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International Journal of Pharmaceutics 137 (1996) 75–84

international journal of pharmaceutics

Fundamental investigation of a novel drug delivery system, a transdermal delivery system with jet injection

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Received 26 December 1995; accepted 8 February 1996

Abstract

A new drug delivery system simultaneously using jet injection and a transdermal delivery system was proposed. After pretreatment of hairless rat skin by a jet injector containing physiological saline without any drug to make a pore in the skin, aqueous solution containing gentamycin sulfate, nicardipine hydrochloride or theophylline was applied on the pore. Absorption clearance (volume flow rate) through the skin (the product of permeability coefficient and application area) was almost the same (0.4 μl/h) with any type of drug and regardless of properties and concentrations. Plasma concentration of gentamycin when the distance between the injector and skin surface was 5 mm was twice that when there was no space between the two. It was found by morphological observation that injection from 5 mm away made a larger pore in the stratum corneum (about 0.3 mm^2) and a clear saline reservoir in the viable epidermis and dermis. Mathematical analysis showed that this larger pore greatly increased the skin delivery (absorption) rate, whereas the saline reservoir increased it little. This high delivery rate continued for over 1 week with theophylline.

Keywords: Jet injection; Skin permeation; Volume flow rate

1. Introduction

Pharmacists working in the clinical field find that there are too few medicines provided for patients who cannot ingest them orally: only a few parenteral dosage forms such as injections and suppositories are available. A new way of easily administering a drug without the professional skill, for example, injections require, is necessary because of the increase in aged patients and the propagation of home therapy. Advantages of the transdermal delivery system (TDS) which is one potential parenteral dosage form can provide continuous delivery of a drug via the skin, avoids critical adverse effects by removing the

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PII S0378-5173(96)04488-2

formulation and is easy to administer. It therefore offers benefits for the patients requiring home therapy. Because the skin permeability of drugs is generally low (Ranade, 1991), much effort has been made to develop skin permeation enhancers (Xu and Chien, 1991). Application of these enhancers has been limited, however, probably due to the skin irritation they cause.

The objective in this study was to establish a methodology for continuous drug delivery through a small pore pierced in the skin. Drug is applied through the pore using a solvent rich TDS. This method is applicable to many drugs that dissolve in a solvent which is biologically inert like water. A jet injector, a high pressure device, was used to pierce the skin (Lindmayer et al., 1986), and has been used in the self injection of insulin. We designed a new drug delivery system as illustrated in Fig. 1. The TDS containing the drug solution is applied to skin, and a drug is loaded by a jet injector, and then the jet injection is performed on the TDS. The jetted drug solution pierces the skin surface as well as the upper and bottom walls of the TDS. The solution is regarded as a loading dose and is more rapidly absorbed than could be achieved by the usual TDS. The drug in TDS supplies a maintenance dose via the small pore pierced by the jet injection. If the upper wall of the TDS is made from an elastic material like rubber used in injection vials, the pore formed by the jetted solution closes in a moment. As the first step in making a drug delivery system which combines jet injection delivery with TDS, we sought to learn: (1) whether an amount of drug maintaining an effective plasma level could be delivered through the pore; understood without repeating, (2) whether the permeability of drug through the pore could be changed by different injection methods; and (3) the duration of drug delivery using this system. Therefore, no drug solution was loaded into the jet injection in this report; the injection was used only to pierce the skin using physiological saline.

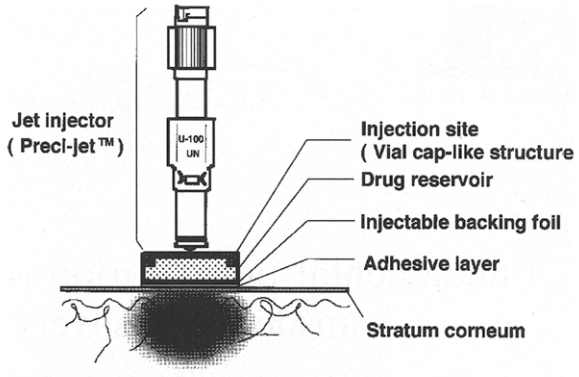

Fig. 1. A novel drug delivery system: transdermal delivery system with jet injection.

2. Experimental

2.1. Jet injector (Preci-Jet™; PJ)

Preci-Jet™ and Medi-Jector™ (Medi-Ject

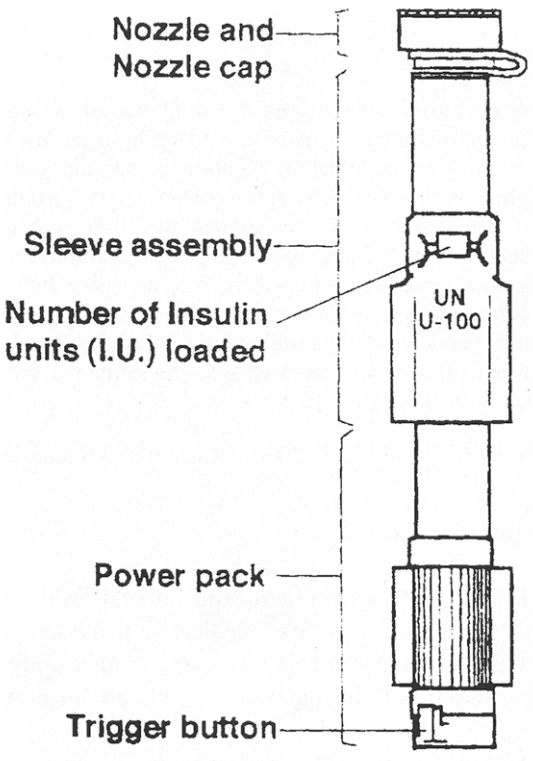

Fig. 2. Typical diagram of Preci-Jet™.

Table 1
Conditions for administration of each drug

	I.v. injection		Topical application	
	Concentration	Dose	Concentration	Volume
GM	0.1% in water 0.5% in water	1.0 mg/kg 5.0 mg/kg	30% in water	1.0 ml
TP	0.5% in water 1.0% in water	5 mg/kg 15 mg/kg	9.26% in water (pH = 9.3)	1.0 ml
NC	0.05% in 10% EtOH 0.3% in 10% EtOH	0.5 mg/kg 3.0 mg/kg	2.0% in 10% EtOH	1.0 ml

Corp., Minneapolis, USA) are available jet injectors. PJ (Fig. 2) was supplied by Kodama, Ltd. (Tokyo, Japan). This device was developed by Advanced Medical Technologies Inc. (Canada) and uses no needle for patient self-injection of insulin. Using PJ at high pressure, the insulin injected solution pierces the skin and spreads into the subcutis. In this report we used the PJ using physiological saline only to pierce the skin as mentioned above.

2.2. Materials

Gentamicin sulfate (GM; 694-733 Da, biochemical grade) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Because of the low solubility of theophylline (TP; 456.5 Da) in water, aminophylline (theophylline-ethylene diamine, titrated as 92.6% of TP, JP grade) was used instead. Nicardipine hydrochloride (NC; 516.0 Da) was a gift from Nissan Chemical Industries, Ltd. (Tokyo, Japan). YC-204, internal standard to assay for NC, was kindly supplied by Yamanouchi Pharmaceutical Co., Ltd. (Tokyo). Other reagents were of analytical grade or HPLC grade.

2.3. Animals

Male WBN/ILA-Ht hairless rats (average weight 250 g, Ishikawa Experimental Animal Laboratory, Saitama, Japan) were used in all animal experiments.

2.4. Intravenous injection

Three model drugs were intravenously injected to the rats to determine their elimination parameters. Doses are shown in Table 1. Each solution was injected into the jugular vein and blood samples (300 μl) were withdrawn from the symmetrical site of the vein at predetermined times. When NC was used, ethanol was added to the solvent at 10% to prevent its adsorption on vials and injection syringes.

2.5. In vivo permeation experiment

A rat was fixed to a plate on its back. The rat gluteal region was selected as application site, because the subcutis tissue of that region is relatively thick. Physiological saline alone (60 μl) was injected by PJ to the skin with and without a hollow cylinder (i.d. = 13 mm) made of Teflon at the lowest injection pressure (6 units-back off). The solutions applied are summarized in Table 1. The hollow cylinder was attached to the gluteal region by Alon Alfa A™ (Sankyo Co., Ltd., Tokyo) prior to injection so that there would be a distance between the injector and the skin surface and to ease topical administration. The drug solution (1 ml) was then promptly placed in the hollow cylinder and the cylinder covered with a cap. The back of the rat was turned upward so that the drug solution remained in contact with the skin. When injection was made without any distance between the injector and the skin surface (without using a cylinder so that the injector

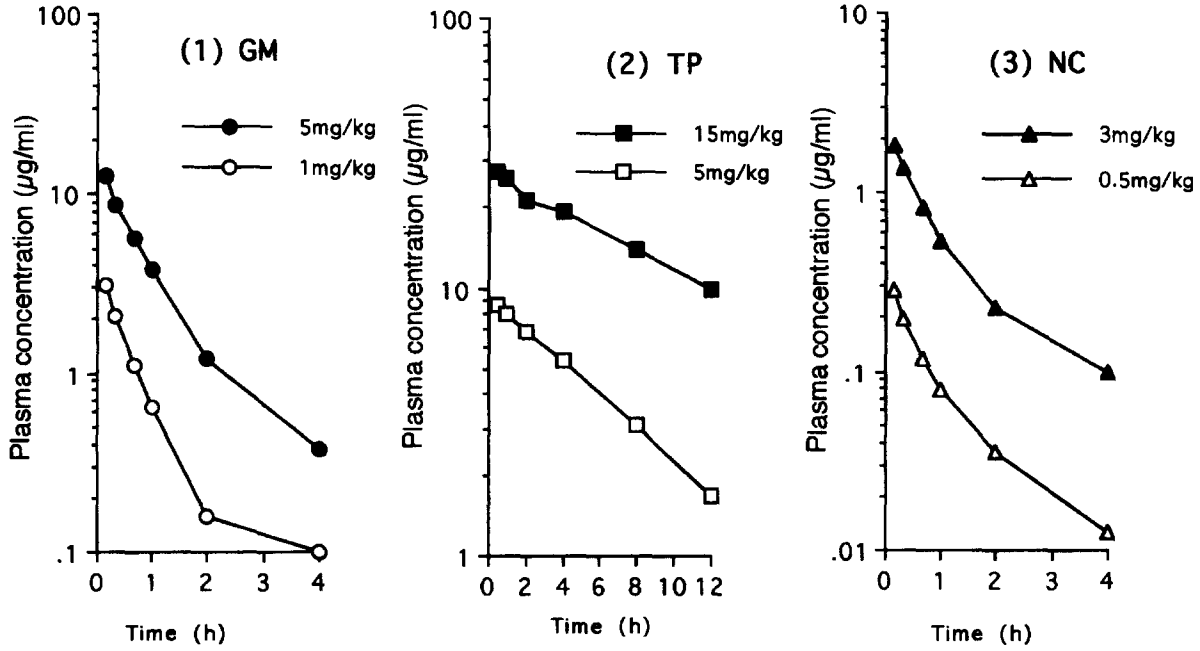

Fig. 3. Time course of plasma concentration of model drugs after intravenous injection.

touched the skin surface), a filter paper (5 mmΦ) adsorbed with saline was put on the skin immediately after the PJ treatment, the cylinder was placed on that, and the drug solution was applied after removal of the filter paper. A PJ non-treated permeation experiment was done for comparison. The effect of the distance between skin surface and PJ orifice (3.4, 4.2, 7.7, 9.5 mm) on the drug permeation rate was investigated by varying the height of the cylinder. Blood samples (300 μl) were withdrawn from the jugular vein at predetermined times.

2.6. Morphological observation of skin

After the in vivo permeation experiment the injection area of skin was excised under anesthesia by sodium pentobarbital (Dainabot Co., Ltd., Osaka, Japan, 50 mg/kg, i.p.). After immersing the skin samples in 20% formalin, they were stained using the hematoxylin-eosine method, microtomed, and observed under a microscope.

2.7. In vitro permeation experiment

PJ was used on the rat gluteal region at a distance of 0 or 5 mm between the injector orifice and the skin surface. The region was then excised under anesthesia by sodium pentobarbital. The skin piece was mounted in a vertical diffusion cell with an effective diffusion area of 0.95 cm^2 (Yukawa et al., 1989). The cell had a water jacket and was connected to a 37°C water bath. Volume of the receiver compartment was 4.5 ml and it was filled with water. The same cylinder used in the in vivo permeation experiment was used as a donor compartment, which was fixed with a clamp. The donor compartment was filled with 3% GM solution. Samples were withdrawn from the receiver compartment at predetermined times and the same volume of water was added. The sampling volume was controlled to maintain a sink condition (Skelly et al., 1987). The in vitro permeation experiment was done also using tape stripped (20 times) skin (Washitake et al., 1973) after the two (contact or 5 mm) PJ treatments referred to above.

2.8. Assay

GM and TP were assayed using the TDX™

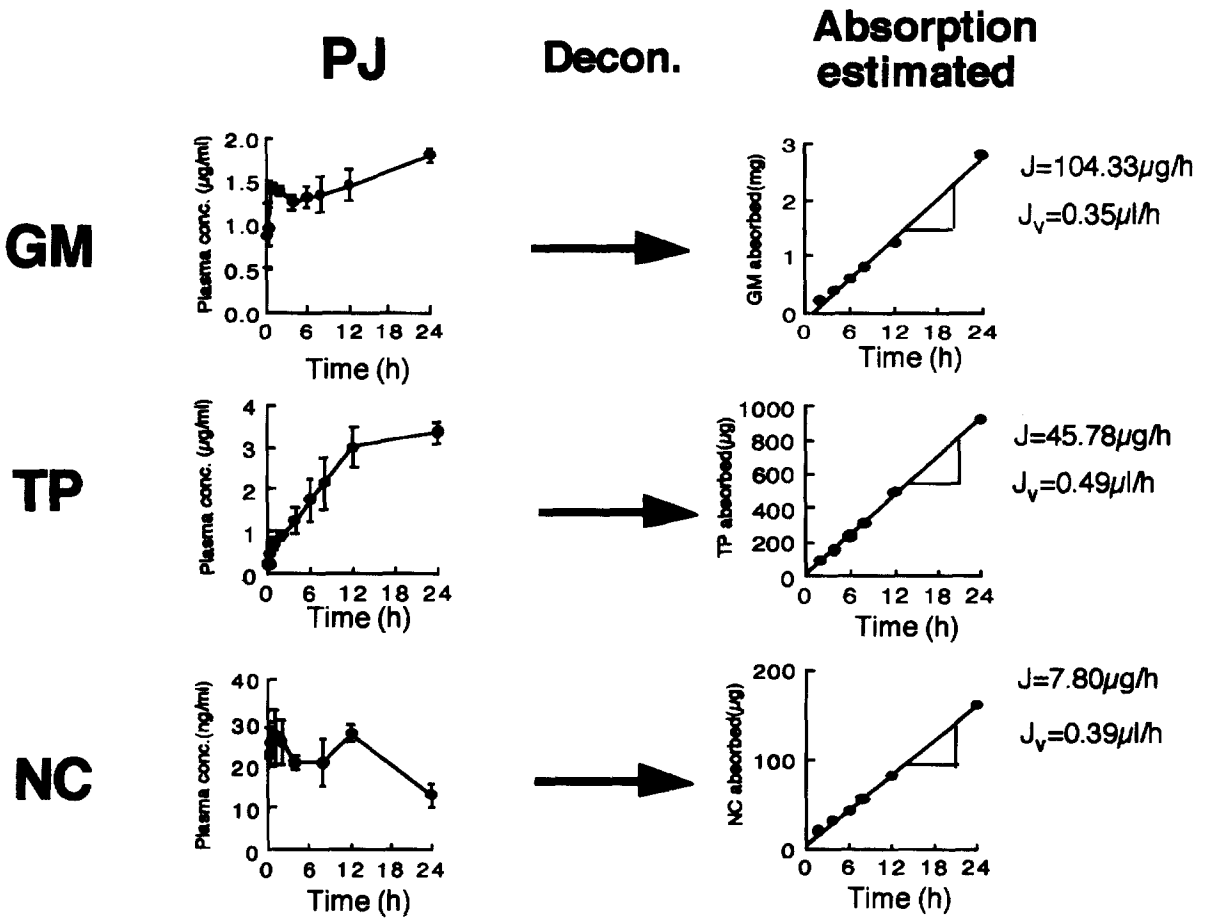

Fig. 4. Time course of plasma concentration of model drugs after topical application with PJ treatment (left) and cumulative amount of skin permeation of model drugs calculated by deconvolution method (right) after application with PJ treatment. Slope of the regression line means the permeation rate.

system (Dainabot, Tokyo). NC was measured according to Kobayashi (1987).

2.9. Estimation of skin absorption rate

Elimination profiles of drugs after intravenous injection were analyzed using MULTI (algorithm; Gauss-Newton method) (Yamaoka et al., 1981). The in vivo absorption rate of drugs from donor compartment into the systemic circulation was estimated using a Convolution and Deconvolution program (Research Institute of TTS Technology, Sakado, Saitama, Japan) (Sato et al., 1988).

2.10. Measurement of pore area in stratum corneum after PJ treatment

After PJ treatment Alon Alfa A was poured into the pore. The mold of the pore was observed and its area was measured under a microscope.

2.11. A week-long in vivo permeation experiment

A week-long in vivo permeation experiment was done to study the continuation of this delivery method using PJ-treated skin. The Teflon cylinder was attached to the skin using a rubber type adhesive (Bond G Clear, Konishi Co., Ltd.,

Osaka). Ten percent aminophylline solution (pH 9.3, equivalent to 9.26% TP) was applied to the rats which were maintained on a normal diet in a holding cage. Blood sampling was done every 24 h.

3. Results and discussion

3.1. Permeation of drugs via PJ-treated skin

Fig. 3 shows the time course of plasma concentration of each drug after intravenous injection. GM and NC were fitted to a linear two compartment model, and TP understood to a linear one compartment model. No dose dependency was found for any of the drugs at the doses used. Equations for the plasma concentration (Cp, μg/ml) and time (t,h) were

$$GM: C_P = D(2.94e^{-9.38t} + 2.85e^{-1.16t})$$

$$TP: C_P = D(1.67e^{-0.09t})$$

$$NC: C_P = D(0.69e^{-2.28t} + 0.16e^{-0.42t})$$

where D (mg/kg) is intravenous dose.

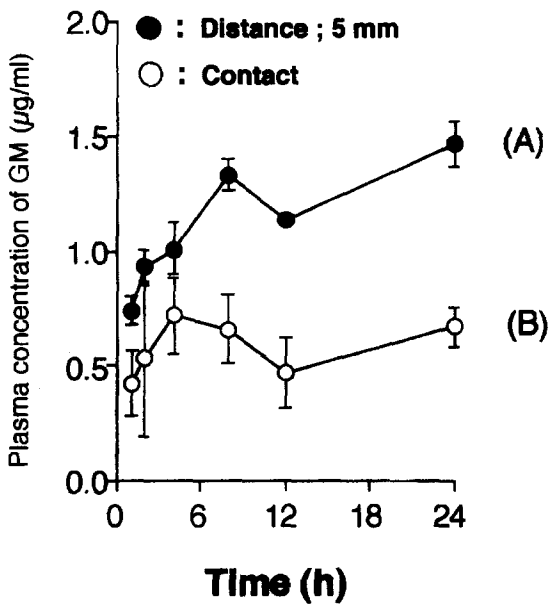

Fig. 5. Plasma concentration of GM after topical application to PJ pretreated skin.

No model drug was found in plasma when topically applied without PJ pretreatment (data not shown), whereas it was found when pretreatment had been made (jetted with a 10 mm distance between the orifice and skin) (Fig. 4). It was not found in plasma even with the PJ pretreatment, however, when the pore formed by PJ was plugged up by Alon Alfa A. It is clear from the results that a new drug skin permeation route was formed by the PJ treatment. Fig. 4 also shows the cumulative amount of drugs permeating through skin, calculated by deconvolution. The slope of the lines in the figure is permeation (or absorption) rate. Although the value was dependent on the species, the rate divided by the applied concentration, namely volume flow rate, was almost the same independent of drug species (0.41 ± 0.041 μl/h). This means that the drug permeation through skin treated by PJ might be explained by solvent drag (Nakashima et al., 1988). From this calculation, if a 50% drug solution were applied in this manner, about 5 mg of drug could be delivered per day. There are several drugs of which the daily dose is less than 5 mg, and with them clinical efficacy could be obtained by this delivery method. Selection of an inert solvent to solubilize the drugs may be the next hurdle.

3.2. Change in the permeability of drug and skin condition with alteration of PJ treatment

It was clear from a preliminary experiment that plasma concentration of a drug by this delivery method was different among the distances between the orifice of PJ and the skin surface. An in vivo permeation experiment was thus carried out using different PJ pretreatments: one pretreatment had a space of 5 mm between the PJ orifice and the skin surface (A) and another had no space (B). A 30% GM solution was applied to skin after the pretreatment. Fig. 5 shows the effect of pretreatments on the time course of plasma concentration of GM: treatment (A) showed higher concentration than (B). Volume flow calculated by deconvolution was: 0.29 ± 0.02 and 0.143 ± 0.034 μl/h for treatment (A) and (B), respectively. Treatment (A) thus gave about a two times higher permeation rate than (B).

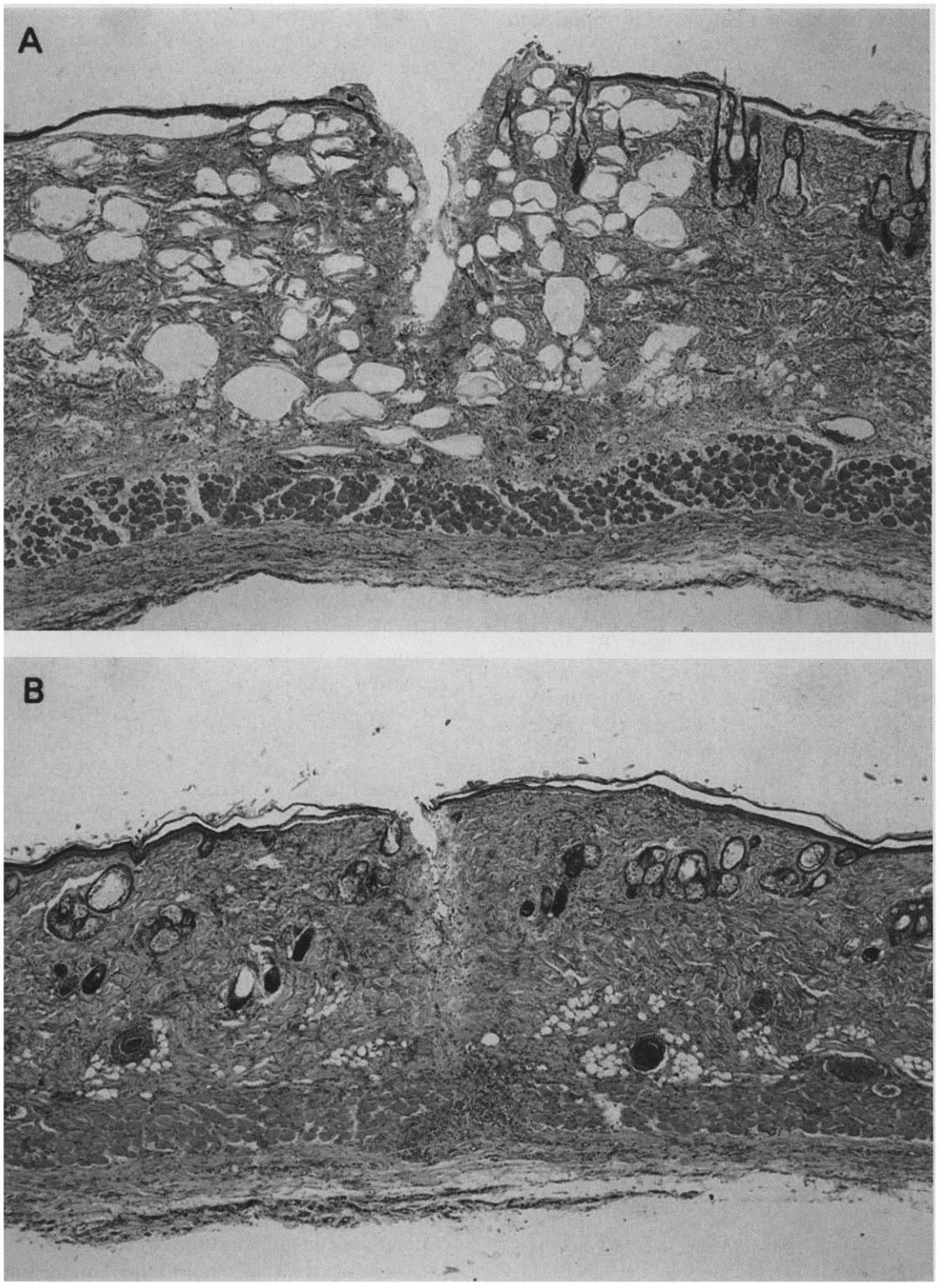

Fig. 6. Microscopic observation of skin after two types of pre-treatment. Saline was injected by PJ with 0 distance (A) or 5 mm away (B).

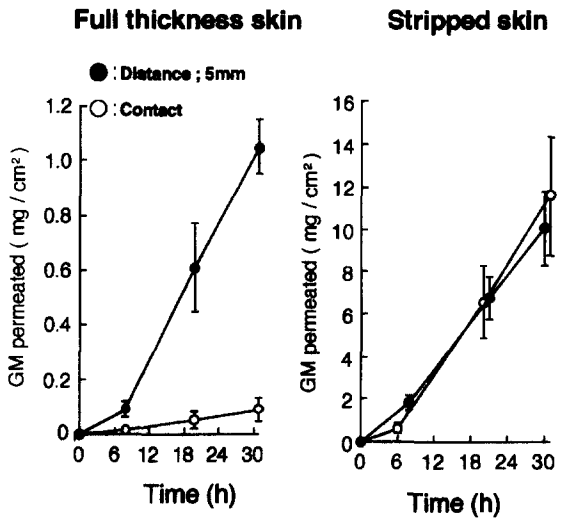

Fig. 7. In vitro permeation of GM through full thickness or stripped skin pretreated with PJ.

The influence of skin conditions after the treatment of (A) and (B) on volume flow rates was determined by microscopically examining the skin pieces (Fig. 6). Treatment (A) formed a larger pore in the stratum corneum than (B). In treatment (A), the jetted solution (saline) was not penetrated through the skin, but made the depot in the skin, whereas in treatment (B) the jetted solution penetrated through the skin.

Both the skin changes of the larger pore in the stratum corneum and the depot in the skin layer were viewed as possible causes of the higher permeability by treatment (A), so the contribution of each change was evaluated. After treatment (A) or (B) in vivo, an in vitro permeation experiment through intact (full thickness) skin or stripped skin was carried out (Fig. 7). The cumulative amount of GM through full thickness skin with treatment (A) was about ten times greater than B (Fig. 7, left), whereas no difference was found between (A) and (B) for stripped skin (Fig. 7, right).

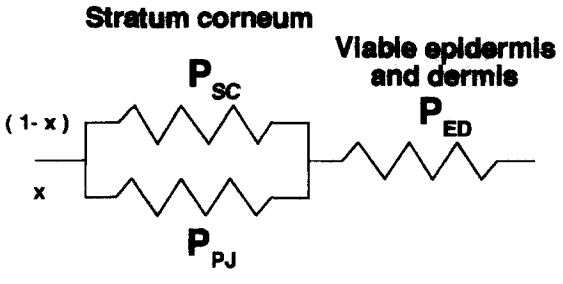

Fig. 8. A model showing drug permeation through skin pretreated by jet injection.

The pore area in the stratum corneum formed by PJ treatment was the primary contributor to the increase in overall permeability through skin. There seemed to be little contribution of the depot in the skin layer. From these results, the drug permeation rate through skin after PJ pretreatment can be calculated from Eq. 1 based on the model described in Fig. 8:

$$P_T = [(1 - X)P_{SC} + XP_{PJ}] \times P_{ED}/[(1 - X)P_{SC} + XP_{PJ} + P_{ED}] \quad (1)$$

where P is the Permeability coefficient (cm/s), X is the area ratio of the pore in the stratum corneum, and subscripts, T, SC, PJ and ED mean full thickness skin, stratum corneum, PJ and viable epidermis and dermis, respectively. Since no GM permeation was observed without PJ treatment, P_{SC} in Eq. 1 could be zero. Then, the equation becomes,

$$P_T = (XP_{PJ}P_{ED})/(XP_{PJ} + P_{ED}) \quad (2)$$

Logarithmic expression of permeability coefficients of GM with treatment (A) through intact and stripped skin in vitro were -6.51 and -5.38, respectively; with treatment (B), the values were -7.38 and -5.37, respectively. Using Eq. 2, XP_{PJ} of treatments (A) and (B) were calculated to be -6.47 and -7.38, respectively, so that XP_{PJ} was about eight times higher with treatment (A).

In this study $XP_{PJ} \ll P_{ED}$, so that Eq. 2 becomes $P_T \approx XP_{PJ}$. Since the P_{PJ} was assumed to be the permeability coefficient of the porous route, P_{PJ} would be the same by treatments (A) and (B). Therefore, the difference in permeability of GM between the two treatments might be explained by the pore area, X.

A higher permeability rate was achieved by a larger pore in the stratum corneum. Thereupon, we investigated the relationships among the dis-

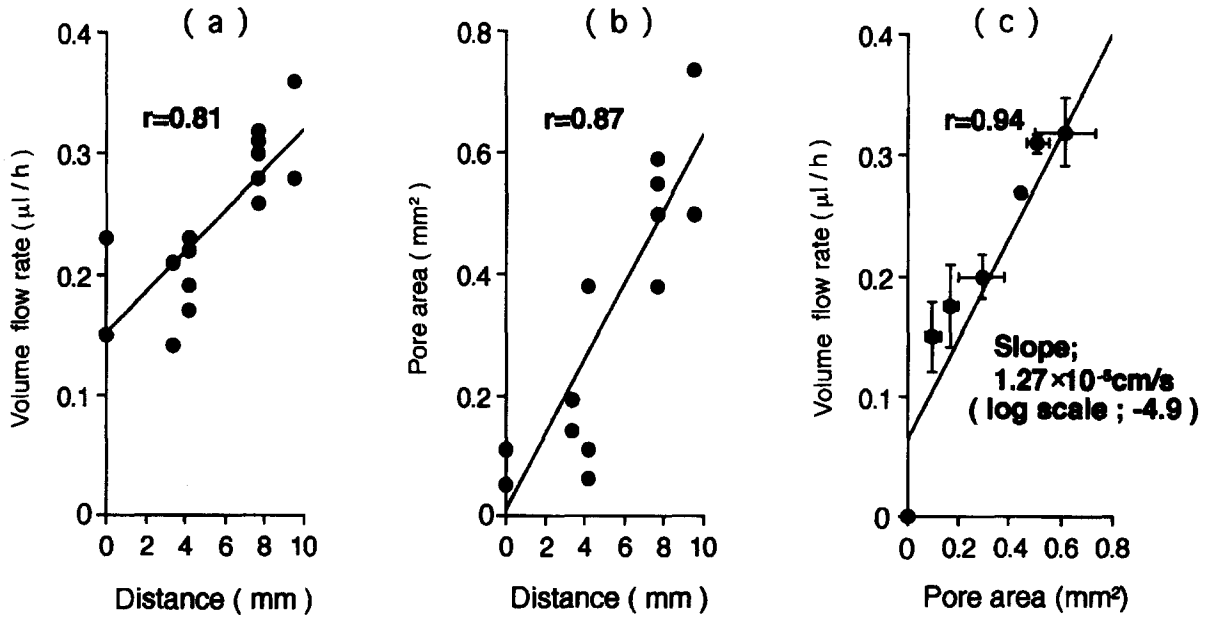

Fig. 9. Correlations between volume flow rate and skin — Jet injection distance (a), pore area and the distance (b) and the flow rate and pore area (c).

tances between the orifice to the skin surface, the pore size in the stratum corneum and the permeation rate. This investigation allowed us to know controlling skin permeation rate. After the PJ treatment at different distances from the orifice to the skin surface ranging from 0 to 9.5 mm, an in vivo permeation experiment was done using 30% GM solution.

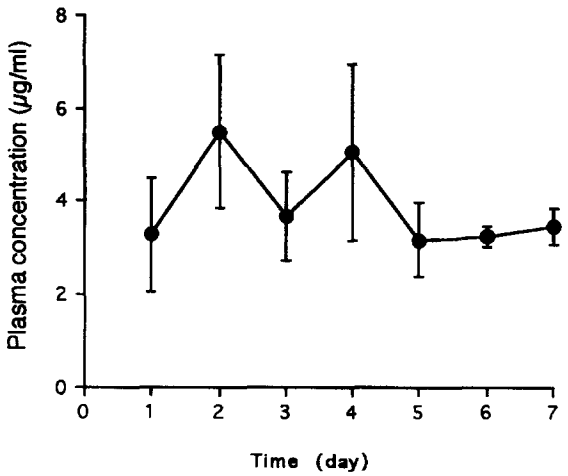

Fig. 10. Plasma concentration of TP during a week-long application.

Fig. 9a, b and c shows the relationships between distance and volume flow rate, distance and pore size, and pore size and volume flow rate, respectively. It was evaluated from the figure that the distance determined the pore size and the volume flow rate depended on the pore size; thus, the permeation rate could be controlled by the distance. It was speculated that the reason for the larger pore with the increase in distance might be due to the spreading of the jetted solution with the increased distance. Further investigations on control of the jetted volume or pressure are needed to confirm this. The slope of the regression line in Fig. 9c was considered as the permeability coefficient (volume flow rate/area) (Hatanaka et al., 1994), and was calculated to be 1.27×10^{-5} cm/s. This value was about the same as the permeability coefficient through stripped skin in hairless rat (Morimoto et al., 1986; Kobayashi et al., 1994). The PJ pretreatment might therefore be seen as a low invasive and practical method to make a tiny area of stripped skin.

Finally, we investigated the continuation of this delivery method. After the PJ treatment, TP solu-

tion was applied to the skin (Table 1), and the rats were kept in the holding cage so that they could not take off the device. Fig. 10 shows the plasma concentration of TP over a 1-week period, although the plasma levels were continued over a week in spite of a little fluctuation. This delivery method might thus be applicable to long-term dosing.

Fundamental investigation of drug permeation through PJ-treated skin was carried out making a new drug delivery system. This system may be applied a broad range of drugs which dissolve in inert solvent.

Acknowledgment

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (07457532).

References

Hatanaka, T., Manabe, E., Sugibayashi, K. and Morimoto, Y., An application of the hydrodynamic pore theory to percutaneous absorption of drugs. *Pharm. Res.*, 11 (1994) 654–658.

Kobayashi, D., Matsuzawa, T., Sugibayashi, K., Morimoto, Y. and Kimura, M., Analysis of the combined effect of l-menthol and ethanol as skin permeation enhancers based on a two-layer skin model. *Pharm. Res.*, 11 (1994) 96–103.

Kobayashi, S., Simple method for the determination of nicardipine in plasma using high-performance liquid chromatography. *J. Chromatogr.*, 420 (1987) 439–444,

Lindmayer, I., Menassa, K., Lambert, J., Moghrabi, A., Legendre, L., Legault, C., Letendre, M. and Halle, J.P., Development of new jet injection for insulin therapy. *Diabetes Care*, 9 (1986) 294–297.

Morimoto, Y., Sugibayashi, K., Hosoya, K. and Higuchi, W.I., Penetration enhancing effect of Azone on the transport of 5-fluorouracil across the hairless rat skin. *Int. J. Pharm.*, 32 (1986) 31–38.

Nakashima, E., Ishikawa, F., Sato, H., Deguchi, Y., Tamai, I., Matsushita, R., Ichimura, F. and Tsuji, A., Kinetics of peritoneal drug transport in rats: an application of the pore theory of transcapillary exchange. *J. Pharm. Sci.*, 77 (1988) 481–488.

Ranade, V.V., Drug delivery systems, 6. Transdermal drug delivery. *J. Clin. Pharmacol.*, 31 (1991) 401–418.

Sato, K., Oda, T., Sugibayashi, K. and Morimoto, Y., Estimation of blood concentration of drugs after topical application from in vitro skin permeation data. I. Prediction by convolution and confirmation by deconvolution. *Chem. Pharm. Bull.*, 36 (1988) 2232–2238.

Skelly, J.P., Shah, V.P., Maibach, H.I., Guy, R.H., Wester, R.C., Flynn, G. and Yacobi, A., FDA and AAPS report of the workshop on principles and practices of in vitro percutaneous penetration studies: relevance to bioavailability and bioeqivalence. *Pharm. Res.*, 4 (1987) 265–267.

Washitake, M., Yajima, T., Anmo, T., Arita, T. and Hori, R., Studies on percutaneous absorption of drugs. III. Percutaneous absorption of drugs through damaged skin. *Chem. Pharm. Bull.*, 21 (1973) 2444–2451.

Xu, P. and Chien, Y.W., Enhanced skin permeability for transdermal drug delivery: physiopathologicai and physicochemical considerations. *Crit. Rev. Therapeut. Drug Carrier Sys.*, 8 (1991) 211–236.

Yamaoka, K., Tanigawara, Y., Nakagawa, T. and Uno, T., A pharmacokinetic analysis program (MULTI) for microcomputer. *J. Pharm. Dyn.*, 4 (1981) 879–885.

Yukawa, J., Sugibayashi, K. and Morimoto, Y., Effect of various additives on the skin permeation of ketoprofen from the film forming transdermal formulation. *Yakuzaigaku*, 49 (1989) 254–262.