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Iontophoretic transdermal absorption of insulin and calcitonin in rats with newly-devised switching technique and addition of urea

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Abstract

The effect of urea and reversing polarity of electrodes (switching technique) in iontophoresis was investigated in order to get a better transdermal absorption of peptide drugs, insulin and calcitonin, and to reduce dermal irritation caused by the iontophoresis. Two cells with an electrode were set on the hair-removed abdominal skin of diabetic or oophorectomized rats. After putting peptide solution into the anode side or both of the cells, an electric current with pulsed rectangular wave form (4 kHz, 50% duty) was passed through the skin for 2 h at 0.075 mA cm^{-2} (insulin) and for 50 min or 2 h at 0.015 mA cm^{-2} (calcitonin). Absorption of insulin and calcitonin was estimated from the reduction of glucose and calcium levels in the plasma of the rats, respectively. When the polarity of electrodes was reversed at intervals of 20 min for insulin and 25 min for calcitonin, absorption of the drug was effectively enhanced. The addition of urea to the insulin solution together with the switching technique brought about a remarkably facilitated absorption of insulin. Moreover, comparison of the skin conditions between switching and non-switching experiments suggested that irritation of skin could be reduced by employment of the switching iontophoresis. © 1997 Elsevier Science B.V.

Keywords: Iontophoresis; Switching technique; Transdermal absorption; Insulin; Calcitonin; Urea; Depolarization; Dermal irritation

1. Introduction

Iontophoresis has been known to enhance the transdermal permeation of ionic compounds. Permeability of nonionic compounds, such as manni-

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tol and alkanols, is also enhanced by iontophoresis via electroosmosis and convective water flow (Sims et al., 1991; Terzo et al., 1989).

Transdermal administration of peptide drugs such as insulin or calcitonin is preferable from the viewpoints of avoidance of first-pass metabolism and pain on injection. Liu et al. (1988) reported on the transdermal absorption of insulin after iontophoretic application for 2 h. However, long term application of electric current may induce the polarization of the skin tissue and result in decrease of flux and skin irritation. Therefore, we devised a new technique (switching technique) in which the polarity of electrodes was reversed with appropriate time intervals during iontophoresis (Tomohira et al., 1989). The aims of the switching iontophoresis are better permeation of drugs, especially peptide drugs such as insulin and calcitonin, as presented in this paper, and reduction of skin irritation. Further, the switching technique can cause both electrode cells be utilized as drug reservoirs at the same time.

On the other hand, Srinivasan et al. (1990) reported that compounds with a molecular weight of more than 1000 could not permeate the skin by application of fixed voltage with no pretreatment of the skin using ethanol. In this work, further, we investigated the effect of urea on the transdermal absorption of insulin and calcitonin via switching or non-switching iontophoresis in rats.

2. Materials

Insulin (calf, 24–25 IU mg^{-1}) and streptozotosin were purchased from Sigma. Thyrocalcitonin (calcitonin, Sigma) was cordially supplied from Teijin. Urea, agar powder, Glucose-B Test kit and Calcium-C Test kit were purchased from Wako Pure Chemical Industry. Other reagents and chemicals used were of purest commercial grade.

3. Preparation of animal models for diabetes and menopause

In the experiments for administration of insulin, Wistar male rats (body weight 250–350 g) were made diabetic by a single intraperitoneal injection of streptozotosin (40 mg kg^{-1}). As a model of menopause, Wistar female rats (2–3 weeks) were used after oophorectomization and subsequent recovery period for more than a month. The menopausal state was confirmed by a vaginal smear test.

4. Preparation of iontophoretic cells

Fig. 1 shows schematically the setting of electrodes in the non-switching (ordinary) iontophoresis. The head part of a 50-ml disposable syringe (Terumo, 29 mm i.d.) was cut 10 mm in height and used as a shell for the iontophoretic cell in the anode side, as shown by cross-sectional view in Fig. 1. A platinum plate 20 mm in diameter and 0.1 mm thick was used as an anode. A cut body of the same disposable syringe was used as cathode. Each electrode was fixed in a cell using a gel layer of 1% agar/0.05% NaCl. The anode side cell has a spare space of 3 ml for drug solution, which was injected through agar gel layer using a syringe, as shown in Fig. 1.

In the switching iontophoresis, a similar cell to the above mentioned anode side cell was used in the cathode side as well as in the anode side.

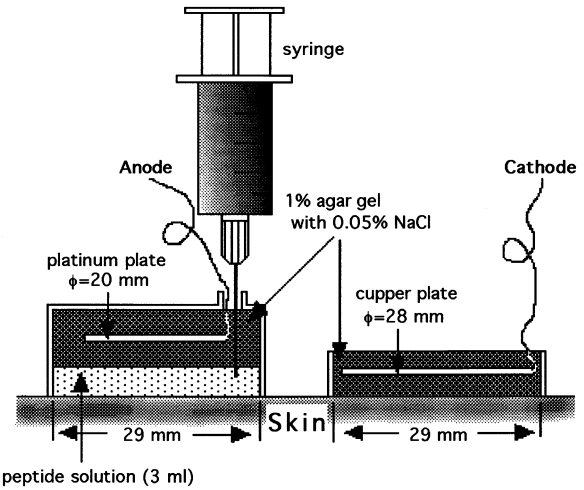

Fig. 1. Schematic illustration of electrodes setting in non-switching iontophoresis. Shell for the iontophoretic cell was shown as cross-sectional view.

5. Preparation of peptide solution

5.1. Insulin solution

Concentration of insulin was fixed at 50 U ml^{-1} by referring to a report by Kari (1986). Insulin solution was adjusted to pH 1.5 using 0.1 N HCl, which should give it a positive charge for the better iontophoretic absorption of insulin, based on a report by Stephan et al. (1984).

5.2. Calcitonin solution

Calcitonin was dissolved with citrate buffer (7.8×10^{-6}M) at concentrations of 1, 3, 6, 6.4 and 16.6 $\mu g\ ml^{-1}$. Buffered solutions used were adjusted to pH 3.5, 4.5 and 5.5 using 0.1 M HCl or 0.1 M NaOH.

6. Studies of iontophoresis in vivo

6.1. Insulin

After a fasting for more than 12 h, diabetic rats were anaesthetized by i.p. injection of urethane (850 mg kg^{-1}) and fixed supinely. Hair of abdominal region was removed by animal hair clipper and skin was wiped several times with 80% (v/v) ethanol. After that, considering the relaxation of the increased blood glucose level induced by fixation, the rats were made repose for 4 h. Subsequently, anode side and cathode side iontophoretic cells were adhered on the abdominal skin of a rat using a surgical adhesive (Aron Alpha A 'Sankyo') with a distance of 10 mm. A 3 ml of insulin solution was injected into the anode side cell. A pulsed electric current of 0.075 mA cm^{-2} (4 kHz, 50% duty) was applied to skin for 2 h.

After iontophoretic application, approximately 0.1 ml of blood was collected from jugular vein and blood collection was repeated every 2 h until 12 h after the start of experiment. Plasma was separated by centrifugation of the blood and kept in freezer until analysis. Plasma glucose level was determined using Glucose-B Test Wako which based on glucose oxidase method. Transdermal absorption of insulin was evaluated by reduction of the plasma glucose level assuming the level at time zero as 100%. Area between the 100% line and plasma glucose level reduction curve was calculated by a trapezoidal rule.

6.2. Calcitonin

Oophorectomized rats were treated in a similar manner as the iontophoretic application of insulin. Iontophoresis was carried out using pulsed electric current at 0.015 mA cm^{-2} (4 kHz, 50% duty) for 2 h or 50 min. Transdermal absorption of calcitonin was evaluated using plasma calcium level determined by Calcium-C Test Wako based on *o*-cresolphtalein-complexon method. Area between the 100% line and plasma calcium level reduction curve was calculated by a trapezoidal rule.

6.3. Switching iontophoresis

In the experiments for switching iontophoresis, a couple of iontophoretic cells equipped with a platinum plate and a chamber was adhered on the abdominal skin of rats. A 3 ml of the peptide solution (insulin, 50 U ml^{-1}, pH 1.5; calcitonin, 1 $\mu g\ ml^{-1}$, pH 4.5) was put into both of the iontophoretic cells. In the case of insulin, pulsed current of 0.075 mA cm^{-2} (4 kHz, 50% duty) was applied for 2 h under switching of every 10, 20 or 30 min. In the case of calcitonin, pulsed current of 0.015 mA cm^{-2} (4 kHz, 50% duty) was applied for 50 min under switching manners of every 10 min, 20–20–10 min, 17–17–16 min, or every 25 min.

6.4. Statistical analysis

Comparisons between two groups were performed by Student's *t*-test.

7. Results and discussion

7.1. Non-switching iontophoresis of insulin

Fig. 2 shows the reduction profile of blood glucose level in diabetic rats after ordinary iontophoretic application of insulin solution (50 U

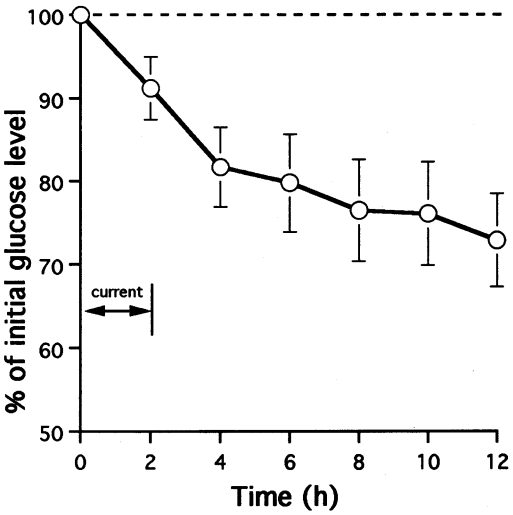

Fig. 2. Reduction profile of blood glucose level after non-switching iontophoresis (0.075 mA cm^{-2}) of insulin (50 U ml^{-1}). Each symbol represents the mean ± S.E. of 4–11 rats.

ml^{-1}) for 2 h. The blood glucose level decreased to approximately 73% at 12 h after the start of experiment. However, redness was observed partially at the skin where the anode side cell was attached. This irritating effect of iontophoresis using pulsed current was guessed to be due to polarization of skin and/or pH change of drug solution caused by continuous application of current using platinum electrode.

7.2. Influence of concentration and pH of calcitonin solution on non-switching iontophoresis of calcitonin across rat skin

Plasma level profiles of calcium after ordinary iontophoretic application of calcitonin for 2 h, with different concentrations of calcitonin at pH 3.5, are shown in Fig. 3. No calcium level reduction was observed in the application of citrate buffered solution as a control with iontophoresis. On the other hand, plasma calcium level was reduced to 83% of the initial one at 6 h after iontophoretic application of calcitonin at 6.4 μg ml^{-1}, and the level was recovered gradually to the initial level at 12 h after the application. Similar calcium level reduction was observed in the case of 16.6 μg ml^{-1} solution but no recovering was observed for 12 h after the application. There was no symptom of irritation in the iontophoresis by 0.015 mA cm^{-2} for 2 h.

The influence of pH of 6.4 μg ml^{-1} calcitonin solution on plasma calcium level reduction under iontophoresis for 2 h is shown in Fig. 4. Differences in minimum calcium levels in pH 3.5, 4.5 and 5.5 were not remarkable, but continuous reduction was observed in the case of pH 4.5. Therefore, buffered solution of pH 4.5 was chosen as a medium in the following studies.

Fig. 5 shows reduction profiles of plasma calcium level after non-iontophoretic and iontophoretic applications of calcitonin in different concentrations and manners. Although no reduction was observed after non-iontophoretic application of the highest concentration of calcitonin (6 μg ml^{-1}) for 2 h, iontophoresis brought about the reduction of calcium level even in the application of 1 μg ml^{-1} solution for 50 min. Rebound of calcium level appeared in both conditions but maximum levels were observed at 6 and 10 h after iontophoretic application of 1 μg ml^{-1} solution for 50 min and 3 μg ml^{-1} solution for 2 h, respectively. The delayed rebound of calcium level in the iontophoretic application of 3 μg ml^{-1}

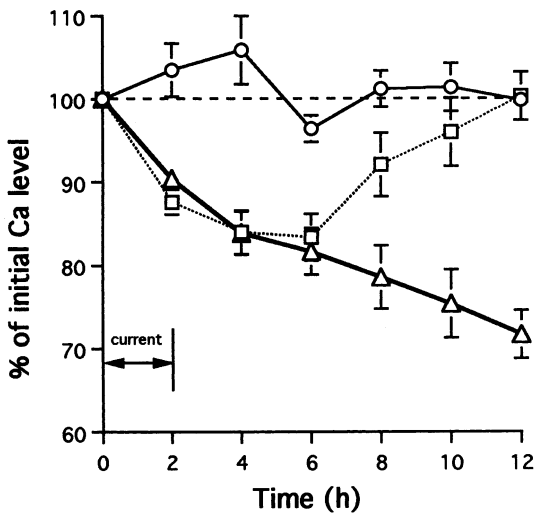

Fig. 3. Plasma calcium level profiles after non-switching iontophoresis (0.015 mA cm^{-2}) of calcitonin at pH 3.5. Each symbol represents the mean ± S.E. of 4–8 rats. 0 μg ml^{-1} (– ○ –), 6.4 μg ml^{-1} (– □ –), 16.6 μg ml^{-1} (– △ –).

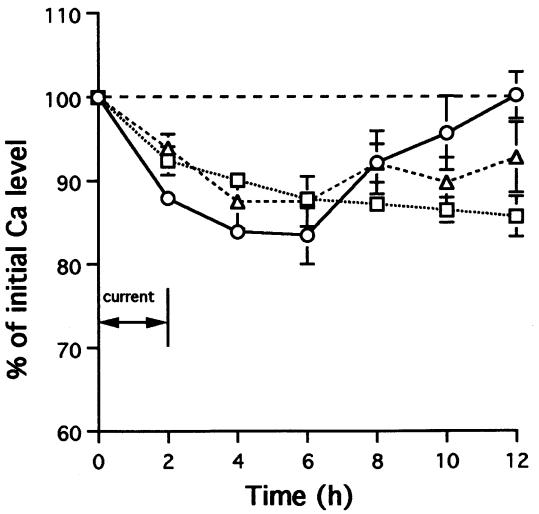

Fig. 4. Influence of pH of calcitonin solution (6.4 μg ml^{-1}) on plasma calcium level reduction under non-switching iontophoresis. Each symbol represents the mean ± S.E. of eight rats. pH 3.5 (– ○ –), pH 4.5 (– □ –), pH 5.5 (– △ –)

solution for 2 h seemed to reflect the higher calcitonin concentration and the longer duration of iontophoresis than in the application of 1 μg ml^{-1} solution for 50 min.

These results suggest that reduction of calcium level was caused by the permeation of calcitonin under iontophoresis. A meaningful reduction of calcitonin level was obtained by the iontophoretic application of 1 μg ml^{-1} solution for 50 min. Therefore, concentration and iontophoresis duration for calcitonin in the following studies were decided as 1 μg ml^{-1} and 50 min, respectively.

7.3. Effect of switching on iontophoretic application of insulin and calcitonin

The irritation of skin was observed in the ordinary iontophoretic application of insulin using 0.075 mA cm^{-2} pulsed current for 2 h, possibly due to polarization and low pH of the insulin solution. Therefore, we devised 'switching iontophoresis' to reduce the skin irritation in the ordinary iontophoresis and to utilize both of the cells as drug reservoirs, in which the polarity of the electrode is reversed periodically. Fig. 6 shows the blood glucose level reduction in iontophoretic application of insulin with switching at intervals of 10, 20 and 30 min comparing with the ordinary (non-switching) iontophoresis. When switching was carried out every 20 min, approximately 55% of reduction level was observed at 4 h after the start of the experiment. However, no apparent effect of switching was observed in the switching with the intervals of 10 and 30 min. Switching can cause a transfer of the drug molecules to an opposite direction, i.e. from skin to drug reservoir. If an interval of switching was not enough for the transfer of the drug into dermis, the drug should be pulled back to the reservoir by the switched current. Therefore, the result seemed to suggests that suitable or optimum switching interval should exist in the switching iontophoresis, possibly relating to a transfer of the drug molecules during the period between each switching.

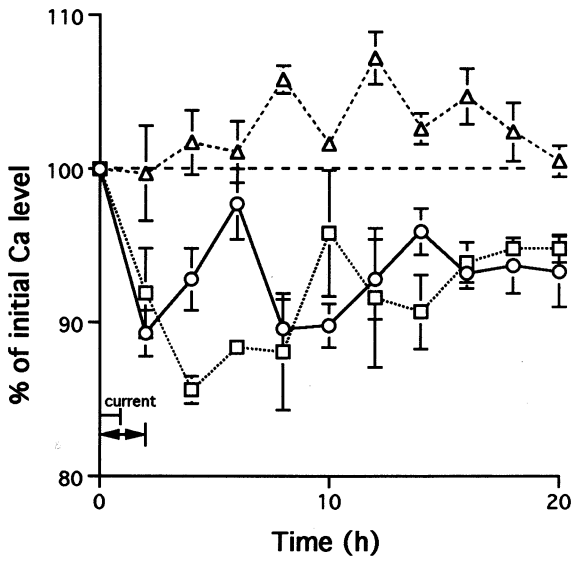

Fig. 5. Reduction profiles of plasma calcium level after non-iontophoretic and non-switching iontophoretic applications of calcitonin in different concentrations and manners. Each symbol represents the mean ± S.E. of 3–8 rats. I.P.-50 min, 1 μg ml^{-1} (– ○ –), I.P.-2 h, 3 μg ml^{-1} (– □ –), non-I.P., 6 μg ml^{-1} (– △ –). 'I.P.' means iontophoresis.

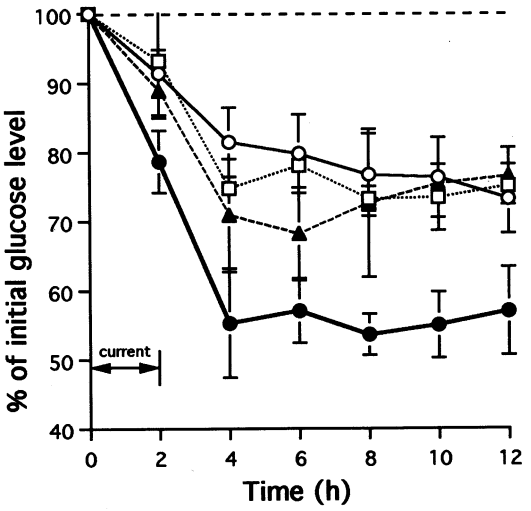

Fig. 6. Comparison of blood glucose level reduction profiles after switching and non-switching iontophoresis of insulin. Each symbol represents the mean ± S.E. of 3–11 rats. non-switching (– ○ –), switching, 10 min (– □ –), switching, 20 min (– ● –), switching, 30 min (– ▲ –).

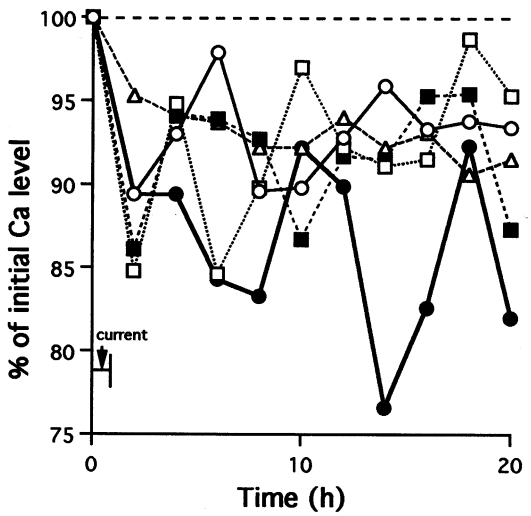

Fig. 7. Comparison of plasma calcium level reduction profiles after switching and non-switching iontophoresis of calcitonin. Each symbol represents the mean of 3–8 rats. Non-switching (– ○ –), switching; every 10 min (– □ –), 17–17–16 min (– ■ –), 20–20–10 min (– △ –), every 25 min (– ● –).

Redness of the skin under both of the cells was observed in all cases, but it was very slight compared with that in the ordinary iontophoresis of insulin.

On the other hand, there was no remarkable effect of switching in the plasma calcium level reduction, as shown in Fig. 7, though a slightly larger reduction was obtained in 25–25 min switching than in the other conditions. The difference between the effect of switching on iontophoretic permeation of insulin and calcitonin seemed to relate mainly to the difference in type and intensity of electric current applied and in physicochemical characteristics such as molecular weight, isoelectric point, etc.

7.4. Effect of urea on non-switching iontophoresis of insulin and calcitonin

Urea has been known to hydrate the stratum corneum. The hydration of stratum corneum seemed to increase the electric conductivity of skin and enhance the iontophoretic transdermal delivery of drug. Therefore, effect of urea on transdermal absorption of insulin and calcitonin under iontophoresis was investigated. Fig. 8 shows the reduction of blood glucose level in iontophoretic application of insulin with and without the addition of urea. The glucose level

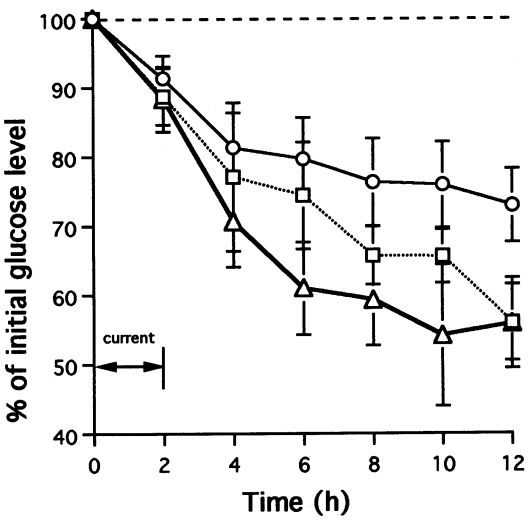

Fig. 8. Reduction profiles of blood glucose level after non-switching iontophoresis of insulin with and without addition of urea. Each symbol represents the mean ± S.E. of 4–11 rats. Without urea (– ○ –), with 5% urea (– □ –), with 10% urea(– △ –).

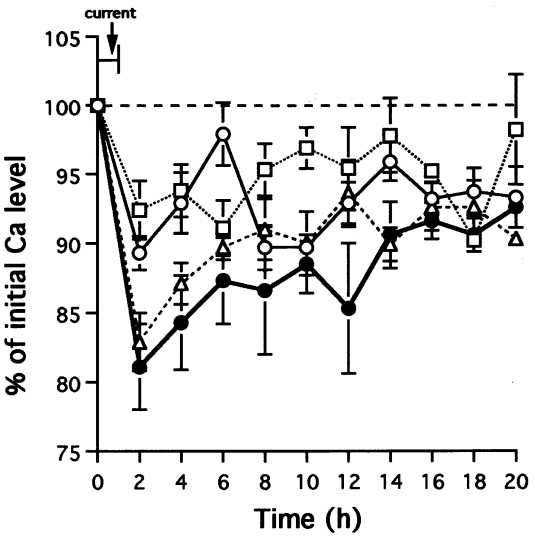

Fig. 9. Reduction profiles of plasma calcium level after non-switching iontophoresis of insulin with and without addition of urea. Each symbol represents the mean ± S.E. of 3–8 rats. Without urea (– ○ –), with 5% urea (– □ –), with 10% urea (– △ –), with 15% urea (– ● –).

was reduced to approximately 73% of the initial level at 12 h after iontophoretic application of insulin alone. On the contrary, addition of urea at a concentration of 5 and 10% resulted in the reduction level to approximately 56% at 12 h after iontophoresis. Though there was no statistically significant difference in the area between each reduction curve and 100% line, the results suggest the possible enhancing effect of urea. This estimation was supported also from the fact that the more severe irritation of the skin, i.e. not only the redness but also small blister was observed under the cathode side cell with the addition of urea.

Effect of urea on plasma calcium level reduction was shown in Fig. 9. The most remarkable reduction was observed in the case of 15% addition of urea at 2 h (70 min after the termination of iontophoresis) and it was almost 80% of the initial calcium level. Statistically meaningful difference was obtained ($P < 0.01$) between without addition and 10 or 15% addition of urea for the area between the 100% line and reduction curve. Contrary to the case of insulin, no skin irritation was found after 50 min iontophoresis. When iontophoresis was carried out for 2 h with the addition of 10% urea, slight redness was observed (data are not shown). The results suggest that weaker current and shorter duration of iontophoresis than in the experiment for insulin (Fig. 8) are the reason why irritation was hardly caused in the experiment for calcitonin (Fig. 9).

Effect of hydration of skin on iontophoretic delivery was supported by Fig. 10, which shows the calcium level reduction by iontophoretic delivery of calcitonin after pretreatment for 30 min with citrate buffer alone or with 10% urea. Until 6 h, there was no remarkable difference in area between 100% line and reduction curve in non-pretreated (Fig. 9) and pretreated iontophoresis. However, pretreatment gave larger area in the result until 20 h. Moreover, pretreatment with citrate buffer alone showed a tendency of prolonged decrement of plasma calcium level, possibly because of hydration of stratum corneum. Contribution of the hydration of skin should be supported also by a recent report of Thysman et al. (1995) in which the hydration of stratum corneum during in vivo iontophoresis was investigated in human volunteers using attenuated total reflectance-FTIR.

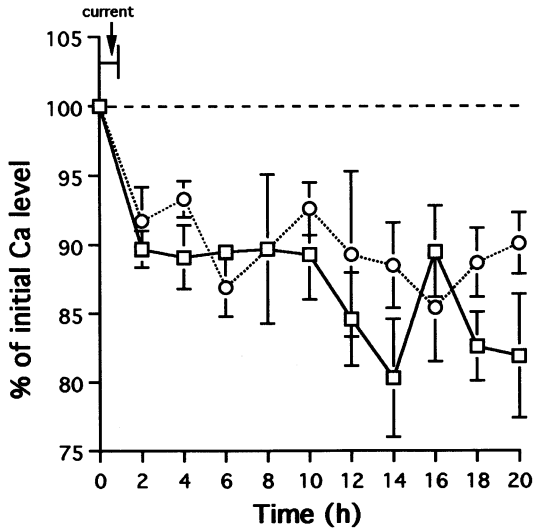

Fig. 10. Plasma calcium level reduction by non-switching iontophoresis of calcitonin after pretreatment for 30 min with citrate buffer alone or with 10% urea. Each symbol represents the mean ± S.E. of 3–4 rats. Citrate buffer alone (– ○ –), with 10% urea (– □ –).

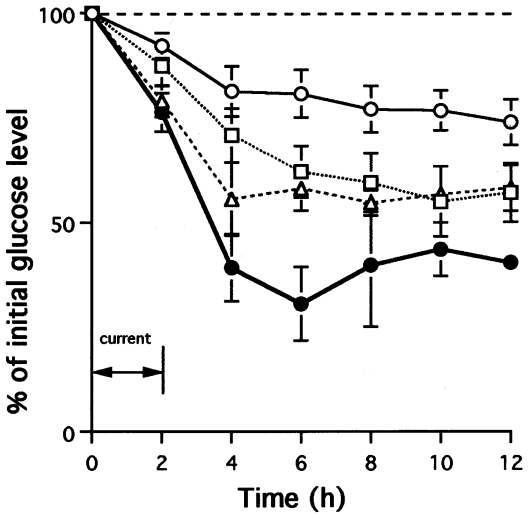

Fig. 11. Comparison of blood glucose level reduction after iontophoretic application of insulin under various conditions. Each symbol represents the mean ± S.E. of 3–11 rats. Control (– ○ –), with 10% urea (– □ –), switching, 20 min (– △ –), with 10% urea and switching, 20 min (– ● –).

7.5. Effect of switching iontophoresis under addition of urea on transdermal absorption of insulin

Both of the switching technique and addition of urea have shown an enhanced absorption of the peptides. Therefore, combination effect of switching and addition of urea was investigated expecting an additive or synergistic effect. As shown in Fig. 11, combination of switching iontophoresis with 20 min intervals and addition of 10% urea brought about a remarkable reduction of blood glucose level, and it reached 30% of the initial level at 6 h after start of experiment. There was statistically meaningful difference ($P < 0.01$) in the area between 100% line and reduction curve until 12 h, between control (non-switching iontophoresis) and switching iontophoresis with addition of urea. Moreover, no difference was observed in the condition of skin after application of switching iontophoresis with and without the addition of urea.

In the results already mentioned, addition of urea in non-switching iontophoresis caused an irritation of skin. The switching seemed to reduce the irritation of skin under the electrode cells, even with the addition of urea. Combination of the switching technique and addition of chemical enhancer such as urea in iontophoretic delivery of the peptide drugs was suggested to be useful.

8. Conclusion

Transdermal absorption of peptide drugs, insulin and calcitonin, under iontophoresis using pulsed current was enhanced by the switching technique and/or addition of urea in the drug solution. Combination of the switching and addition of chemical enhancer such as urea should be useful from the viewpoint of absorption enhancement and avoidance of irritation on skin. Recently, Santi and Guy (1996) reported that no adverse effect of periodic alternation of electrode polarity, i.e. switching, was observed in the overall efficiency of extraction process in 'reverse iontophoresis'. This report encourages our study to clarify the usefulness of 'switching iontophoresis'. Further investigation concerning the mechanism of the enhancing effect and less irritation in the switching iontophoresis should be done.

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