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Pretreatment effects of moxibustion on the skin permeation of FITC-dextran

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Abstract

This study was conducted to evaluate the pretreatment effects of different *in vivo* moxibustion on the permeation of a model high molecular compound, FITC-dextran, with a mean molecular weight of 4 kDa (FD-4), through excised hairless rat skin. Direct or indirect moxibustion (0.10 g moxa) was pretreated consecutively 4 times every 5 min on the abdomen of hairless rats, and the permeation of FD-4 was determined through the excised skin over 8 h from 30 min after starting the first moxibustion. This consecutive moxibustion pretreatment showed a significant increase in the skin temperature as well as skin permeation of FD-4 compared with the control group (no moxibustion pretreatment). Quantitative parameters showed an increase in skin temperature and skin permeation: the area under the skin temperature over control temperature–time curve during one burning cycle (5.0 min) (AUC_{temp}) or the maximum skin temperature during moxibustion (T_{max}) and the cumulative amount of FD-4 permeated through skin over 8 h (Q_8) or steady-state flux were increased by moxibustion pretreatment. Then, the effect of pedestal thickness (distance from the moxa cylinder and skin surface), shape of the moxa cylinder (5 mm diameter, 13 mm height or 9 mm diameter, 7 mm height), burning materials (moxa or aromatic incense), pedestal component (paper, potato or ginger) and moxibustion pretreatment method (direct or indirect moxibustion) was evaluated on the AUC_{temp} or T_{max} and Q_8 or flux. The amount of protein leached from the skin surface was also determined as an inflammatory index by this moxibustion pretreatment. When the skin temperature was increased to 60 °C, the Q_8 or flux as well as the amount of protein leached were markedly increased. When the skin temperature was controlled to 42 to 45 °C by an adequate selection of pedestal thickness, shape of the moxa cylinder, burning materials, pedestal component and moxibustion pretreatment method, on the other hand, protein leaching remained unaltered, but the Q_8 or flux significantly increased with the T_{max}. This study thus provides credible evidence that moxibustion pretreatment increases the skin permeation of high molecular compounds.

Keywords: Moxibustion; Skin permeation; Permeation enhancement; Skin temperature; Protein leaching; Skin barrier impairment

1. Introduction

Skin permeability of a variety of therapeutic drugs can be modified by several means, such as chemical enhancers, iontophoresis (Herndon, 2007), electroporation (Mori et al., 2003; Tokudome and Sugibayashi, 2004; Tokumoto et al., 2006), phonophoresis (Mitragotri and Kost, 2004), and microneedles (Martanto et al., 2006; Wu et al., 2006, 2007). Such chemical and physical strategies have been applied to increase the transdermal delivery of macromolecules as well as low molecular drugs by modifying the barrier properties of skin, especially in the stratum corneum (Elias, 2005). Only a few means have been clinically applied, however, due to high cost, cumbersome systems, low efficacy and so on. Heat treatment, such as moxibustion, is a physical technique and may be relevant in predicting therapeutic efficacy.

Moxa is a natural medicine that consists of several natural plants and is known to contain heptatriacontane and tannins having catechol derivatives (Kobayashi, 1988). Moxa has been used for a long time as a folk medicine for its bactericidal and antifungal properties, especially in moxibustion treatment, an oriental traditional physical therapy. It has also been utilized for muscle pain relief. The treatment is about 2000 years old in China and about 1000 years in Japan. Moxibustion therapy induces medicinal actions, especially by stimulating acupoints through the skin. Recently, moxibustion has been re-evaluated from a

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pharmacological point of view (Chiba et al., 1997; Uchida et al., 2003). After long-term stimulation with direct moxibustion to the acupoint tsu-san-li, immunohistological changes of high endothelial venules could be observed in the moxa-stimulated acupoint dermis (Tohya et al., 2000). Moxibustion induced various inflammatory responses, such as blood vessel reaction and enhancement of microvascular permeability (Okazaki et al., 1990). A clinical diagnosis can be used to determine if the skin was engorged with blood and whether there are skin blisters after moxibustion treatment. There are two different processes of moxibustion; *i.e.*, direct moxibustion and indirect moxibustion. Typical models of direct and indirect moxibustion systems are shown in Fig. 1a–c. No barrier materials, except air, pass through the hole of the pedestal between the moxa and skin surface in direct moxibustion (Fig. 1a), so the components directly encounter the skin surface through the hole in the pedestal. In contrast, the moxa is separated from the skin by a solid pedestal, although there are many small pores in the pedestal in the indirect process (Fig. 1b and c), so the components gradually reach the skin through the pedestal wall.

Moxibustion may be useful to increase the skin permeation of drugs, as well as the chemical and physical effects explained above. In particular, topical formulations containing therapeutic drugs may be applied at the site of moxibustion pretreatment. Thus, the purpose of this study was to evaluate the pretreatment effects of moxibustion on the permeation of a model hydrophilic and high molecular compound, FITC-dextran, with a mean molecular weight of 4 kDa (FD-4) through excised hairless rat skin. FD-4 was selected because it is hardly permeates the skin and its molecular size is similar to many bioactive petides and nucleotides. Moxibustion was performed *in vivo* and skin permeation was performed *in vitro*. Two shapes of moxa cylinder (5 mm in diameter and 13 mm in height, and 9 mm in diameter and 7 mm in height) were used. Several pedestals of different thicknesses and made of paper, ginger or potato were evaluated. Aromatic incense was also used for comparison with moxa.

2. Materials and methods

2.1. Animals

Healthy male hairless rats (WBN/ILA-Ht, 200–230 g of body weight) were purchased from the Life Science Research Center, Josai University (Sakado, Saitama, Japan), and all the animals used in the study were treated under the guidelines of the Life Science Research Center, Josai University.

2.2. Chemicals

FD-4 was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Phosphate-buffered saline (PBS) of pH 7.4 was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The moxa in SennenQ mini was supplied by Senefa Co. (Nagahama, Shiga, Japan). Aromatic incense (Senefa with a fruity fragrance) from Senefa Co. was also used for comparison with moxa. The moxa or aromatic incense, containing 0.10 g powdered moxa or a clayed aromatic preparation, of 5 or 9 mm diameter and 13 or 9 mm height, respectively, were made in our laboratory (Fig. 1). Drug wrapping paper (Hakuai Co., Tokyo, Japan) was used to wrap 0.10 g of moxa or aromatic preparation. The fireproofed paper pedestal of SennenQ mini was used, although one side of the pedestal with pressure-sensitive adhesive (PSA) was not applied to the skin surface, but the other side to avoid a stripping effect of the stratum corneum by PSA on the skin permeation of FD-4. The thickness of the pedestal was adjusted to 1, 2 or 5 mm by piling or cutting the paper pedestal. Pedestals 2 or 5 mm thick were also made in our laboratory using fresh ginger and potato. A bicinchoninic acid (BCA) assay kit was obtained from Pierce Biotechnology, Inc. (Rockford, IL,

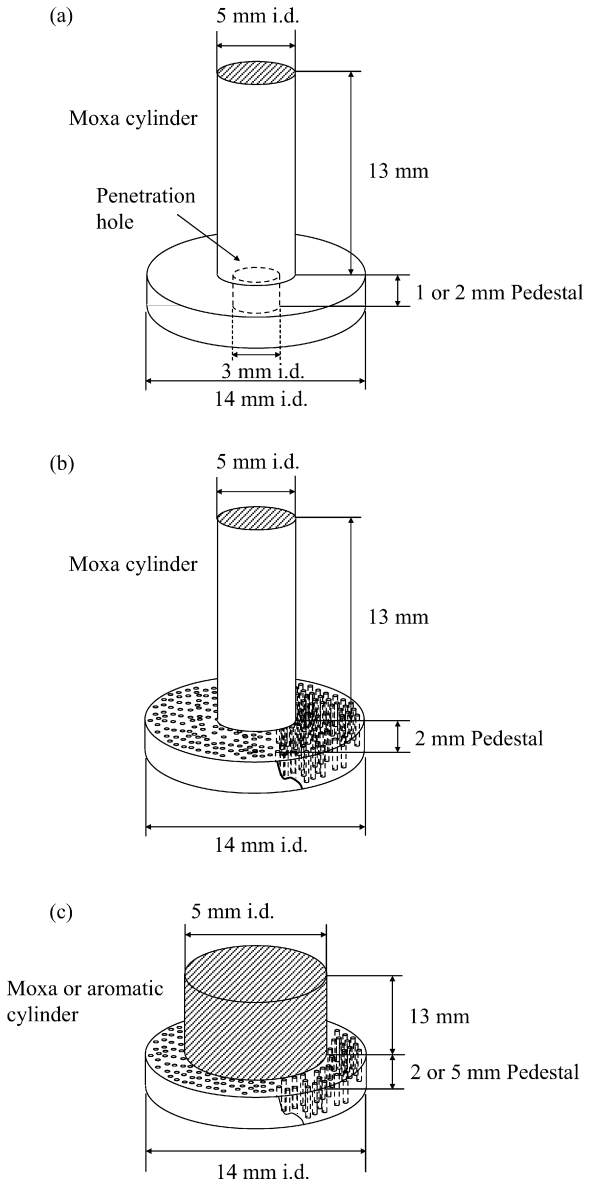

Fig. 1. Schematic representation of moxa samples used in direct moxibustion (a) and indirect moxibustion (b and c). (a) Direct moxibustion with a penetration hole of 3 mm diameter in paper or ginger pedestals (#1, 2, 3 and 7); (b) indirect moxibustion with many small holes in paper or ginger pedestals (#4 and 8); and (c) indirect moxibustion or aromatic incense burning with many holes in paper, ginger or potato pedestals. Different shapes of moxa or aromatic incense cylinder from (a) and (b).

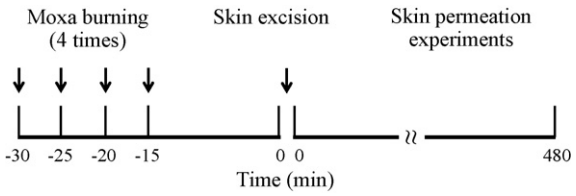

Fig. 2. Experimental schedule of moxa pretreatment and subsequent skin permeation and protein leaching experiments.

USA) to determine the amount of protein leached from the skin surface.

2.3. Skin preparation and skin permeation experiments

The abdominal skin of hairless rat (3 mm above the umbilicus in the midline) was treated 4 times consecutively for 5.0 min with moxa or aromatic incense burning by fixing the animals on their back under anesthesia by i.p. injection of pentobarbital. The pretreatment area of skin was carefully excised and any underlying fat or muscle tissue was removed. The oppositely untreated side of the skin sample along the abdomen midline for each rat (10 mm below the umbilicus) served as a control. The excised skin was sandwiched between two half-diffusion cells with an effective diffusion area of 0.95 cm^2 and a cell volume of 2.5 mL (Okumura et al., 1989). The skin permeation experiment of FD-4 was started 30 min after starting the first moxa or aromatic burning. The experimental schedule is shown in Fig. 2.

The stratum corneum side of the skin faced the FD-4-filled compartment, while the dermis side faced the sampling compartment. The donor compartment was filled with 2.5 mL of 0.25 mM FD-4 in PBS, and the sampling compartment was filled with 2.5 mL PBS. Each compartment was magnetically stirred. An aliquot (500 μL) was withdrawn from the sampling compartment at predetermined time intervals, and fresh PBS was replaced after each sampling to keep the cell volume constant. The temperature of the whole set was regulated at 32 °C by warm water circulation.

Several moxa or aromatic incense samples (Table 1) were evaluated for the skin permeation of FD-4 over 8 h in the present study.

2.4. Protein leaching from skin

Excised hairless rat skin with or without moxibustion (or aromatic incense) pretreatment was sandwiched between two half-cells (0.95 cm^2 in diffusion area). Both cells were filled with 2.5 mL of PBS. An aliquot (500 μL) was withdrawn from stratum corneum-side cells to measure protein leaching by BCA protein assay. The time course of protein leaching was followed over 8 h.

2.5. Analytical method

FD-4 concentration was determined by fluorescence intensity at an excitation wavelength of 495 nm and an emission wavelength of 515 nm, using a spectrofluorophotometer (RF-5300 PC, Shimadzu, Kyoto, Japan).

The amount of protein leached into samples was assayed using the BCA protein assay based on the colorimetric detection of total protein.

3. Results

3.1. Effects of pedestal thickness

Since the thickness of the pedestal on the moxa cylinder may be greatly related to skin temperature as well as to moxibustion therapy, it may also affect the skin permeation of FD-4 after moxibustion pretreatment. Thus, an *in vitro* permeation experiment was performed to assess the effect of the pedestal thickness of the moxibustion system on the skin permeation of FD-4. First, direct moxibustion using a cylinder 5 mm in diameter and 13 mm in height was evaluated using a paper pedestal, and the thickness of the pedestal was set at 1.0 or 2.0 mm, since most moxibustion systems are this shape for direct moxibustion (Fig. 1a). This moxibustion system directly delivers volatile essential oils con-

Table 1
Experimental groups in the present study

Number	Burning material	Cylinder size (diameter × height, mm)	Pedestal			Figure (symbol)
			Material	Height (mm)	Type	
#0	No material					Figs. 3–6 (▲)
#1 and 2	Moxa	5 × 13	Paper	1.0	Direct	Fig. 3 (◯, ●)
#3	Moxa	5 × 13	Paper	2.0	Direct	Fig. 3 (◊)
#4	Moxa	5 × 13	Paper	2.0	Indirect	Fig. 4 (●)
#5	Moxa	9 × 7	Paper	2.0	Indirect	Fig. 4 (◯)
#6	Aroma	9 × 7	Paper	5.0	Indirect	Fig. 4 (◊)
#7	Moxa	5 × 13	Ginger	2.0	Direct	Fig. 5 (◊)
#8	Moxa	5 × 13	Ginger	2.0	Indirect	Fig. 5 (◯)
#9	Moxa	9 × 7	Ginger	2.0	Indirect	Fig. 6 (●)
#10	Moxa	9 × 7	Potato	2.0	Indirect	Fig. 6 (◯)
#11	Aroma	9 × 7	Ginger	5.0	Indirect	Fig. 6 (♦)
#12	Aroma	9 × 7	Potato	5.0	Indirect	Fig. 6 (◊)

"Aroma" means aromatic incense.

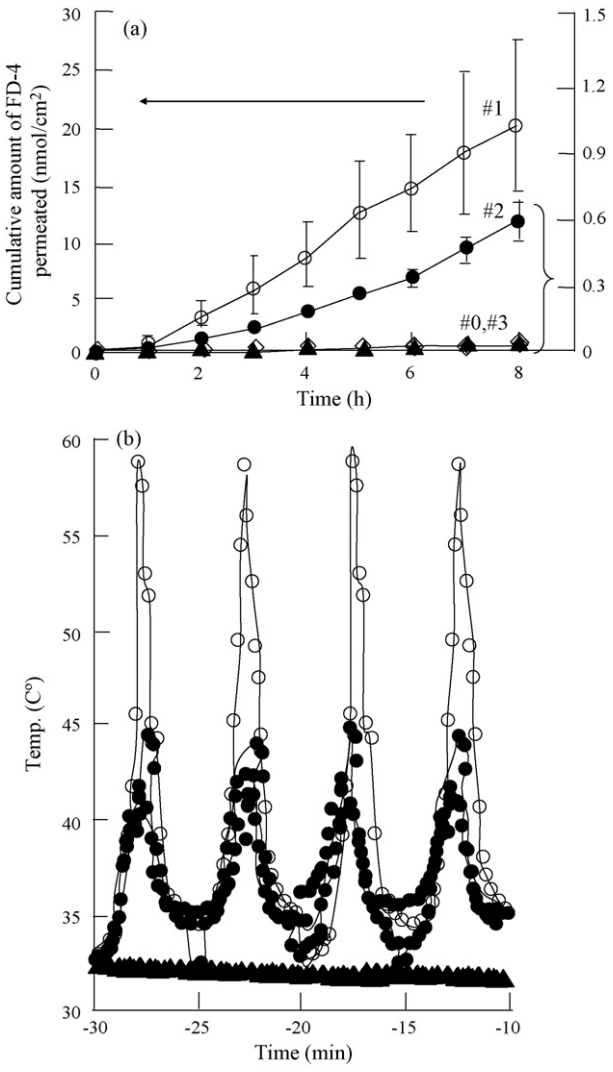

Fig. 3. Effect of pedestal thickness on the time courses of the amount of FD-4 that permeated through excised hairless rat skin (a) and skin temperature (b) following and during direct moxibustion treatment, respectively, to hairless rat abdominal skin. Symbols (◯) and (●) 1.0 mm pedestal, (◯) skin blister found, and (●) no blister found (#1 and 2 in Table 1), (◊) 2.0 mm pedestal (#3), and (▲) control (without moxibustion, #0). Each data point shows the mean ± S.D. of 4–7 rats.

tained in moxa to the skin surface through the penetration hole in the pedestal (Fig. 1) (Zhou, 2003). The experimental schedule of moxa pretreatment and the following skin permeation study are shown in Fig. 2.

Fig. 3a shows the time courses of the cumulative amount of FD-4 that permeated through the excised hairless rat skin following consecutive moxibustion pretreatment to the abdomen of hairless rats. When the 2.0 mm pedestal was used (#3 in Table 1), no enhancement effect of moxibustion pretreatment was observed on the skin permeation of FD-4 compared with the control group (no moxibustion pretreatment, #0). When the 1.0 mm pedestal was used, on the other hand, a marked enhancement effect was observed (#1 and 2). Unfortunately, large variations were found in the skin permeation of FD-4 when using the 1.0 mm pedestal. This was due to the occurrence or absence of skin blisters on the skin surface by moxibustion treatment. This group was divided into two sub-groups; one with blisters (#1) and the other without blisters (#2) at the pretreatment site of moxibustion. Significantly different skin permeations of FD-4 were observed between the two sub-groups, as shown in Fig. 3a.

The corresponding time course of the skin temperature of hairless rats during 4 moxibustion treatments is shown in Fig. 3b. The skin temperature before moxibustion varied from 32 to 35 °C among animals. Since the mean value (±S.D.) was about 32.6 °C (±0.5), data before moxibustion were normalized to this mean value. When moxibustion was applied to the skin surface, the skin temperature increased during burning. The maximum temperature was observed about 2.0 min after starting the burning, and skin temperature recovered to almost the initial value or a little higher about 3.5 min after starting the burning. We then decided to consecutively apply moxibustion 4 times every 5 min (see Fig. 2). Almost the same temperature profiles at the skin surface were observed for each step of moxibustion in every case. When the 2 mm pedestal was used for moxibustion (#3), the increase in skin temperature was only slight, as shown in Fig. 3b; the highest temperature was about 44 °C. When the 1 mm pedestal was used, on the other hand, a big variation was found in the skin temperature. The highest temperature (T_{max}) was about 59 °C if a skin blister was found (#1, Fig. 3), whereas it was about 45 °C without blistering (#2, Fig. 3). The area under the skin surface temperature over the initial temperature for the first moxibustion period, AUC_{temp}, was determined using a trapezoidal rule. T_{max} or AUC_{temp} may be used as an index for the increase in skin permeation of FD-4 after moxibustion, as shown in Table 2.

3.2. Effects of burning materials, cylinder size and indirect burning

Aromatic incenses are already marketed for relaxation or spiritual feeling, as in aromatherapy or aromachology. In order to identify the usefulness of increased skin temperature to increase the skin permeation of FD-4, aromatic incense burning was also evaluated for comparison with moxibustion. A moxa cylinder or aromatic incense with a paper pedestal was burned on the skin surface in the present study. Since the increase in skin temperature by burning aromatic incense is usually much higher than that by moxibustion, the thickness of paper pedestal was 5.0 and 2.0 mm for aromatic incense and the moxa cylinder, respectively. Indirect burning was applied in this experiment, since direct aromatic incense is not appropriate. The cylinder size (9 mm diameter, 7 mm height) was different from in the experiments in Fig. 3 (5 mm diameter, 13 mm height), although the amount of moxa (0.10 g) was the same. Indirect moxibustion using a 5 mm × 13 mm cylinder was also evaluated for comparison.

Fig. 4a and b show changes in the cumulative amount of FD-4 that permeated through the excised skin with indirect moxibustion or aromatic incense pretreatment and skin temperature during moxa (#4 and 5 in Table 1) or aromatic incense (#6) burning. The skin permeation of FD-4 after moxibustion pre-

Table 2
Summary of skin permeation, skin temperature and protein leaching experiments

Number	Skin permeation of FD-4			Skin temperature			Figures
	Flux (nmol/cm^2/h)	Lag time (h)	Q_8 (nmol/cm^2)	AUC_{temp} (°C min)	T_{max} (°C)	Protein leaching (μg/cm^2)	
0	0.004 ± 0.002	3.52 ± 0.67	0.024 ± 0.007	0	0	0.69 ± 0.09	Figs. 3–6 (▲)
1	3.185 ± 0.654	1.06 ± 0.72	21.508 ± 6.861	111.3 ± 9.2	58.8 ± 1.0	18.56** ± 13.69	Fig. 3 (◯)
2	0.101 ± 0.026	2.97 ± 0.33	0.595 ± 0.087	48.2 ± 1.1	45.1 ± 0.2	1.49 ± 0.18	Fig. 3 (●)
3	0.005 ± 0.002	2.71 ± 0.15	0.025 ± 0.003	68.1 ± 1.9	41.7 ± 0.4	1.32 ± 0.00	Fig. 3 (◊)
4	0.009 ± 0.003	2.93 ± 0.21	0.050 ± 0.018	68.1 ± 2.3	41.6 ± 0.5	1.32 ± 0.00	Fig. 4 (●)
5	0.101 ± 0.024	2.77 ± 0.28	0.591 ± 0.091	86.1 ± 1.8	44.4 ± 0.7	1.49 ± 0.12	Fig. 4 (◯)
6	0.029 ± 0.006	2.86 ± 0.22	0.166 ± 0.063	88.7 ± 2.4	41.8 ± 1.1	1.49 ± 0.18	Fig. 4 (◊)
7	0.004 ± 0.001	2.95 ± 0.23	0.029 ± 0.005	79.0 ± 1.2	42.9 ± 0.8	1.14 ± 0.18	Fig. 5 (◊)
8	0.017 ± 0.004	2.55 ± 0.20	0.111 ± 0.025	80.5 ± 1.2	42.7 ± 0.1	1.40 ± 0.09	Fig. 5 (◯)
9	0.070 ± 0.023	2.86 ± 0.10	0.465 ± 0.062	211.6 ± 4.8	44.9 ± 0.4	1.49 ± 0.53	Fig. 6 (●)
10	0.042 ± 0.011	1.86 ± 0.07	0.295 ± 0.057	76.7 ± 2.4	44.3 ± 0.7	1.14 ± 0.18	Fig. 6 (◯)
11	0.035 ± 0.010	2.77 ± 0.35	0.231 ± 0.018	131.2 ± 2.5	44.5 ± 0.4	1.49 ± 0.53	Fig. 6 (♦)
12	0.018 ± 0.005	2.68 ± 0.1	0.118 ± 0.021	141.2 ± 1.9	43.0 ± 0.9	1.48 ± 0.13	Fig. 6 (◊)

Each value represents the mean ± S.D. of 4–7 experiments. AUC_{temp} and T_{max} are the values for the first moxibustion.
** Significant difference ($p < 0.01$).

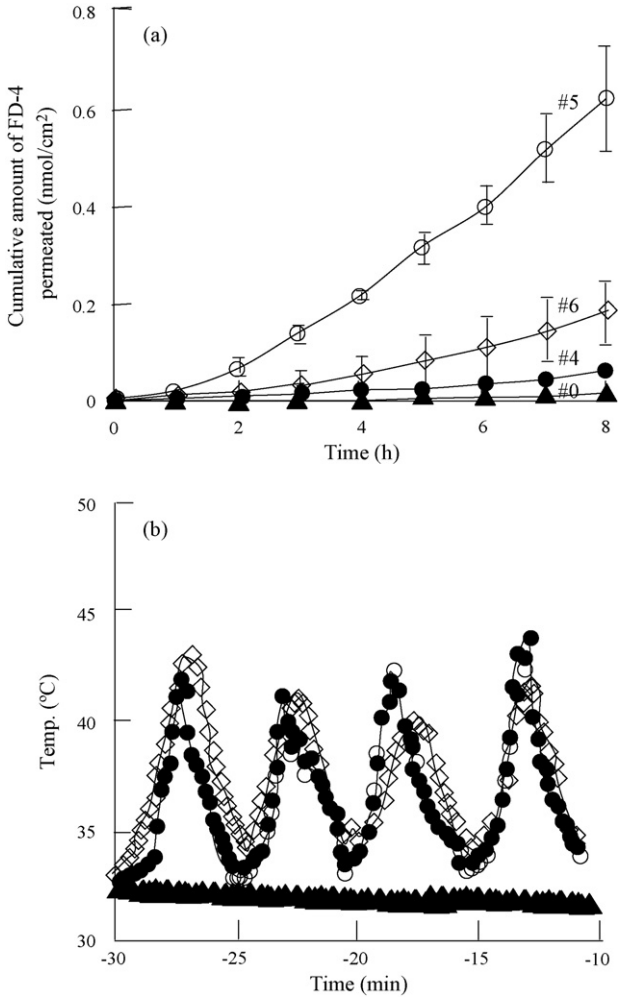

Fig. 4. Effect of indirect moxibustion or aromatic incense burning with different shapes of burning cylinder on the time course of the amount of FD-4 that permeated through the excised hairless rat skin (a) and skin temperature (b) following and during indirect burning, respectively, to hairless rat abdominal skin. Symbols (●) moxa (5 mm × 13 mm, #4), (◯) moxa (9 mm × 7 mm, #5), (◊) aromatic incense (9 mm × 7 mm, #6), and (▲) control (as in Fig. 3, #0). Each data point shows the mean ± S.D. of 4–6 determinations.

treatment (#5) was 3 times higher than that after aromatic incense (#6), and the skin temperature during moxibustion was higher than that during aromatic incense burning. Interestingly, cylinder size (9 mm diameter, 7 mm in height) (#5) showed more than 10 times higher skin permeation than the cylinder of 5 mm in diameter and 13 mm in height (#4). When comparing direct moxibustion (#3, Fig. 3) with indirect moxibustion (#4, Fig. 4), indirect moxibustion showed higher skin permeation as well as higher skin temperature.

3.3. Effects of a ginger pedestal

Ginger and potato pedestals in addition to the paper pedestal were evaluated for skin permeation and skin temperature by pretreatment with 0.10 g moxa and aromatic incense. Fig. 5a and b show changes in the cumulative amount of FD-4 that permeated the excised skin and skin temperature during direct (#7) and indirect (#8) moxibustion using a ginger pedestal. Indirect moxibustion (#8) was more effective to increase the skin permeation of FD-4 than direct moxibustion (#7). In addition, the ginger pedestal (#8) was more effective than the paper pedestal (#4) (Fig. 4) under the same conditions except for the pedestal material.

Fig. 6 shows the effect of moxibustion and aromatic incense burning on the skin permeation of FD-4 and skin temperature. Indirect moxibustion using a cylinder of 9 mm in diameter and 7 mm in height was used in this experiment, since it was more effective than the correspondent direct moxibustion using a cylinder 5 mm in diameter and 13 mm in height. The cylinder with a ginger pedestal (#9) was extremely effective compared with the potato pedestal (#10), but almost the same as the paper pedestal (#5) (Fig. 4). Moxibustion using a ginger pedestal (#9) showed similar T_{max} but higher AUC_{temp} compared with a potato pedestal (#10).

Aromatic incense burning (#11 and 12) showed lower skin permeation than the corresponding moxibustion (#9 and 10, respectively). These results may be related to the effect of volatile compounds in moxa.

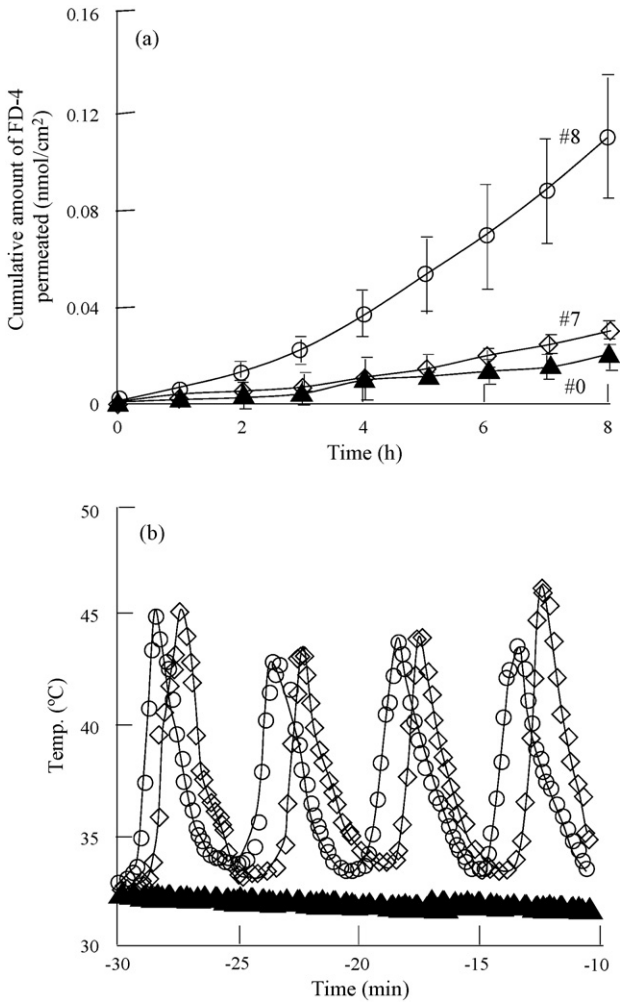

Fig. 5. Effect of ginger pedestal on the time course of the amount of FD-4 that permeated through the excised hairless rat skin (a) and skin temperature (b) following and during direct or indirect moxibution treatment, respectively, with a 2 mm pedestal. Symbols (◊) direct moxibution treatment with moxa cylinder with a 2 mm ginger pedestal (#7), (◯) indirect moxibution treatment with a moxa cylinder having a 2 mm ginger pedestal (#8), and (▲) control (as in Figs. 3–5, #0). Each data point shows the mean ± S.D. of 4–6 determinations.

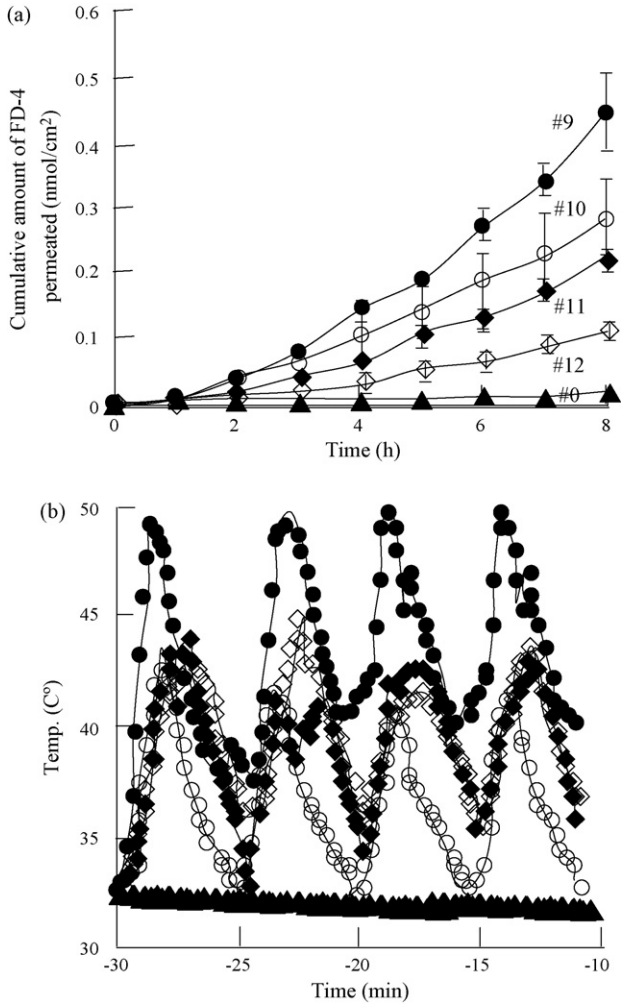

Fig. 6. Comparison of the effect of the ginger pedestal with a potato pedestal on the time course of the amount of FD-4 that permeated through the excised hairless rat skin (a) and skin temperature (b) following and during indirect moxibustion or aromatic incense burning, respectively. Symbols (●) moxa with ginger pedestal (#9), (◯) moxa with potato pedestal (#10), (♦) aromatic incense with ginger pedestal (#11), (◊) aromatic insense with potato pedestal (#12), and (▲) control (as in Figs. 3–5, #0). Each data point shows the mean ± S.D. of 4–6 determinations.

3.4. The relationship between skin permeation of FD-4 and skin temperature

To understand the effect of several variances in moxibustion pretreatments on skin permeation, the correlations between the cumulative amount of skin permeation of FD-4 over 8 h (Q_8), pseudo-steady-state flux of FD-4 (flux) and reciprocal lag time to pseudo-steady-state flux (1/lag time), and maximum skin temperature (T_{max}) and changes in the area under the temperature vs. time over 5.0 min (AUC_{temp}) were evaluated. These results are shown in Fig. 7.

When the T_{max} was over 42 °C, the Q_8 and flux of FD-4 increased with an increase in T_{max} (Fig. 7a and c). In contrast, no clear relationships were found between these skin permeation parameters and AUC_{temp} (Fig. 7b and d). These results suggest that the increase in the skin permeation of FD-4 was related to the high skin temperature but not to the average temperature. The reciprocal of lag time (1/lag time) was slightly increased by T_{max} and AUC_{temp} (Fig. 7e and f). The value of 1/lag time is directly related to the diffusivity of FD-4 in the skin barrier. Thus, skin diffusivity was not so affected by moxibustion pretreatment.

3.5. Skin damage

We evaluated protein leaching from the skin surface using the BCA protein assay as an index of safety for the moxibustion. This experiment was carried out under the same conditions as for the skin permeation experiments in the experimental schedule, as shown in Fig. 2. The obtained results for protein leaching are shown in Table 2. Marked differences in the cumulative amount of protein leached over 8 h were found between control and treatment groups.

Fig. 8a and b show the relations between the cumulative amounts of protein leached over 8 h and T_{max} or AUC_{temp}, respectively. A higher cumulative amount of protein leached

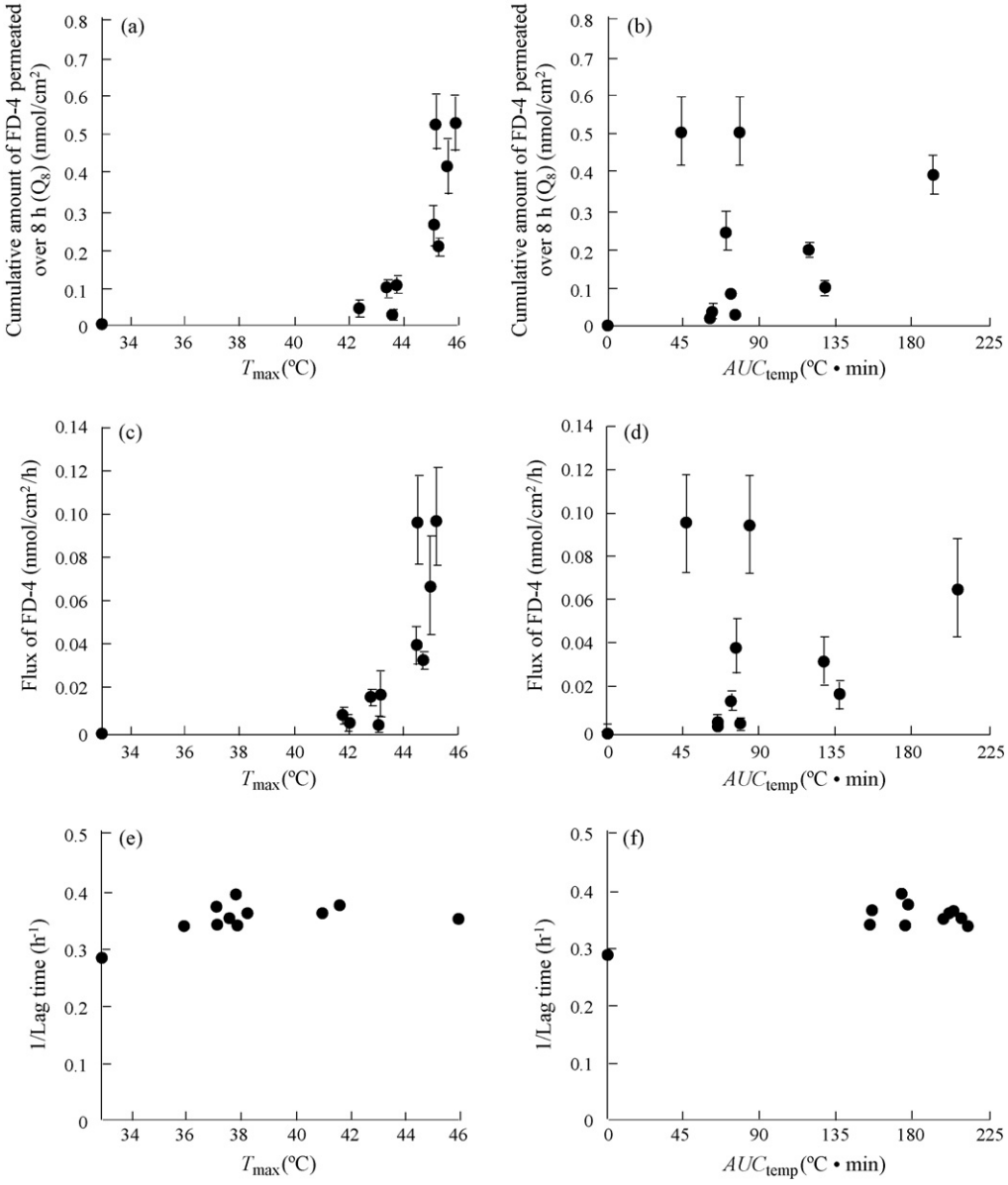

Fig. 7. Relationship between the permeation parameters of FD-4 through the excised hairless rat skin (cumulative amount permeated 8 h in (a) and (b); flux in (c) and (d); and 1/lag time in e and f) and the skin temperature (T_{max} in a, c and e; AUC_{temp} in b, d and f) following direct or indirect treatment with moxa or aromatic incense with ginger, paper or potato pedestal. Each data point shows the mean or mean ± S.D. of 4–6 determinations.

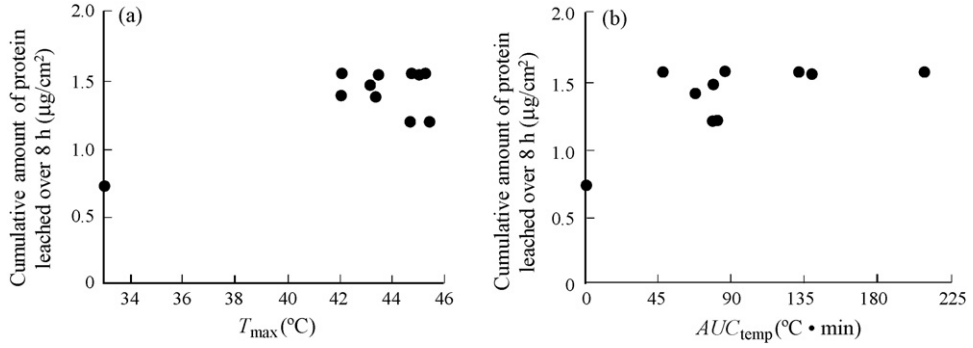

Fig. 8. Relationship between the cumulative amount of protein leached from excised hairless rat skin over 8 h and the temperature profiles (T_{max} is left figure and AUC_{temp} is right figure, respectively) following direct or indirect treatment with moxa or aromatic incense with ginger, paper or potato pedestal. Each data point shows the mean of 4–6 groups.

was observed with a higher skin temperature. The figure suggests that the AUC_{temp} may be a better index than the T_{max} for predicting the cumulative amount of protein leached.

3.6. Relationship between skin permeation and protein leaching

Fig. 9a–c show the relationship among the Q_8, flux and 1/lag time, respectively, and the cumulative amount of protein leached from the skin surface over 8 h. No good relationship was found between the Q_8 or flux and protein leaching, as shown in Fig. 9a and b; however, 1/lag time for each treatment group was increased by an increase in protein leaching (Fig. 9c). It is interesting to note that the increase in the diffusivity of FD-4 in the skin barrier may be greatly related to the cumulative amount of protein leached. The increase in diffusivity must be also related to the skin permeation of FD-4. Changes in the stratum corneum barrier by heat were involved in increased FD-4 permeation through the skin.

4. Discussion

The main factor to increase skin permeation must be the modified barrier function in the stratum corneum by heating the tissue by moxibustion. Chiba et al. (1997) suggested that the thermal effect of indirect moxibustion was mainly dependent on the spacing distance between the moxa and skin. The thickness of the pedestal of the moxa cylinder was very important for the effect of moxibustion on the skin permeation of FD-4 (Fig. 3a). The efficacy for heating the skin was higher when using a thinner pedestal (Fig. 3b). The use of an extremely thin pedestal, however, caused severe skin damage. Large variations were found in the skin permeation of FD-4 as well as skin temperature when using a 1.0 mm paper pedestal. This was due to the occurrence or absence of blistering on the skin surface (blistering was found in some cases, but not others). When this group was divided into two sub-groups according to the existence or absence of skin blisters, significantly different skin permeations of FD-4 and skin temperatures were observed between them. T_{max} can be used as an index to prevent skin blisters.

Ginger and potato pedestals in addition to the paper pedestal were evaluated for skin permeation and skin temperature. The ginger pedestal was more effective than paper and potato pedestals (Fig. 6). Ginger had already been used in the moxibustion therapy (Xiaoxiang, 2006), and a high therapeutic moxibustion effect was reported using a ginger pedestal in the Chinese literature (Liu and Wang, 2006). Potato pedestal was used as a control as well as paper pedestal to evaluate the effect of ginger pedestal in our experiment. Increased T_{max} by moxibustion may be related to the effectiveness of the skin permeation of FD-4. Most of the skin permeation-enhancement data were explained by the increase in T_{max} (Fig. 7a and c). However, we cannot explain the similar skin permeation between the paper (#5) and ginger (#9) pedestal. The details should be studied scientifically in the future.

A cylinder size of 9 mm diameter and 7 mm height showed markedly higher skin permeation than that of 5 mm diameter and 13 mm height. Although this is due to different attached areas of the moxa to the skin surface, a nonlinear relation was observed between the area and enhancing ratio of skin permeation of FD-4. Indirect moxibustion showed higher skin permeation and higher skin temperature than direct moxibustion. This is also related to the heating area of the skin. In addition, this was probably due

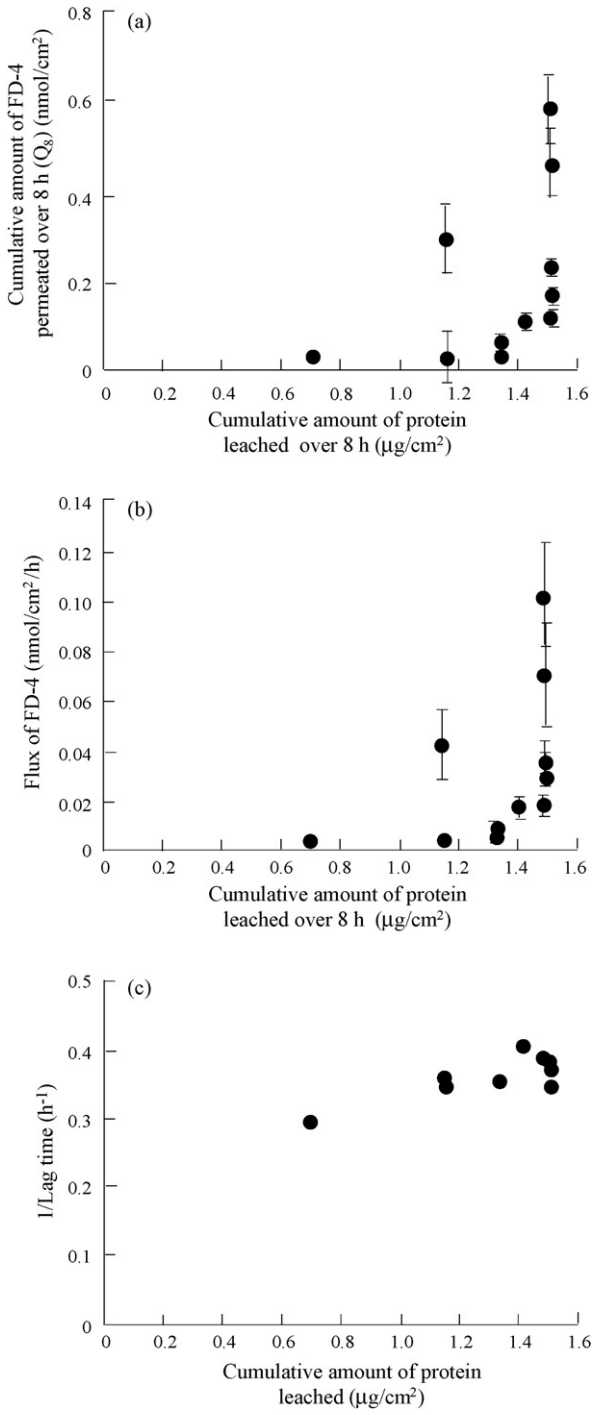

Fig. 9. Relationship between the permeation parameters of FD-4 through excised hairless rat skin (cumulative amount permeated 8 h in (a); flux in (b); 1/lag time in c) and the cumulative amount of protein leached over 8 h following direct or indirect treatment with moxa or aromatic incense with ginger, paper or potato pedestal. Each data point shows the mean or mean ± S.D. of 4–6 groups.

to easier heat transport through the paper pedestal with small pores than through the pedestal hole.

When the T_{max} was over 42 °C, the Q_8 and flux of FD-4 were increased with the increase in T_{max}. In contrast, no clear relationships were found between these skin permeation parameters and AUC_{temp}. These results suggest that the increase in the barrier structure may be modified at temperatures above 42 °C and the degree of barrier function may be dependent upon skin temperature above 42 °C. The reciprocal of lag time (1/lag time) was proportional to the diffusivity of the penetrant in the skin barrier and the 1/lag time was slightly increased by T_{max} and AUC_{temp}. Thus, the change in the barrier function is probably caused by high skin temperature over 42 °C.

Safety point of view is very important in techniques to enhance the skin permeation of drugs. Changes in the lipid structure of the stratum corneum were reported after heating the skin barrier (Bouwstra et al., 1994, 1995). The extent of protein leaching from the skin surface can be used as an index of lipid structure change or corresponding skin damage produced by moxibustion. Goates and Knutson (1994) and Thewalt et al. (1992) reported that lipid motion was more restricted in the solid lipid environment than that in the conventional gel-phase bilayer, when the skin surface temperature was below about 42 °C.

5. Conclusion

The present work is a feasibility study to increase the skin permeation of drugs by moxibustion pretreatment. Since moxibustion is well known to be safe from its long history in Japan and China, moxibustion pretreatment can be used as a new technique to increase the skin permeation of therapeutic drugs. The present data for protein leaching from the skin surface supported the safety of moxibustion.

This means to increase skin permeation of drugs is much different from the present physical skin permeation-enhancement systems such as iontophoresis, electroporation, phonophoresis and microneedle application. We may apply a dermal patch on the skin which was pretreated by moxibustion to have enhanced skin permeation with moxibustion effect. Thus, moxibustion treatment is a unique and useful technique to increase or improve the skin permeation of malabsorptive drugs.

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