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Iontophoresis of Insulin Through Rabbit Skin

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이온토포레시스에 의한 가토 피부를 통한 인슐린 전달

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It has been indicated that problems associated with insulin iontophoresis are low bioavailability, slow absorption rate and the use of high dosage. Pretreatment of skin as a method of solving these problems was used in alloxan-induced diabetic white rabbits. Skins were treated with skin needle, electric razor, knife razor and scotch tape. Transport data shows that insulin delivery was enhanced significantly by the treatment which disrupt the barrier properties of stratum corneum. The data also shows that insulin absorption lasted for several hours after the cessation of iontophoresis. The degree of skin treatment was estimated by measuring the electrical resistance of skin. When the skins were treated with skin needle and electric razor, the standard deviations of resistance were small, which suggests the possibility of uniform delivery of insulin . The dermal responses after the invasive delivery were evaluated in accordance with OECD Guideline. It seems that electrical resistance of the skin correlate well with the dermal irritation.

Keywords—Iontophoresis, Insulin, Invasive delivery, Treated skin, Electrical resistance.

Past works on iontophoresis of drugs have been mainly aimed at the localization of drugs in surface tissues for local diseases.[1] Recent works have been focused on the systemic transdermal delivery of drugs (both charged and uncharged drug molecules with small molecular weight), due to the various advantages of iontophoresis over conventional delivery (injection, oral and passive transdermal delivery), such as the ease of on/off, high degree of control over the drug delivery rate in wide range, reduced systemic side effect, bypass the hepatic first-pass effect and patient compliance. Since late 1980's, with the advancement in biotechnology, many peptides and protein drugs became available as therapeutic agents. Iontophoresis has gained great interests as a method to deliver these drugs. Iontophoretic delivery of various peptides and proteins (e.g. LHRH, TRH, vasopressin, calcitonin, insulin) have been studied, and the results indicated that only peptides with small molecular weight could be delivered to the desired amount.

Numerous studies have dealt with the development of a iontophoretic insulin delivery system. Iontophoresis of insulin was examined in young swine, but failed to deliver effective amount of insulin due to the small number of charges on the insulin molecules and to the large portion of molecules existing as polymeric forms.[2] To increase the amount of in-

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sulin delivered, iontophoresis after removing stratum corneum has been carried out. The results showed that the treatment of skin can reduce blood glucose level effectively in diabetic rabbits.[3] A pulse direct current with small current density was also employed with success to deliver insulin in diabetic rabbits.[4] Iontophoretic conditions (frequency, current, on/off ratio, wave form) has been found to play an important role.[4] Electro-osmosis also contributes to the flux of insulin through the intact skin of albino rabbits.[5]

Though the possibility of iontophoretic insulin delivery has been demonstrated, iontophoretic insulin delivery through transdermal route has met with such limitations as follows: i) because insulin molecules are large(MW ~5800 dalton) and mainly exist as polymeric structure in aqueous solution, transdermal transport through intact skin is minimal. ii) because the pH of skin is similar to the isoelectric point of insulin, insulin molecules are precipitated in skin and lose their bioactivity by enzymatic degradation before they are absorbed into the systemic circulation.[6] iii) lipid matrix domain, where most drug molecules are expected to pass through, is hydrophobic in nature, and thus prevent the flow of insulin and current.[7,8] Due to these problems, the clinical feasibility of iontophoresis as a method for insulin delivery still remains doubtful.

In this paper, we used invasive technique to maximize the iontophoretic flux and to minimize the lag time. The invasive treatment of skin was carried out using such devices as skin needle, electric razor, knife razor and scotch tape. The degree of skin treatment was monitored by the measurement of electrical resistance of skin. Blood glucose and serum insulin levels of diabetic rabbits were measured. The degree of skin irritation which may appear after iontophoresis of the treated skin was also monitored.

Materials and Methods

Alloxan-induced Diabetes Mellitus

Diabetic mellitus was induced in white rabbits (weighing 2-3 kg) by intravenous injection (125 mg/Kg) of alloxan (Sigma Chemical Co., St. Louis) as a 10% solution in normal saline. The rabbits were allowed free access to food and water. Blood glucose levels were assayed 1 and 3 days after alloxan treatment to determine which rabbits had become diabetic. Rabbits with blood glucose levels of 〉 250 mg/dl were used in this study.

Skin Treatments

Hair was removed from the back of a rabbit with electric clippers. After 2 days, stratum corneum was disrupted in various ways using such devices as skin needle, electric razor, knife razor and scotch tape. Skin needle has been used in oriental acupuncture and it is consisted of 80 stainless steel needles/cm^2 (diameter : 0.1 mm, penetration depths : 0.2 mm). Skin was treated with skin needle held perpendicular to the skin by gentle pressing.[9] Treatments by electric razors were carried out by gentle scraping of skin for 10 seconds in order to partially remove stratum corneum. Treatments by knife razor were carried out by gentle moving with slight pressure for 5 times. Treatment by scotch tape was made by stripping the skin for 5 times.[10] After treatment, electrical resistance of the skin was measured for the estimation of uniform treatment.[11] Electrical resistance was measured immediately after the treatment of skin. Electrical resistance was determined by applying an constant current between two Ag/AgCl electrodes (2×2 cm plate) which were placed on top of the treated skin and the potential difference between two electrodes was measured. The electrical resistance of skin was usually in the range of 16-27 KW.

Iontophoresis

Patch type iontophoretic device was developed.

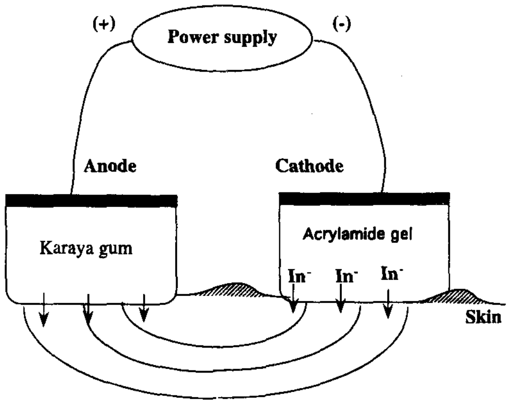

Figure 1—Schematic diagram of the iontophoretic delivery system used in this work. In- denotes negatively charged insulin molecule in the polyacrylamide gel.

The electronic part of the device was consisted of a small battery (6V) and electronic components. Using 1 MΩ resistor in series with the skin, constant current was supplied to the circuit. Nonelectronic part of the iontophoresis patch was consisted of cathodal and anodal chamber filled with gel matrices. Cathodal chamber was filled with polyacrylamide gel. The concentration of the bovine insulin (Diosythn Co., Netherland) in the gel was 4 iu per 1 ml of gel. The pH of the gel was adjusted to 8.0 using sodium salicylate, in order to make the insulin charged negatively. Anodal chamber was filled with karaya gum matrix without insulin.[12] The volume and thickness of the chamber (both anode and cathod) were 2 m*l* and 2 mm, respectively. Contact area with skin was 16 cm^2 (8 cm^2 each). Iontophoresis was carried out for 10 minutes with 0.012 mA/cm^2 pulsed current (on/off ratio=2, frequency 2000 Hz). Figure 1 shows schematic diagram of insulin iontophoresis. The patch was applied directly to the treated skin and secured with surgical tape.

Measurement of Glucose and Insulin Concentration

Blood samples were taken from ear vein. The concentration of glucose was determined using GLUCOSCOT® (Daiichi Kagaku Co., Kyoto, Japan). Serum insulin concentration was determined by a radioimmunoassay kit (Coat-A-Count® Insulin) obtained from Diagnostic Products Corporation (Los Angeles, CA).

Evaluation of Skin Irritation

Skin irritation was evaluated in accordance with No. 404 "Acute Dermal Irritation-Corrosion" of the OECD Guideline for the test of chemicals. Approximately 24 hours prior to the application of the test, hair was removed carefully from the back with electric clippers. Both control and treatment experiments were carried out using the same rabbit. After the test site was treated using electric razor, knife razor, skin needle or scotch tape, insulin patch was applied. Gauge patch soaked with distilled water was applied to the site of control experiment. After the patches were removed, the skin was washed with warm water. Skin irritation was examined for the next 24 hours. Dermal irritation was assessed using the prescribed numerical system for erythema and oedema formation; 0-no formation, 1-very slight formation, 2-well-defined formation, 3-moderate to severe formation, 4-severe formation.

Results and Discussion

The Transport of Insulin

The change of blood glucose and serum insulin level in diabetic rabbits were observed for 24 hours (Table I). In the skin treated with knife razor, the standard deviation of glucose level was large. Whereas, in the skin treated with electric razor and skin needle, it was small. Standard deviation of serum insulin levels shows similar trend as that of glucose levels. Difficulties in uniform treatment by knife razor seems to be the reason for the large standard deviation. Electric and knife razor treatments seems to be more effective in removing stratum corneum layer, because the drop in glucose level

Table I—*Blood Glucose and Serum Insulin Level in the Diabetic Rabbits. Relative Resistance after Treatment is also Shown (n=5).*

Treatment	Blood glucose(mg/dl)		Serum insulin(μ in/ml)		Relative Resistance(%)
	Initial	Minimum	Initial	Maximum	
Electric razor	550±56	82±16	<5	32± 5	14.2± 1.6
Knife razor	450±41	115±55	<5	25±12	16.6±10.8
Skin needle	482±50	144± 9	<5	15± 4	19.7± 1.8
Scotch tape	498±38	230±14	ND*	ND*	17.4± 4.0
Untreated	410±45	271±17	<5	<5	100 ±15.2

* ND: not determined.

Diabetic rabbits were given insulin by iontophoresis using a 0.012 mA/cm^2 pulsed current (on/off ratio=2, frequency 2000 Hz) for 10 minutes. Relative resistance represents the percentage of 21.5 kW, which is the average value of resistance before treatment.

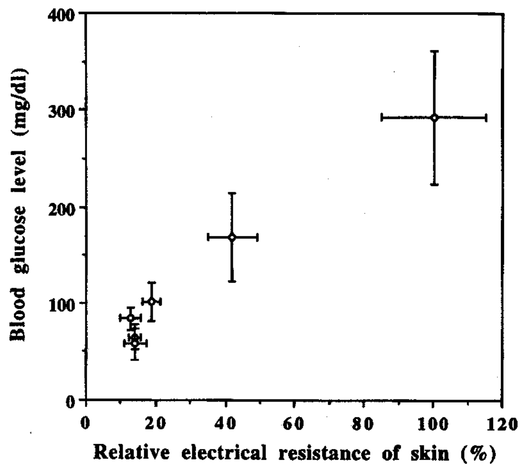

Figure 2—The change of blood glucose level in the diabetic rabbits as a function of the electrical resistance of the skin.

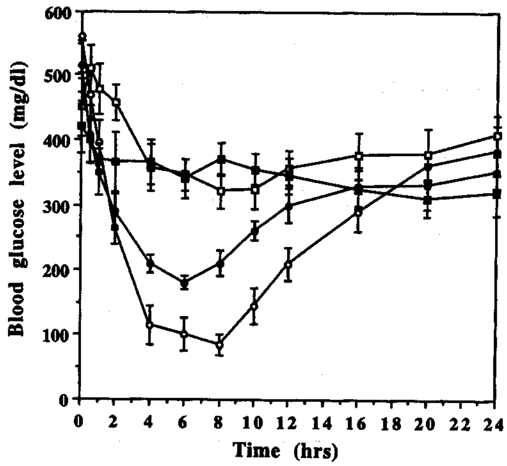

Figure 3—Postiontophoresis blood glucose level of the diabetic rabbits treated with skin needle or electric razor. Postiontophoresis blood glucose level in rabbits without skin treatment (control 1) and the blood glucose level in rabbits with neither skin treatment nor iontophoresis (control 2) (n=5) are also shown.
Key : ○; electric razor, ●; skin needle, □; control 1, ■; control 2

(or the increase in serum insulin level) was larger. These results are in good agreement with the electrical resistance data of the treated skin. As the electrical resistance decreases, transdermal insulin flux increased.

Figure 2 shows the change of blood glucose levels against the electric resistance of skin. Skin was treated by electric razor and the magnitude of resistance was controlled by the number of treatment. When compared to treated skin, untreated skin shows larger distribution of electrical resistance. It seems that, as the treatment of skin increases, the magnitude of electrical resistance falls in a narrower range. This result suggest the possibility of uniformer insulin delivery through the skin, when the skin is treated.

Insulin patch was applied to both treated (skin needle and electric razor) and untreated skin (control 1). After applying insulin patch on skin, the change of glucose level was monitored for 24 hours. Figure 3 shows the change in blood glucose level as a function of time after 10 minutes iontophoresis to both treated and untreated skin. The change in glucose level in untreated rabbits without iontophoresis is also shown (control 2). Initial blood glucose level of diabetic rabbit was in the range of 400-500 mg/dl. When the skin is not treated, the time profile of blood glucose level after iontophoresis seems similar to that obtained from untreated rabbits without iontophoresis. The minimum blood glucose level reached was about 270 mg/dl. When the skin was treated, blood glucose level decreased markedly after iontophoresis. It decreased rapidly for the first 4 hours (in the rate of 80-100 mg/dl), and then it decreased further slowly for the next 4 hours. After 8 hours, blood glucose level recovered slowly to normal value for the next 16 hours. It is quite interesting that iontophoresis for only 10 minutes can induce lower blood glucose level for nearly ten hours. It seems that skin works as a reservoir for insulin molecules, and release them slowly after iontophoresis for the next several hours. When the skin was treated by electric razor, the rate of decrease in blood glucose level after iontophoresis was larger than that observed when the skin was treated by skin needle, suggesting that the removal of the barrier layer (stratum corneum) is more effective when the skin is treated by electric razor. The magnitude of decrease in blood glucose level was also larger when the skin was treated by electric razor. This means that the rate and amount of insulin molecules transported into the rabbit is larger when the skin was treated by electric razor. This implication can be further supported by the data in Figure 4. When the skin was treated by electric razor, serum insulin level increased sharply from 5 iu/ml to 32 iu/ml within 2 hours after iontophoresis, and then decreased slowly to normal serum insulin level in 12 hours. In treated skin by skin needle, the serum insulin level showed peak (16 μiu/ml) at 4 hours after iontophoresis, and then returned to normal serum insulin level in 10 hours. When iontophoresis was applied without any skin treatment, there was minimal fluctuation in serum insulin levels (control 1), which is similar to that obserevd from the untreated rabbits without iontophoresis (control 2). These results also correspond well with the blood glucose level shown in Figure 3. Figure 5 shows the typical fluctuation of blood glucose level after insulin administration by 10 minute iontophoresis per day for 6 days. Normal rabbits (none diabetic, electric razor treated) were used for this experiment. The rabbits were not allowed to have food and

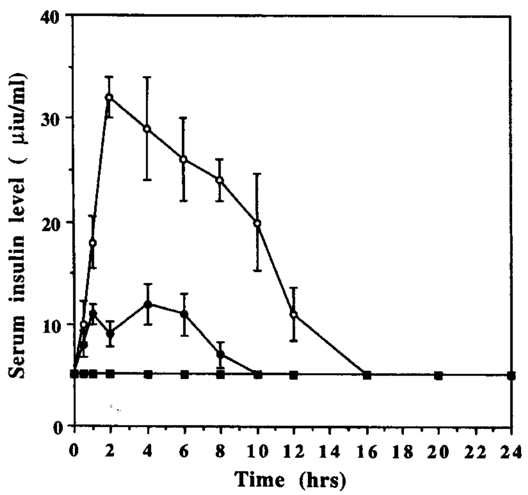

Figure 4—Postiontophoresis serum insulin level (R I A method) of the diabetic rabbits treated with skin needle or electric razor. Postiontophoresis serum insulin level in rabbits without skin treatment (control 1) and the serum insulin level in rabbits with neither skin treatment nor iontophoresis (control 2) (n=5) are also shown.
Key : ○ : electric razor, ● : skin needle, □ : control 1, ■ : control 2

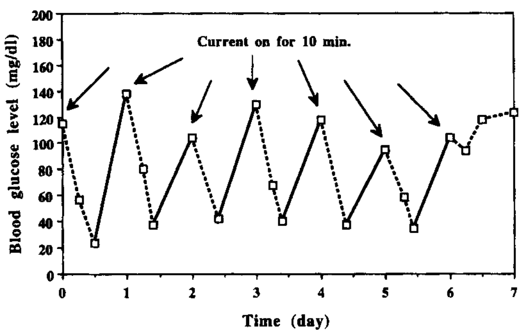

Figure 5—The fluctuation of blood glucose level by the on/off cycle of current (n=5) (········ : reduction of blood glucose level. ——— : recovery of blood glucose levels.)

water for 12 hours after iontophoresis. By 10 minute iontophoresis per day, blood glucose level fluctuated between 140 mg/dl and 20 mg/dl. The magnitude of the drop in blood glucose level seems decreased slightly with time, probably due to the healing of the treated skin.

Skin Irritation Study

The invasive treatment of skin by electrical razor or skin needle may cause severe skin irritation or corrosion. The results for skin irritation test are shown in Table II. There were no signs of toxicity or ill health in any rabbits during the examination period. Transient erythema and oedema were recorded at the treated skin sites for all testing animals after removal of the patch. The skin treated with electric razor or knife razor elicited well-defined erythema and very slight oedema. The skin treated with skin needle or scotch tape elicited only well-defined erythema. All dermal irritations observed in iontophoresed skin with treatment had resolved in 2 days. Slight erythema was recorded at control group with iontophoresis (control 1). This irritation (remark: 1) was resolved in 12 hours. No irritation was observed when insulin patch was attached to the untreated skin without iontophoresis (control 2). These results indicate that skin irritation is induced not by the the patch, but mainly by the skin treatment and iontophoretic current.

These results suggest that skin irritation is directly related to the degree of skin treatment (damage to stratum corneum), which can be reflected as the magnitude of electrical resistance (Table I, Figure 2). In order to test this point more clearly, skin irritation test were conducted in skins treated by electric razor for different duration of time and the results are shown in

Table II—*Results of the First Skin Irritation Test (n=3). Application Time of Iontophoresis was 10 Minutes and the Degree of Skin Irritation was Measured after 24 Hours.*

Treatment	Relative Resistance(%)	Test results Erythema/Oedema	Remark (0~4)
Electric razor	14.2±1.6	Erythema/Oedema	2/1
Knife razor	16.6±10.8	Erythema/Oedema	2/1
Skin needle	19.7±1.8	Erythema	2
Scotch tape	17.4±4.0	Erythema	2
Untreated [a]	100±19.0	Erythema	1
Untreated [b]	100±15.2	None	0

[a] control 1 : with iontophoresis and without skin treeatment.
[b] control 2 : neither iontophoresis nor skin treeatment.
Relative resistance represents the percentage of 21.5 kW, which is the average value of resistance before treatment.

Table III—*Results of the First Skin Irritation Test. Skin was Teated for Different Duration of Time by Electric Razor. Application Time of Iontophoresis was 10 Minutes and the Degree of Skin Irritation was Measured after 24 Hours.*

Duration of treatment (sec.)	Relative Resistance	Test results Erythema/Oedema	Remark (0~4)
0	100±19.0	None	0
3	41.9±6.9	Erythema	1
6	18.9±2.5	Erythema	2
10	14.2±1.6	Erythema/Oedema	2/1
20	14.2±3.2	Erythema/Oedema	2/2
40	12.6±3.0	Erythema/Oedema	3/2

Relative resistance represents the percentage of 21.5 kW, which is the average value of resistance before treatment.

Table III. When the treating time was 3 or 6 second, erythema with remark 1 or 2, respectively, developed. When the treating time was 10 seconds, oedema began to develope. Up to this duration of treatment, the electrical resistance of skin decreased rapidly as the duration of treatment increased. When the treating time longer than 10 seconds, both erythema and oedema developed and the remark increased as the duration of treatment increased. These results clearly demonstrate that the magnitude of electrical resistance can be used as the indicator for the degree of skin treatment and the potential for irritation development .

Conclusion

The iontophoretic delivery of insulin was studied after the dorsal side of rabbit skin was treated by various methods. When the skin was treated with skin needle and electric razor, the standard deviations of electrical resistance were small, which indicates the possibility of uniform delivery of insulin .When the skin was treated by electric razor or skin needle, significant delivery of insulin could be achieved with large decrease in blood glucose level. However, these treatments elicited well-defined erythema and/or very slight oedema. Iontophoresis on the skin with no treatment seemed not affecting the blood glucose level, indicating minimal or no delivery of insulin.

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