (Upjohn as Zanosar injection; SZ) were used during in situ studies. The blood glucose levels were monitored using a glucose analyzer (YSI-model 27, range 1000 mg/dl) of Yellow Springs Instrument Co., OH. Tritiated water [2.5E(6) dpm/g] was purchased from Packard Instrument Co., OH.

Biofluor (New England Nuclear, MA) was used as liquid scintillation cocktail. A liquid scintillation counter (1214, LKB, Gaithersburg, MD) was used for radioactive counting. All other reagents were of analytical grade. The analytical techniques have also been reported previously (Siddiqui et al., 1987).

Induction of diabetes

Hairless rats were made diabetic by intraperitonial (i.p.) (SZ: 40 mg/kg) injection of streptozotocin (SZ). The animals were allowed free access to food and water. Animals were found to become diabetic after 1–3 daily injections (Siddiqui et al., 1987).

Preparation of reservoir and receptor electrodes

The insulin solution of 250 IU/ml adjusted to pH 3.6 was filled into the reservoir electrodes. The reason to have the pH of insulin solution adjusted to 3.6 in the reservoir was based on the observations made in previous work that transdermal iontophoretic delivery of insulin in hairless rats is likely to be more effective at pH 3.6 (Siddiqui et al., 1987). The receptor electrode was prepared to contain 10% polyacrylamide and 0.05% NaCl as a conductive base.

At the end of the experiment the pH of the reservoir was measured. The reservoir electrode was made to act as an anode since insulin molecules carry a positive charge at pH 3.6 (the isoelectric point of insulin is 5.1–5.3). Experiments without insulin in the reservoir electrodes were also carried out. The effects of fasting and anaesthesia on the blood glucose levels were also studied.

Iontophoresis in situ

Hairless rats were anaesthesized with i.p. injection of urethane (as 25% aqueous solution, 1.5 mg/g) prior to the beginning of iontophoretic procedure. At the end of each experiment, the rats were sacrificed by intravenous injection of Euthanasia solution.

Both the reservoir (surface area = 6 cm^2) and the receptor electrodes were applied on the abdominal site on the diabetic rats (Fig. 3). Blood samples were collected in heparinized test tubes

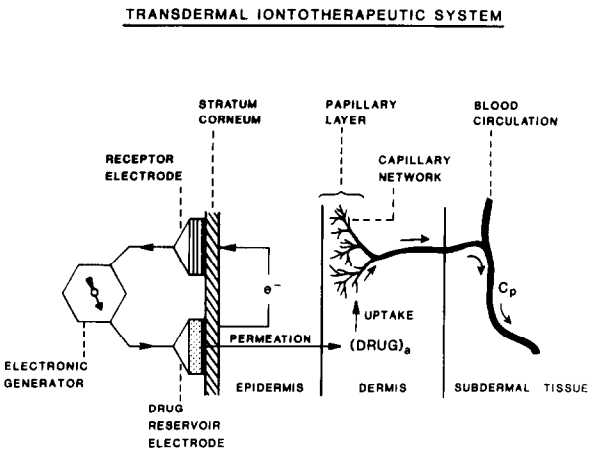

Fig. 3. Schematic illustration of the experimental set-up for transdermal periodic iontophoretic delivery of drugs.

by cutting the tip of the tail. Iontophoresis was carried out using a prototype transdermal periodic iontophoretic system (TPIS) in order to provide the required direct current with desired pulse modes, which can be adjusted by varying the waveform, frequency, on/off ratio and magnitude of ampere (Fig. 2). The current profiles were monitored on an oscilloscope. Duplicate runs were conducted in each experimental conditions.

The integrity of the stratum corneum was examined at the end of the run by measuring the disappearance of tritiated water from the donor compartment of a Franz diffusion cell for 2–3 h as described previously (Siddiqui et al., 1987).

Results and Discussion

It was found in the previous work that the reduction of blood glucose levels was less than 5% when insulin was transdermally delivered in the absence of iontophoresis (Siddiqui et al., 1987). Fig. 4 also shows that neither fasting, anaesthesia nor TPIS without insulin treatment influenced the hyperglycemic condition of hairless rats.

Transdermal delivery of insulin: DC vs pulse

In order to express the effectiveness of blood glucose reduction by the transdermal delivery of insulin, the % change in blood glucose level, de-

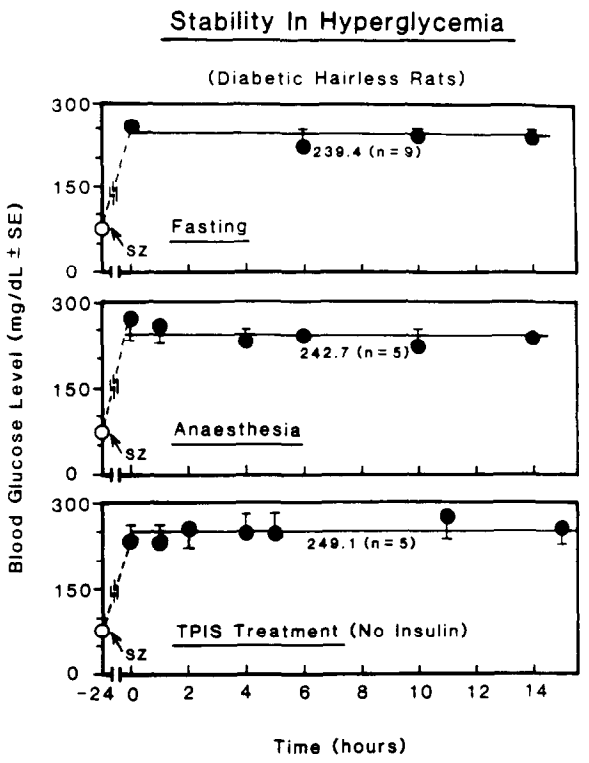

Fig. 4. Effect of fasting, anaesthesia and current (1 mA, 2000 Hz, 1:1, 40 min) on the hyperglycemic condition of diabetic rats.

fined as the percentage blood glucose level of time zero (beginning of the treatment) is used to present the data.

Fig. 5 shows the comparative effect of pulse current and simple direct current, in delivering insulin, on the reduction of hyperglycemic states. The results indicate that the blood glucose levels did not change considerably when insulin was delivered iontophoretically using simple DC mode at a current density of up to 0.33 mA/cm^2 (2 mA/6 cm^2 = 0.33 mA/cm^2). On the other hand, the blood glucose level was markedly reduced when pulse DC was applied at the same current density, and was maintained at much lower levels for a longer period of time before going back to the original hyperglycemic state.

Parameters affecting transdermal delivery of insulin

Intensity of current and duration of treatment. The rate and extent of charged molecules which

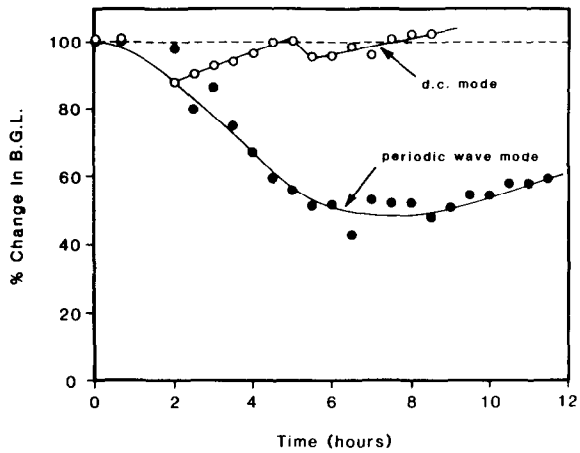

Fig. 5. Effect of delivery mode on blood glucose levels (BGL) in diabetic hairless rats treated with transdermal periodic iontophoretic system at 2 mA (0.33 mA/cm^2) for 40 min; ○, simple DC, ●, pulse DC (2000 Hz, 4:1). Each data point represents the mean of two individual runs and line was drawn by eye.

may penetrate through the skin are expected to be theoretically proportional to the intensity of current and the duration of treatment for a transdermal iontophoretic delivery process. The results in Fig. 6 indicate that the blood glucose levels in diabetic rats were reduced at approximately the same rate, at current intensities of either 1 (0.17 mA/cm^2) or 2 mA (0.33 mA/cm^2); while the

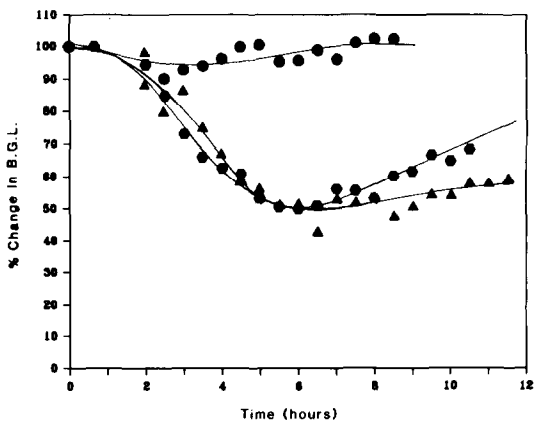

Fig. 6. Effect of current intensity on the blood glucose control in diabetic hairless rats (treatment duration = 40 min). Each data point represents the mean of two individual runs and line was drawn by eye: ●, 2 mA, DC; ●, 1 mA, 2000 Hz, on/off = 4:1; ▲, 2 mA, 2000 Hz, on/off = 4:1 (n = 2).

current intensity of 2 mA is able to reduce the blood glucose levels for a comparatively longer time. This phenomenon could be attributed to the possible aggregation of insulin molecules at physiological pH and the reservoir function of the stratum corneum. It is speculated that the insulin monomers (at pH 3.6) will be self-associated once they are driven into the skin since the pH of physiological environment would be higher (Klostermeyer, 1966). These aggregates would be expected to remain in the stratum corneum due to their larger dimension and then slowly diffuse into the systemic circulation. The strong interactions between insulin and the skin tissues (insulin/skin interactions) will also trap the insulin molecules in the stratum corneum. Consequently, more insulin molecules were driven into the skin at 2 mA, and the same onset rate was observed at current intensities of 1 and 2 mA. The blood glucose levels were, however, reduced for a longer period of time at 2 mA as more insulin molecules were delivered into the stratum corneum and ultimately into micro-circulation in the skin.

Fig. 7 shows that the length of hyperglycemic control was dependent upon the duration of the application of transdermal periodic iontophoretic system containing insulin. It was found that the increase in treatment time prolonged the control

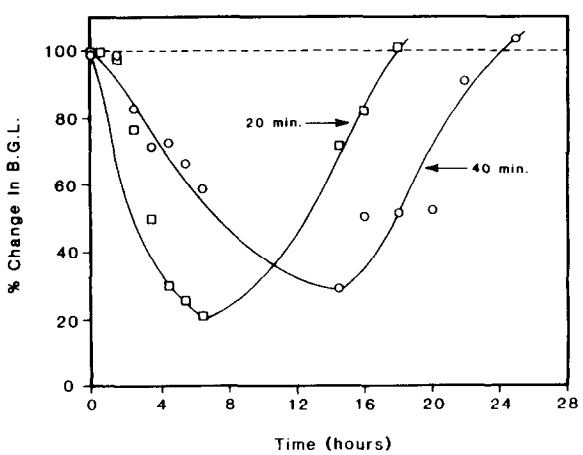

Fig. 7. Effect of treatment duration on the glucose reduction of diabetic hairless rats (1 mA or 0.17 mA/cm^2, 1000 Hz, on/off = 1 : 1). Each data point represents the mean of two individual runs and line was drawn by eye: □, 20 min; ○, 40 min.

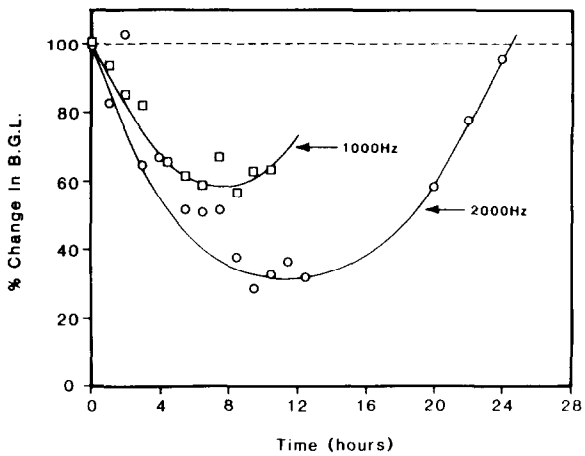

Fig. 8. Effect of frequency on the blood glucose reduction in the diabetic hairless rats (1 mA, 40 min, on/off = 1 : 1). Each data point represents the mean of two individual runs and line was drawn by eye: □, 1000 Hz, ○, 2000 Hz (n = 2).

of blood glucose levels in the reduced blood glucose state.

Frequency. The effectiveness of current frequency in delivering insulin was studied using a frequency range of 50–2000 Hz. No blood glucose reduction was observed when a frequency of below 1000 Hz was used. Fig. 8 suggests that higher frequency offers better blood glucose reduction when the same average current intensity is used. This phenomenon can be explained by the decrease in skin impedance with the increase in current frequency (Yamamoto and Yamamoto, 1976). More data are presently being generated to substantiate the frequency effect.

On / off ratio. Three different on/off ratio settings were used to investigate the effect of the relative proportion of polarization and depolarization process on the efficiency of transdermal iontophoretic insulin delivery. As can be seen in Fig. 9, the on/off ratio of 1 : 1 yields the best glucose control when the frequency is at 2000 Hz (0.5 ms/cycle). These observations suggest that at a cycle duration of 0.5 ms, the duration for the depolarization process should be at least the same as that for the polarization process, i.e. 0.25 ms/0.25 ms, for more efficient transdermal iontophoretic delivery of insulin. Apparently, any residue polarization in the skin from the previous

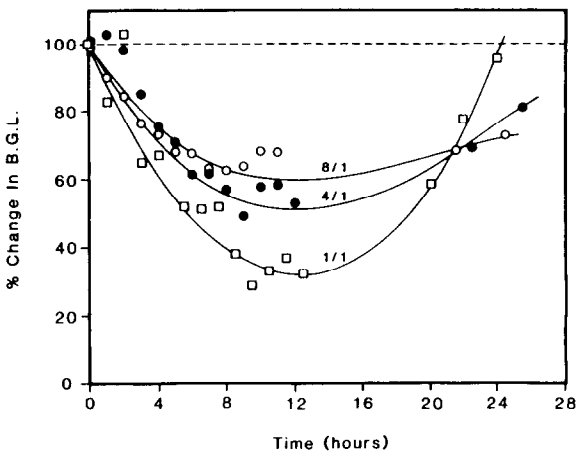

Fig. 9. Effect of on/off ratio on the blood glucose reduction in the diabetic hairless rats (1 mA, 40 min, 2000 Hz) Each data point represents the mean of two individual runs and line was drawn by eye: ○, 8:1; ●, 4:1; □, 1:1 ($n = 2$)

cycle would reduce the effectiveness of insulin delivery, resulting in less reduction in blood glucose levels as shown at an on/off ratio of 4:1 (0.4 ms/0.1 ms) and 8:1 (0.44 ms/0.056 ms).

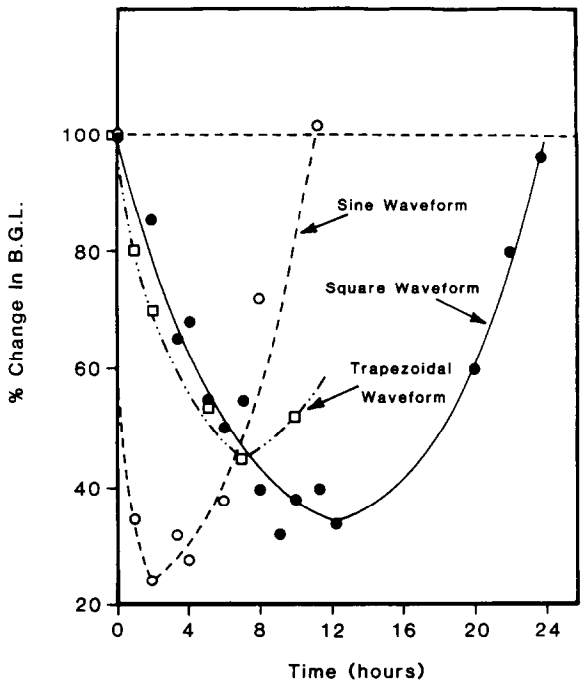

Fig. 10. Effect of waveform on the blood glucose reduction in diabetic hairless rats: ●, square; ○, sine; □, trapezoidal.

Waveform. The effect of various waveforms including square, sinusoidal and trapezoidal waves on the blood glucose reduction was also studied (Fig. 10). The results suggest that the time required to attain the maximum reduction in the blood glucose levels and the duration of its effectiveness appears to be dependent upon the waveform used.

Integrity of the stratum corneum

Exposure of the stratum corneum of the rat's abdominal skin to the reservoir electrode with iontophoresis did not produce any effect on the rate of percutaneous penetration of tritiated water. The reduction in the radioactive counts of tritiated water from the donor compartment of the Franz diffusion cell was about 5% for both the control and the treated skin within 2–3 h.

pH change

The pH in the reservoir electrode was found to show negligible change after the iontophoretic treatment for formulation used in this study.

Conclusions

A non-parenteral method for the delivery of macromolecules such as insulin was developed by using a pulse DC mode transdermal iontophoretic technique. Low current intensity and short treatment duration were found to be adequate to obtain equivalent blood glucose control compared with that using conventional DC iontophoresis. The intensity of current, frequency, on/off ratio and mode of waveform were found to play an important role in the transdermal iontophoretic delivery of insulin. More extensive investigations on various aspects of this system are necessary to obtain optimum parameters of pulse DC mode for transdermal iontophoretic delivery of insulin.

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