Pretreatment Effects of Moxibustion on the Skin Permeation and the Skin and Muscle Concentration of Drugs

甲第42号

2008

曹 殿 秀
Dianxiu Cao

Pretreatment Effects of Moxibustion on the Skin Permeation and the Skin and Muscle Concentration of Drugs

2008

曹 殿 秀

Dianxiu Cao

Contents

Abstract

This study was conducted to evaluate the *in vivo* pretreatment effects of moxibustion on the *in vitro* skin permeation of weak ionized macromolecular compound FITC-dextran (mean molecular weight of 4 kDa, FD-4) and ion compound sodium salicylate, and on *in vivo* distribution of ion drug sodium salicylate. The direct and indirect moxibustion or indirect aromatic incense (0.10 g moxa or aromatic cylinder) were pretreated consecutive four times once every 5 minutes on the abdominal surface of hairless rats, and the skin permeation of FD-4, sodium salicylate and the tissue concentration of sodium salicylate over 8 hours from 30 min after starting the first pretreatment was determined using *in vitro* diffusion method and *in vivo* evaluation method, respectively. This consecutive moxibustion pretreatment makedly increased skin temperature and cumulative amount of FD-4 and sodium salicylate permeated through skin over 8 h (Q_8) compared with the control group (without moxibustion treatment), and the alteration of sodium salicylate distribution in tissue in treatment group was also much higher than in control group. The effects of the pedestal thickness (distance from the moxa cylinder to skin surface), burning materials (moxa or aromatic incense), pedestal component (paper, potato or ginger) and moxibustion pretreatment method (direct or indirect treatment) on the $AUC_{temp\text{-}t}$ (areas under the skin temperature-time curve over 20 minutes of period of moxa cone burning) or T_{max} (The maximum temperature) and Q_8 were evaluated. There is a nonlinear relation between the Q_8 and $AUC_{temp\text{-}t}$. All skin permeation experiments were carried out at approximately 42 ~ 45°C for highest temperature and demonstrated by skin surface protein leaching determining.

The present work is a feasibility study to increase the skin permeation and absorption of drugs by moxibustion pretreatment. The present data obtained after following moxibustion pretreatment based on the skin surface safety condition suggests that the moxibustion treatment on skin surface may not only be used to increase the skin permeation of FD-4 and sodium salicylate but also significantly alter the systemic absorption and distribution of the sodium salicylate given transdermally or administrated by *i.v.* injection. The moxibustion treatment technique may by useful in developing a transdermal delivery system with enhanced systemic penetration, absorption and distribution.

General Introduction

Skin permeability of a variety of therapeutic drugs can be modified by several means, such as chemical enhancers (Purdon *et al.*, 2004), iontophoresis (Herndon, 2007), electroporation (Mori *et al.*, 2003; Tokudome *et al.*, 2004; Tokumoto *et al.*, 2006), phonophoresis (Mitragotri, 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 has a long history of about 2000 years 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 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.

Moxibustion can initiate a non-specific healing reaction by warm regions or acupuncture points with the intention of continuous stimulating and hence influencing the blood flow qualities of through the body. When the body is in the natural position, the thermal energy passages allow the internal organs to be directly reached by moxibustion and heat therapies. The energy passages are connected to the life forces of the organs and their vital functions. There are various moxibustion techniques. Generally, moxibustion is classified into four parts: moxibustion with moxa stick, moxibustion with moxa cone, moxibustion with instrument and needle warming with moxibustion. A widely used type in moxibustion technology is moxibustion with moxa cone, and this type of moxibustion is further categorized into direct and indirect moxibustion. A series of pharmacological effectiveness was generated by the intense heat of moxibustion. These may include: prevention of disease, activation of body's immune system, inhibition of cytotoxicity, and other therapeutic actions and promising modalities. There has been a heavy reliance on the scarring moxibustion in the traditional moxibustion technique. However, the scarring moxibustion would be inappropriate for some sensitive spot, especially used for investigating the changes in the lipid structure of the stratum corneum after performing moxibustion. The burning of moxa is believed to expel cold and warm the meridians, which leads to smoother blood flow. Moxibustion practice was based on the physical formation of natural energy passages. The second law of the thermodynamics states that the energy transformed from heat may be freed

for useful work in lipid or any reaction mixture. The knowledge gained from temperature enhancer mechanism in understanding the kinetic studies of the bilayer phase transitions with temperature also has been immense. The resulting moxibustion treatment will be generally identified as a new penetration enhancement technology of drugs through skin.

In the present study, we conducted to evaluate the *in vivo* pretreatment effects of moxibustion on the *in vitro* skin permeation of a weak ionized macromolecular compound, FITC-dextran (mean molecular weight of 4 kDa, FD-4) and an ionic compound, sodium salycylate (SA-Na), and the on *in vivo* skin distribution of the ionic compound, SA-Na..

Chapter 1

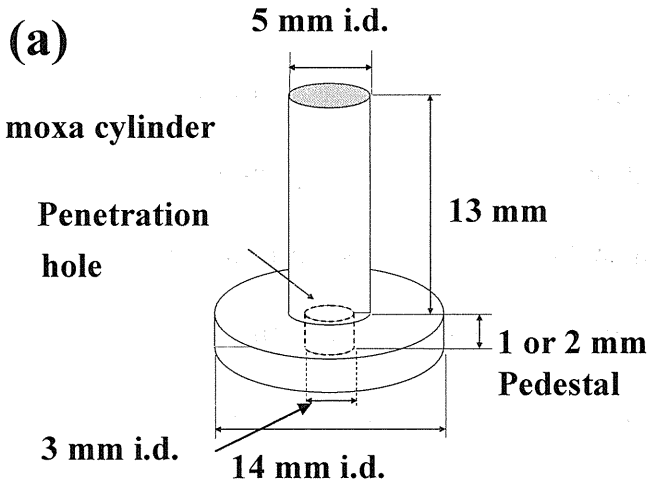
Pretreatment Effects of Moxibustion on the Skin Permeation of FITC-Dextran

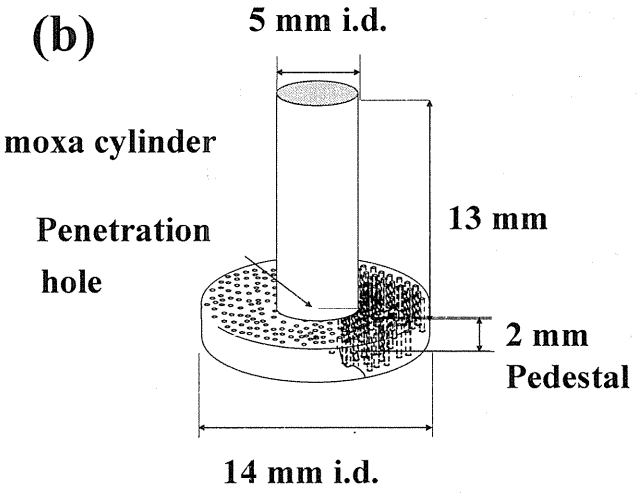
1.1. Introduction

There are two types of moxibustion with moxa cone (see general introduction) ; *i.e.*, direct moxibustion and indirect moxibustion. Typical models of direct and indirect moxibustion systems are shown in Fig. 1a, b, c. No barrier materials, except air, pass through the hole of the pedestal between the moxa and skin surface in the 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, c), so the components gradually reach the skin through the pedestal wall.

When the goal of a study is to assess the mechanism of drug absorption and distribution within the skin, the complex natural barrier structure of stratum corneam of skin is encountered with drug diffusion across the skin. Various skin permeation enhancement techniques are utilized in the rational design and optimization of transdermal drug delivery systems. As early as in nineteen seventies, drug transport across the skin barrier has been mentioned to be influenced by dermal temperature (Riegelman, 1974). Heat may accelerate thermal movement of molecules and increase the diffusion coefficients, improve the slow

kinetic processes of drug dissolution, and increase the solubility in the donor solution and the concentration gradient (Tojo *et al.*, 1987, and Jain *et al.*, 2003). Alterations of temperature have been used in clinical therapy, including cancer therapy (Koichi *et al.*, 2006). But there were seldom studied about using the alteration of temperature to influence the skin permeation, absorption and distribution of drug in the skin barrier after performing the moxibustion technology therapy.





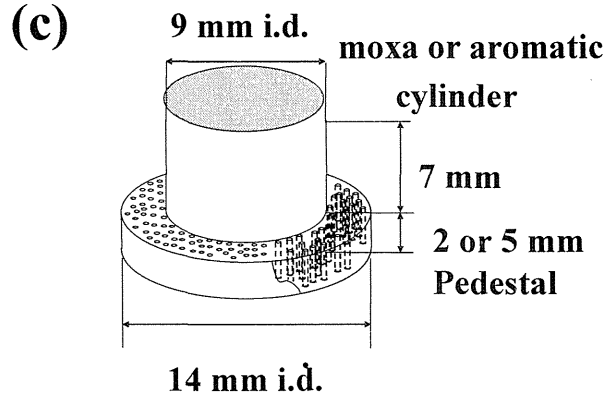


Fig. 1 Schematic representation of moxa samples used in direct moxibustion (a) and indirect moxibustion (b and c)

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.

As clearly understood, the thermal energy passages is directly reached to skin by moxibustion as like in the Passport system. PassPort System is a new investigational device designed to deliver drugs through the skin. The PassPort System creates a series of minute pores (micropores) through the outer layer of the skin (SC) by the instrantaneous and non-traumatic application of a pulse of thermal. The PassPort System is comprised of a single-use disposable PassPort patch (Fig. 2a) and a re-useable handheld Applicator (Fig. 3). The former consists of a conventional transdermal patch attached to an array of metallic

filaments (porator) (Fig. 2b). Pressing the activation button of the Applicator release a single pulse of electrical energy to the porator, where it is converted into thermal energy. The rapid conduction of this thermal energy into the surface of the skin painlessly ablates the SC under each filament to create microchannels by vaporizing microscopic amounts of dead cells from the SC, when the Applicator is removed a simple fold-over design aligns the transdermal patch with the newly formed microchannels. A patch containing medicine is then placed over the microchannels, which are deep enough to allow the entry of the drugs into the skin, but shallow enough that the pain receptors in the dermis, the layer of skin below the stratum corneum, are unaffected. So the whole method is painless. This system is somewhat resemble to the moxibustion.

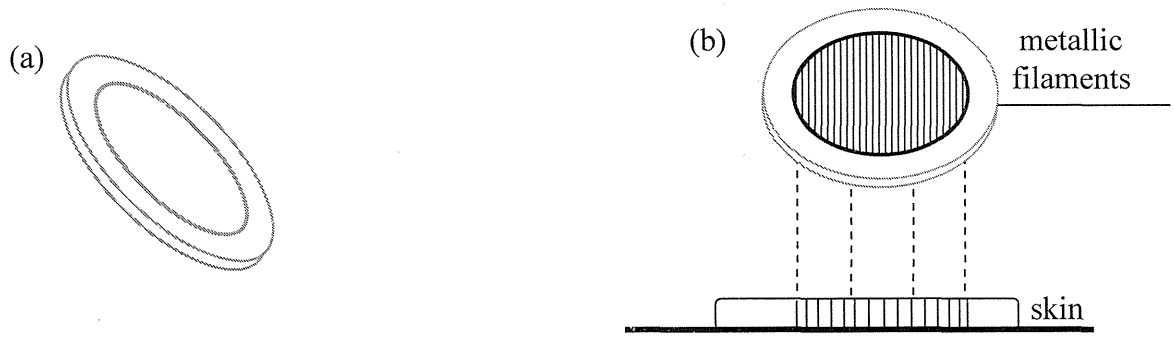


Fig. 2 The PassPort patch (a) and the array of filaments (b) on the PassPort™ system

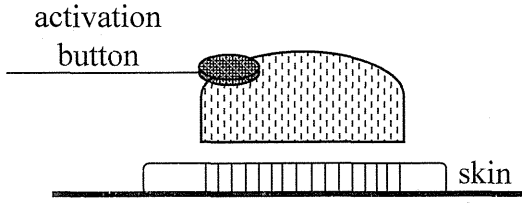


Fig. 3 Handheld Applicator on the PassPort™ system

Moxibustion may be useful to increase the skin permeation of drugs, as well as the chemical and physical effects explained as 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, FD-4, with a mean molecular weight of 4 kDa 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 slice were evaluated. Aromatic incense was also used for comparison with moxa.

1.2. Materials and methods

1.2.1. 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.

1.2.2. Chemicals

FD-4 was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, U.S.A.). 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 firep-roof 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, U.S.A.) to determine the amount of protein leached from the skin surface.

1.2.3. 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. 4.

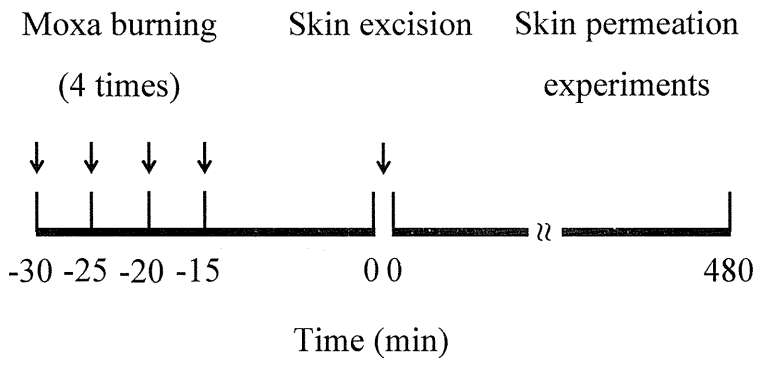


Fig. 4 Experimental schedule of moxa pretreatment and subsequent skin permeation and protein leaching experiments

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 hours in the present study.

Table 1 Experimental groups in the present study

#	Burning material	Cylinder size (diameter × height, mm)	Pedestal			Figure (symbol)
			material	height (mm)	type	
#0	no material					Fig. 3-6 (▲)
#1,2	moxa	5 × 13	paper	1.0 mm	direct	Fig. 3 (○,●)
#3	moxa	5 × 13	paper	2.0 mm	direct	Fig. 3 (◊)
#4	moxa	5 × 13	paper	2.0 mm	indirect	Fig. 4 (●)
#5	moxa	9 × 7	paper	2.0 mm	indirect	Fig. 4 (○)
#6	aroma	9 × 7	paper	5.0 mm	indirect	Fig. 4 (◊)
#7	moxa	5 × 13	ginger	2.0 mm	direct	Fig. 5 (◊)
#8	moxa	5 × 13	ginger	2.0 mm	indirect	Fig. 5 (○)
#9	moxa	9 × 7	ginger	2.0 mm	indirect	Fig. 6 (●)
#10	moxa	9 × 7	potato	2.0 mm	indirect	Fig. 6 (○)
#11	aroma	9 × 7	ginger	5.0 mm	indirect	Fig. 6 (♦)
#12	aroma	9 × 7	potato	5.0 mm	indirect	Fig. 6 (◊)

"Aroma" means aromatic incense.

1.2.4. 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 hours.

1.2.5. 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.

1.3. Results

1.3.1. 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 contained 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.

Figure 5a 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 (un-pretreatment, #0). When the 1.0 mm pedestal was used, on the other hand, a marked enhancement effect was observed (#1, 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. 5a.

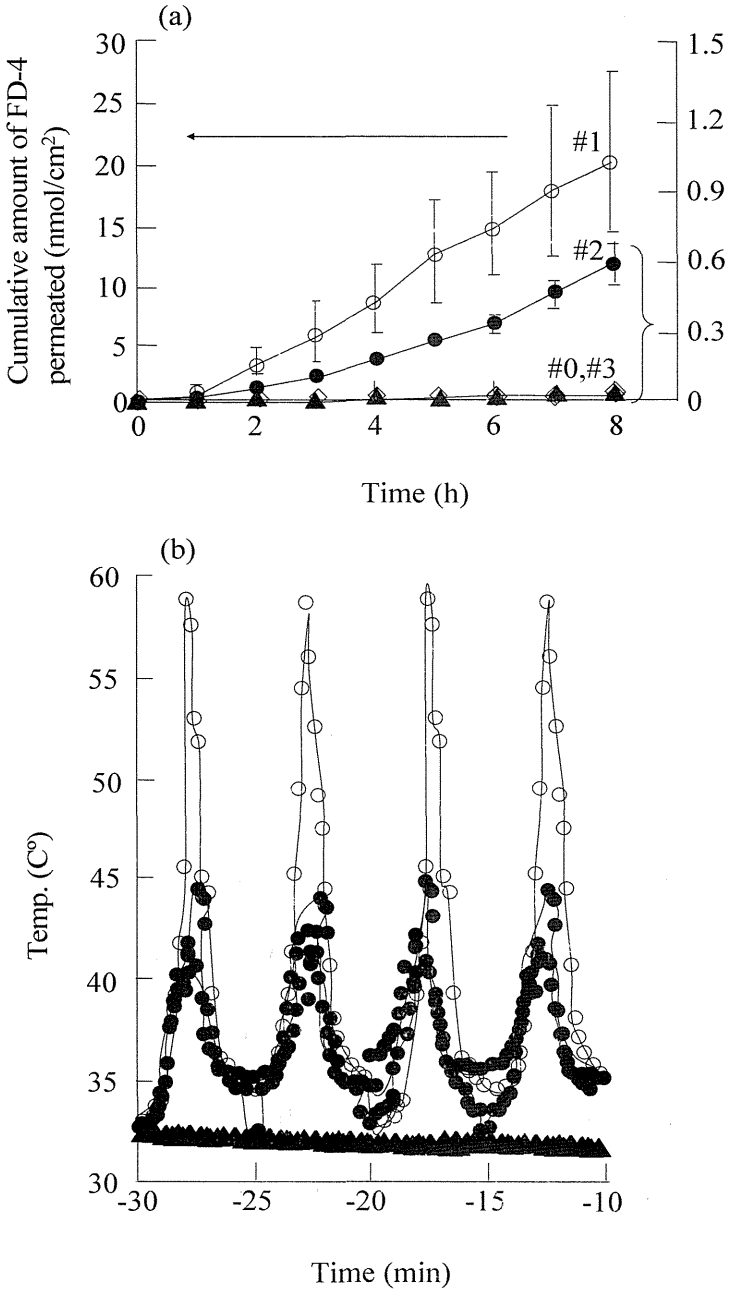


Fig. 5 Effect of pedestal thickness on the time courses of the amount of FD-4 that permeated through excised hairless rat skin (a) and skin temperature (b) following and during direct moxibustion treatment, respectively, to hairless rat abdominal skin

Symbols: ○ and ●: 1.0 mm pedestal (○: skin blister found, and ●: no blister found.), #1, 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.

The corresponding time course of the skin temperature of hairless rats during 4 moxibustion treatments is shown in Fig. 5b. 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. 4). 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 5b; 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. 5), whereas it was about 45°C without blistering (#2, Fig. 5). 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.

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

#	Skin permeation of FD4			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 ($\mu g/cm^2$)	
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. ** Significant difference ($p < 0.01$)

AUC_{temp} and T_{max} are the values for the first moxibustion.

1.3.2. 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 mm 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. 5 (5 mm diameter, 13 mm height), although the amount of moxa (0.10 g) was the same. Indirect moxibustion using a 5 x 13 mm cylinder was also evaluated for comparison.

Figure 6a 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 pretreatment (#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. 5) with indirect moxibustion (#4, Fig. 6), indirect moxibustion showed higher skin permeation as well as higher skin temperature.

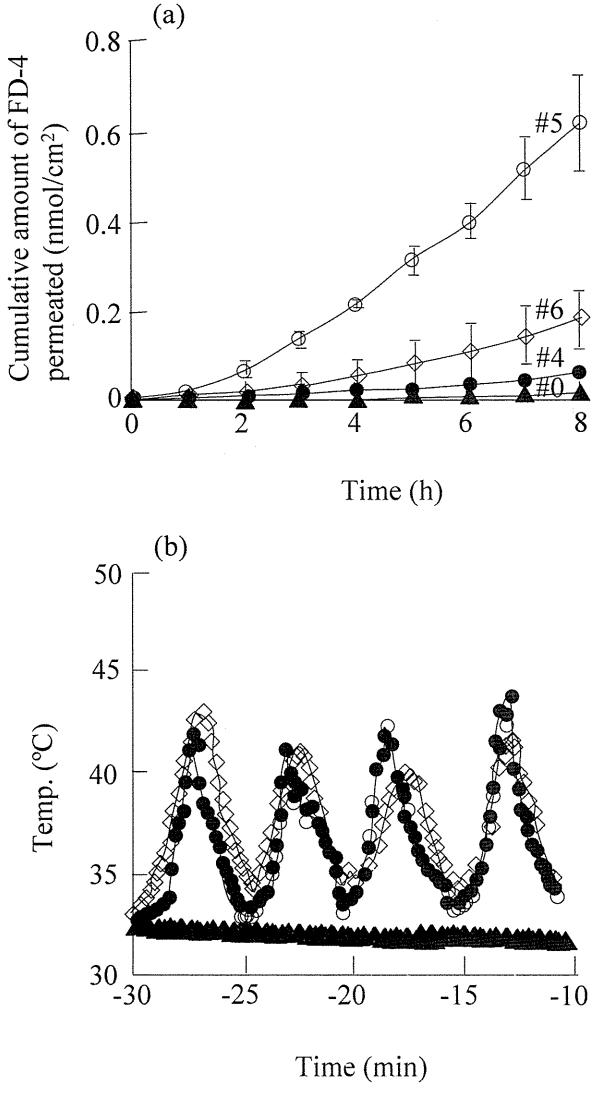


Fig. 6 Effect of indirect moxibustion or aromatic incense burning with different shapes of burning cylinder on the time course of the amount of FD-4 that permeated through the excised hairless rat skin (a) and skin temperature (b) following and during indirect burning, respectively, to hairless rat abdominal skin

Symbols: ●: moxa (5 × 13 mm, #4), ○: moxa (9 × 7 mm, #5), ◊: aromatic incense (9 × 7 mm, #6), and ▲: control (as in Fig. 3, #0)

Each data point shows the mean ± S.D. of 4 – 6 determinations.

1.3.3. 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. Figure 7a 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. 6) under the same conditions except for the pedestal material.

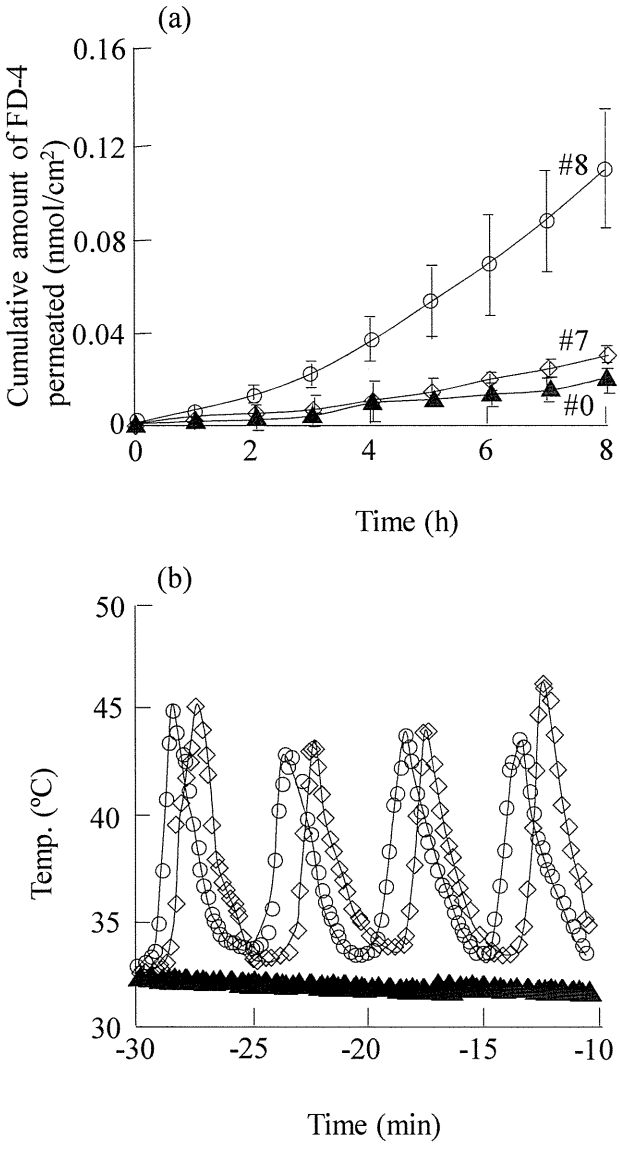


Fig. 7 Effect of ginger pedestal on the time course of the amount of FD-4 that permeated through the excised hairless rat skin (a) and skin temperature (b) following and during direct or indirect moxibustion treatment, respectively, with a 2 mm pedestal

Symbols: ◊: direct moxibustion treatment with moxa cylinder with a 2 mm ginger pedestal (#7), ○: indirect moxibustion treatment with a moxa cylinder having a 2 mm ginger pedestal (#8), and ▲: control (as in Figs. 3, 4 and 5, #0)

Each data point shows the mean ± S.D. of 4 – 6 determinations.

Figure 8 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. 6). 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.

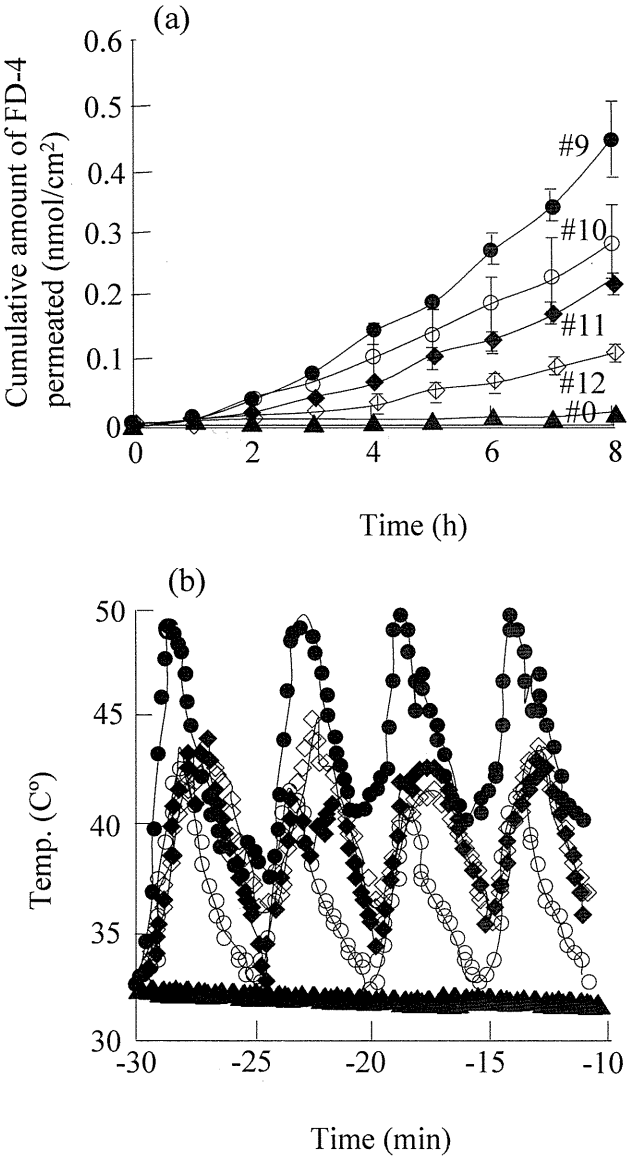


Fig. 8 Comparison of the effect of the ginger pedestal with a potato pedestal on the time course of the amount of FD-4 that permeated through the excised hairless rat skin (a) and skin temperature (b) following and during indirect moxibustion or aromatic incense burning, respectively.

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, 4, 5, #0))

Each data point shows the mean ± S.D. of 4 -- 6 determinations.

1.3.4. 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. 9.

When the T_{max} was over 42°C, the Q_8 and flux of FD-4 increased with an increase in T_{max} (Fig. 9a and c). In contrast, no clear relationships were found between these skin permeation parameters and AUC_{temp} (Fig. 9b 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. 9e 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.

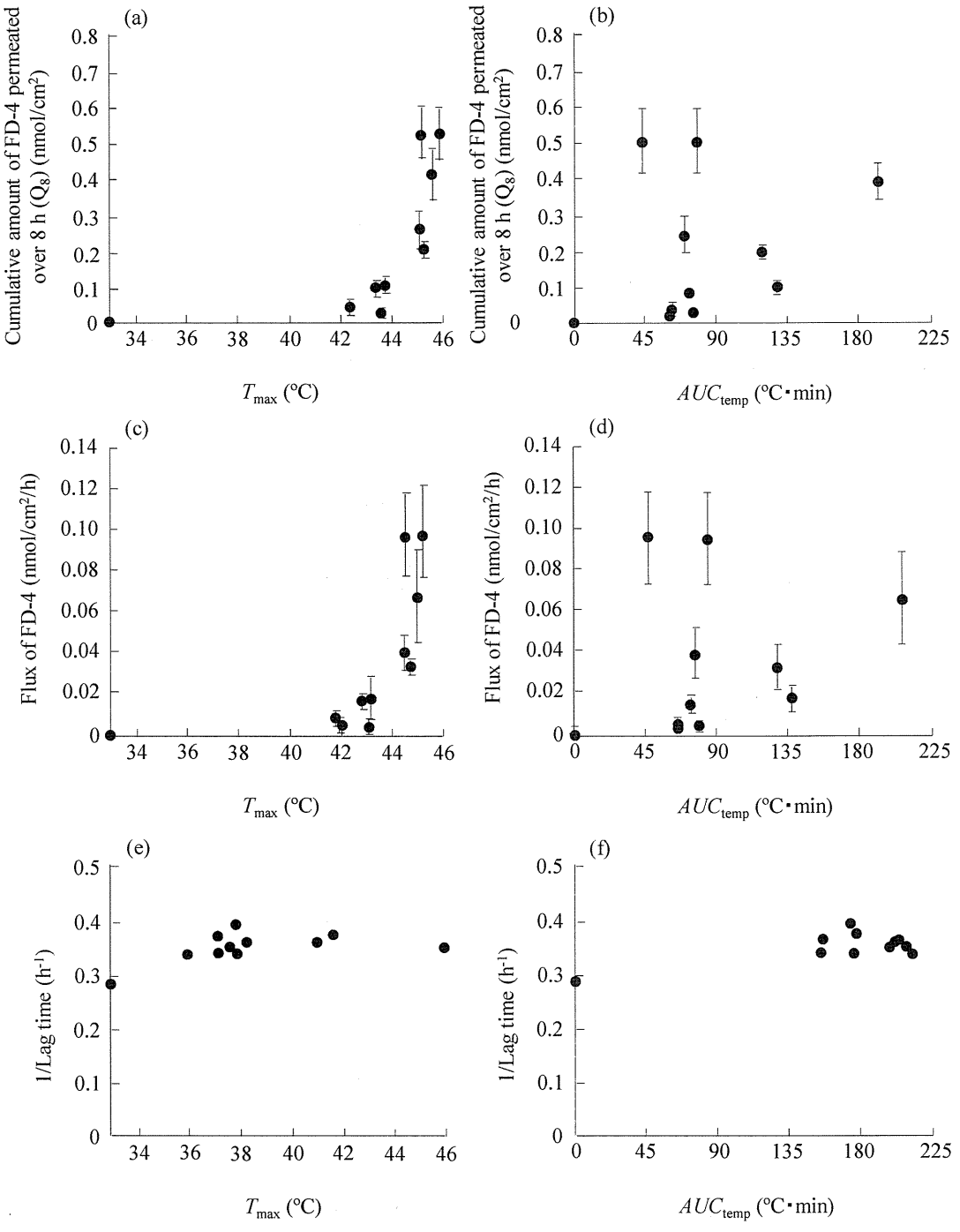


Fig. 9 Relationship between the permeation parameters of FD-4 through the excised hairless rat skin (cumulative amount permeated 8 h in a and b; flux in c and d; and 1/lag time in e and f) and the skin temperature (T_{max} in a, c and e; AUC_{temp} in b, d and f) following direct or indirect treatment 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.

1.3.5. 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. 4. The obtained results for protein leaching are shown in Table 2. Marked differences in the cumulative amount of protein leached over 8 hours were found between control and treatment groups.

Figure 10a and b show the relations between the cumulative amount of protein leached over 8 hours and T_{max} or AUC_{temp}, respectively. A higher cumulative amount of protein leached 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.

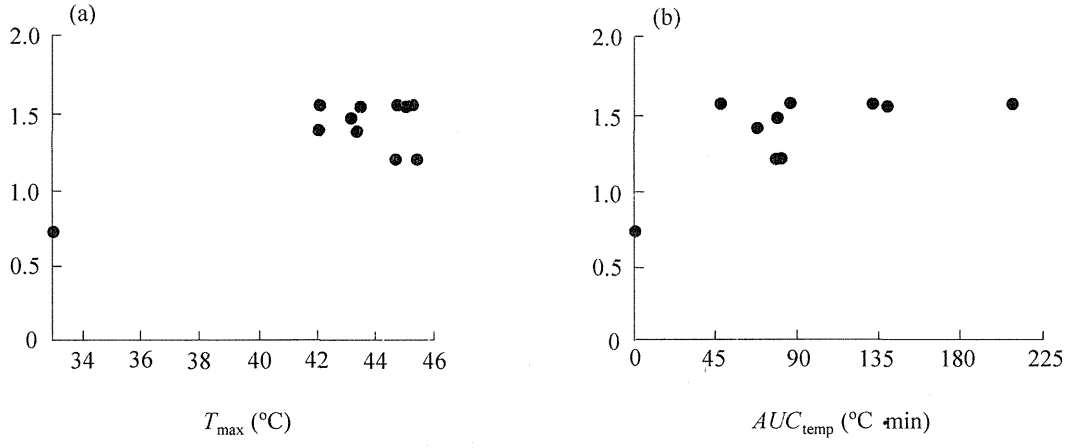
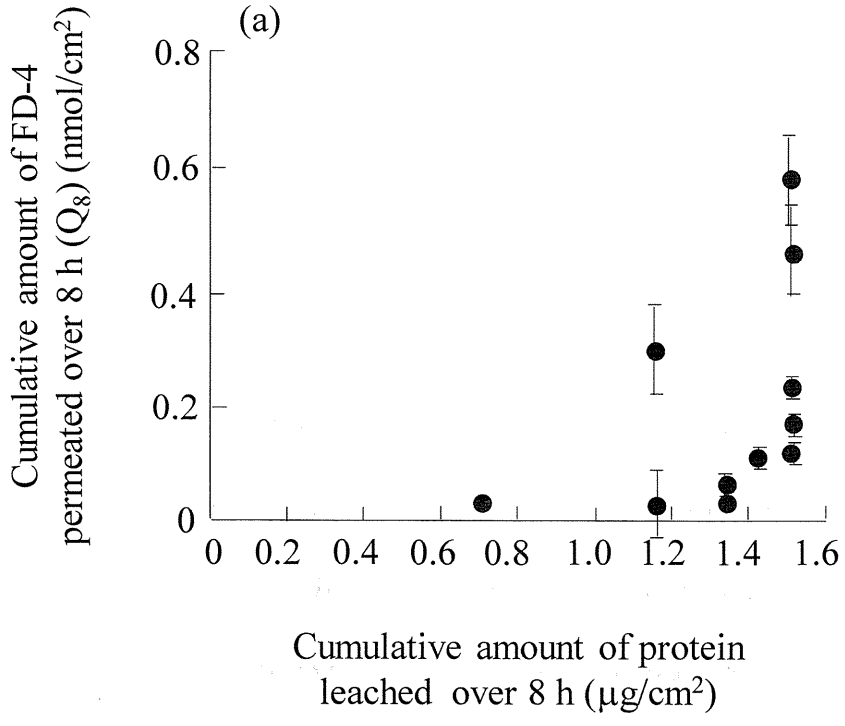


Fig. 10 Relationship between the cumulative amount of protein leached from excised hairless rat skin over 8 h and the temperature profiles (T_{max} is left figure and AUC_{temp} is right figure, respectively) following direct or indirect treatment with moxa or aromatic incense with ginger, paper or potato pedestal.

Each data point shows the mean of 4 – 6 groups.

1.3.6. Relationship between skin permeation and protein leaching

Figure 11a, b and 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 hours. No good relationship was found between the Q_8 or flux and protein leaching (Fig. 11a and b), however, 1/Lag time for each treatment group was increased by an increase in protein leaching (Fig. 11c). 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.



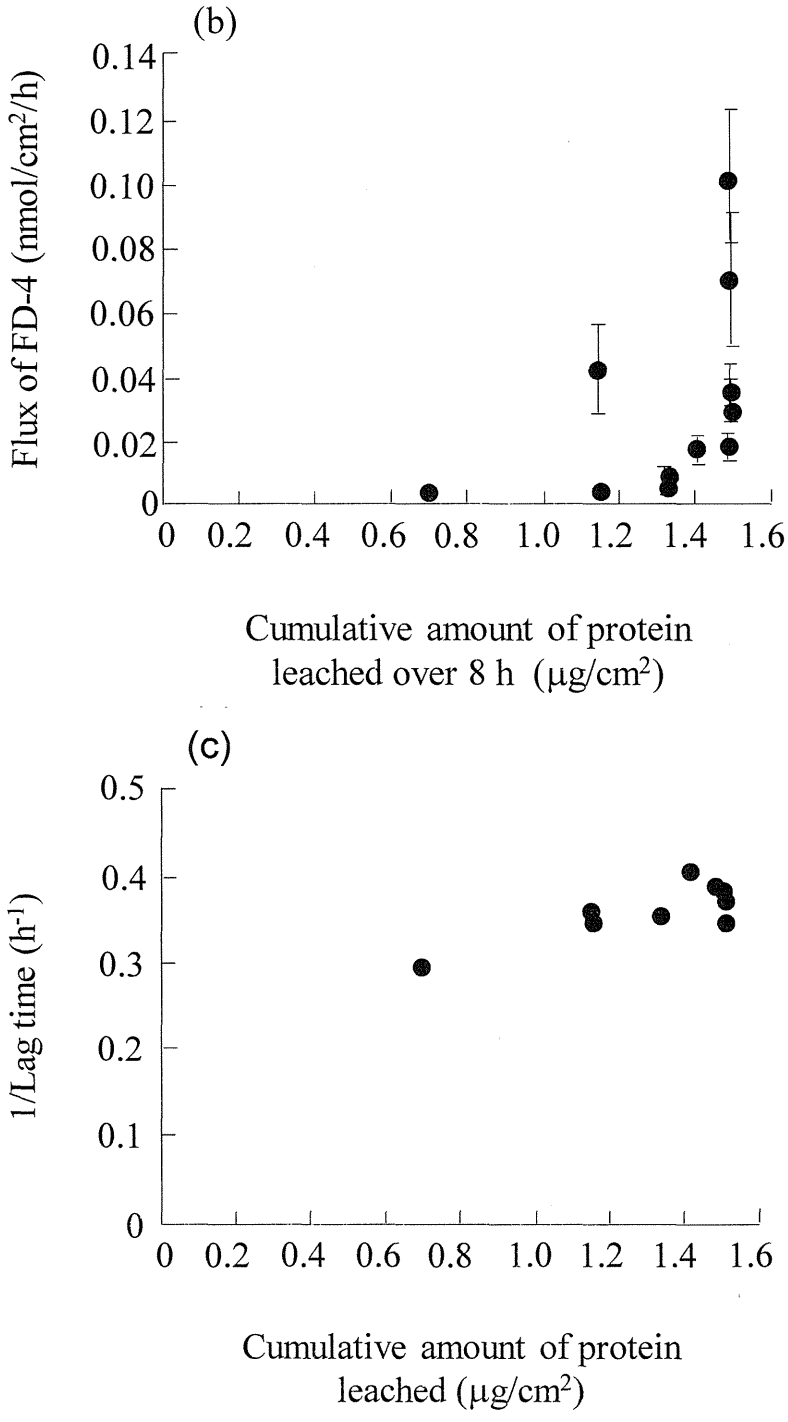


Fig. 11 Relationship between the permeation parameters of FD-4 through excised hairless rat skin (cumulative amount permeated 8 h in a; flux in b; 1/lag time in c) and the cumulative amount of protein leached over 8 h following direct or indirect treatment with moxa or aromatic incense with ginger, paper or potato pedestal.

Each data point shows the mean or mean ± S.D. of 4 – 6 groups.

1.4. 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. 5a). The efficacy for heating the skin was higher when using a thinner pedestal (Fig. 5b). 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. 8). 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 *et al.*, 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-enhancemnet data were explained by the increase in T_{max} (Fig. 9a 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 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.

1.5. Chapter 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 method 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, the moxibustion treatment is a unique and useful technique to increase or improve the skin permeation of malabsorptive drugs.

Chapter 2

Pretreatment Effects of Moxibustion on the Skin Permeation and Skin and Muscle Concentration of Salicylate

1.1. Introduction9y

Moxibustion therapy may affect to the blood flow in the cutaneous and or muscular tissues as well as the stratum corneum barrier, where the latter has already been explained in the Chapter 1. In this chapter (Chapter 2), rats were treated with moxibustion, sodium salicylate (SA-Na) solution (Fig. 10) was applied on the pretreated skin, and skin permeation of the drug was measured *in vitro* and *in vivo* to evaluate the moxibustion effect on the distribution of the drug in the skin and muscle underneath the application site of moxibustion.

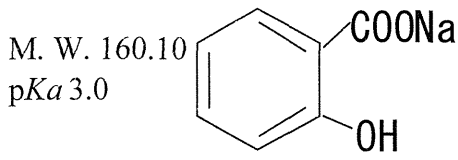


Fig. 12 Chemical structure of sodium salicylate (SA-Na)

Moxibustion may have an effect on the local vasodilation response, because the skin temperature is increased by moxibustion. When the body is in warm environment, the vein and capillary were more dilated than that in cold environment, and the blood flow was higher

in warm than in cold environment. Drug permeability through the endothelial membrane of blood vessel and capillary become higher in warm than in cold environment (Fig. 13a). In addition, if inflammation is observed in the tissues, an EPR (enhanced permeation retention) (Fig. 13b) effect may be obtained. In other words, tight junction in the endothelial membrane of blood vessels and capillary is enlarged in the inflamed condition. Then, moxibustion may increase the skin and muscle concentration of topically applied drug.

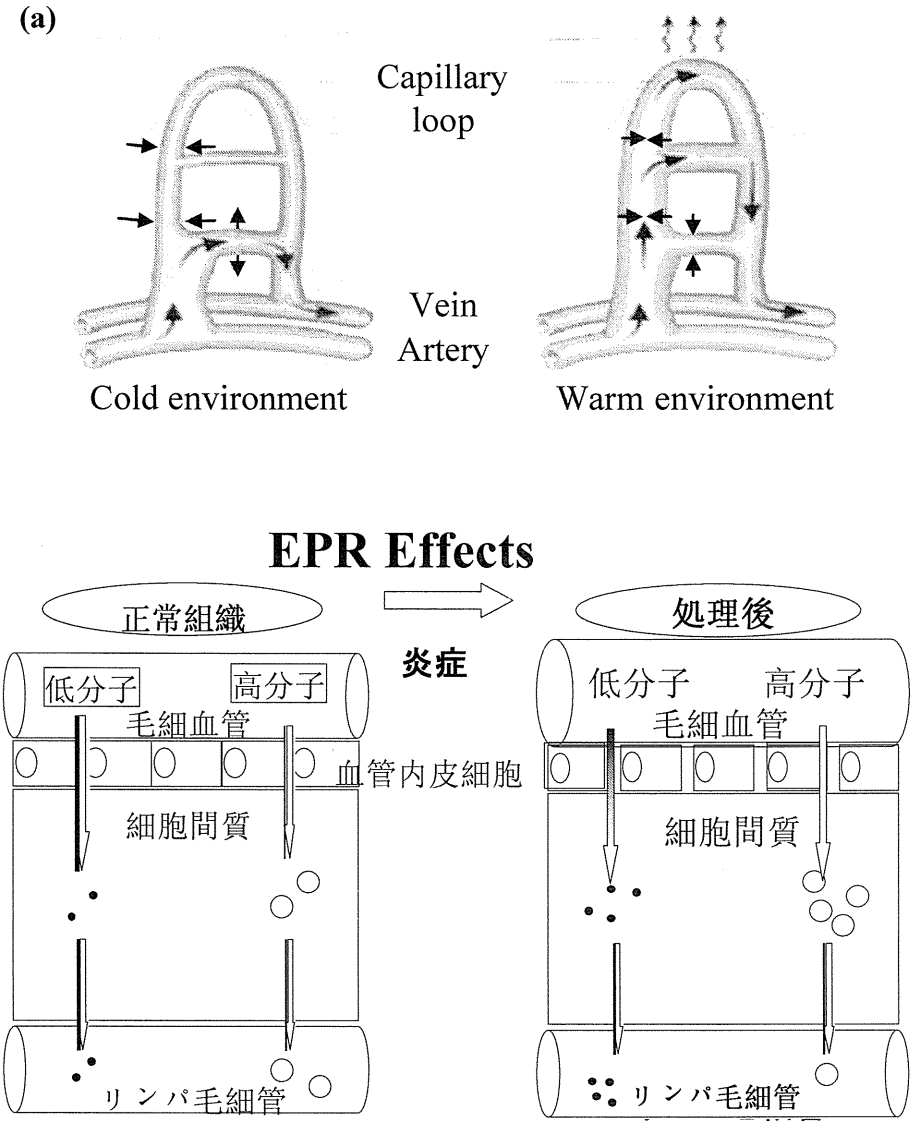


Fig. 13 Schematic representation of effects of moxibustion on vein and EPR

2.2. Materials and methods

2.2.1. Materials and animals

SA-Na and Evans blue were purchased form Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other chemicals and solvents were of reagent grade and were used without further purification.

SennenQ-off regular Q Ibuki was supplied by Senefa Co. (Nagahama, Shiga, Japan). This moxibustion device has a moxibustion cylinder (5 mm diameter × 9 mm length) and a pedestal. In the present experiment, only this cylinder was used. Pedestal was made in the experiment having a diameter of 25 mm and a thickness of 1 – 3 mm with a hole having a diameter of 4 mm, as shown in Fig. 14 (direct moxibustion).

Healthy male hairless rats (WBN/ILA-Ht, 180-230 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.

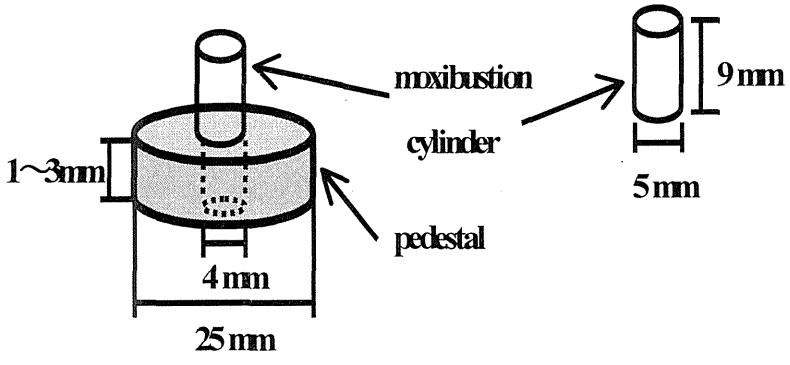


Fig. 14 View showing a frame format of moxibustion

2.2.2. Measurement of skin temperature

Skin temperature was determined as the same in Chapter 1.

2.2.3. In vitro experiment

The abdominal skin of hairless rat (3 mm above the umbilicus in the midline) was treated 1 time or 3 times (The place was very closed, but not the same place. See in Fig. 12) consecutively for 5.0 min with moxa 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 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, as the same in Chapter 1. The skin permeation experiment of SA was started 30 min after starting the first moxa burning.

The stratum corneum side of the skin faced SA-Na filled compartment, while the dermis side faced the sampling compartment. The donor compartment was filled with 2.5 ml of 3% SA-Na 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.

2.2.4. In vivo experiment

2.2.4.1. Intravenous injection

The abdominal skin of hairless rat (3 mm above the umbilicus in the midline) was treated 3 times consecutively for 5.0 min with moxibustion by fixing the animals on their back under

anesthesia by *i.p.* injection of urethane (1.0 g/kg). Figure 15 shows the illustration of a rat in the experiment. During the *in vivo* experiment, rats were kept on the water bed kept at 37°C. SA-Na (5 mg/kg) was intravenously injected through the left jugular vein 30 min after starting the first moxibustion. Blood sampling was done periodically from the right jugular vein. The obtained blood sample was centrifuged at 4 °C to get plasma. At the end of experiment (8 h after injection of sodium salicylate), the skin and muscle having the 2.5 cm diameter under the moxibustion site. The tissue and samples were kept in a freezer until analysis. The control experiment was done without moxibustion.

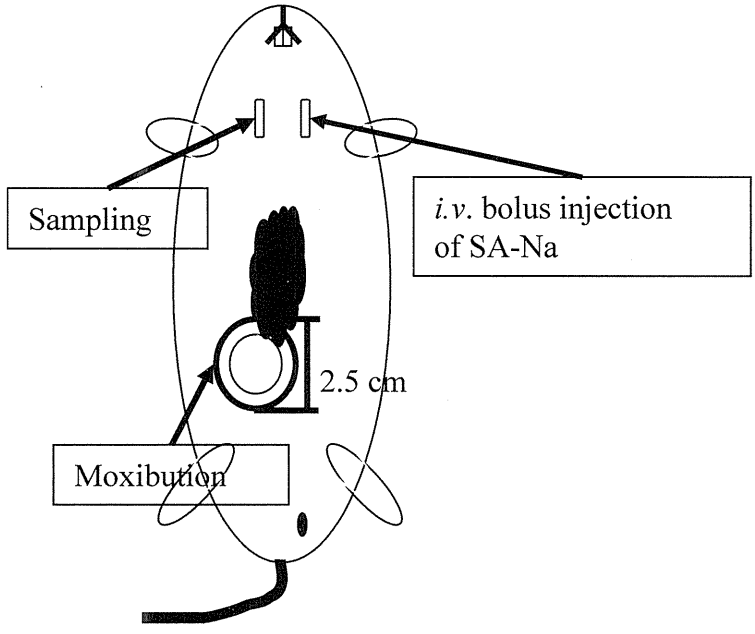


Fig. 15 Method for *in vivo* injection study

2.2.4.2. Skin application

A Glass diffusion cell having an effective diffusion area of 0.95 cm^2 was applied on the skin surface where the same moxibustion treatment was carried out. SA-Na solution at a

concentration of 20% (1.5 mL) was applied 30 min after starting the first moxibustion. Plasma, skin and muscle were obtained as the same as in the experiment for the intravenous injection.

2.2.4.3. Tissue distribution experiment

The right abdominal skin of hairless rat was treated 3 times consecutively for 5.0 min with moxa by fixing the animals on their back under anesthesia by *i.p.* injection of urethane (1.0 g/kg). Then, Evans blue (50 mg/kg) was intravenously injected, followed by taking picture on the moxibustion-application site as well as the control left abdomen.

2.2.5. Determination methods

In vitro experiment:

The sampling buffer solution (100 μL) was added to acetonitrile (200 μL), and centrifuged to obtain supernatant. The supernatant (20 μL) was injected to an HPLC.

In vivo experiment:

2.2.5.1. Plasma: The obtained plasma (100 μL) was added to acetonitrile (200 μL), and centrifuged to obtain supernatant. The supernatant (20 μL) was injected to an HPLC.

2.2.5.2. Skin and muscle sample:

The minced skin and muscle sample was added to acetonitrile (5.0 mL) containing a standard compound and physiological saline (4.0 mL), and homogenized by a Polytron (PT3000, Kinematic AG、Switzerland) under the iced solution. The homogenate was

centrifuged at 4°C, and the aliquot (20 μL) was injected into an HPLC.

HPLC system used and assay conditions are shown in Tables 3 and 4.

Table 3 HPLC system

Pump	LC-10A (Shimadzu, Kyoto, Japan)
Auto-injector	SIL-10A$_{XL}$ (Shimadzu, Kyoto, Japan)
System Controller	SLC-10A (Shimadzu, Kyoto, Japan)
UV detector	SPD-10A (Shimadzu, Kyoto, Japan)
Recorder	C-R3A (Shimadzu, Kyoto, Japan)

Table 4 HPLC condition for the analysis of salicylate (SA)

Mobile phase	methanol : 0.1% phosphate water = 55 : 45 (v/v)
Flow rate	0.7 ml /min
Column	LiChroCART 250 × 4 mm (MERCK KgaA, Darmstadt, Germany)
Column temperature	40°C
UV Wavelength	225 nm
Internal standard	propyl- *p*-hydroxybenzoate

2.3. Results and Discussion

2.3.1. Skin temperature

Figure 16 shows the time course of temperature at the skin surface and subcutaneous tissue after moxibustion. Smoke was disappeared within 1.5 min, but the maximum temperatures were obtained 4 min after starting the moxibustion. The maximum skin surface temperature and subcutaneous tissue temperature were 43.7, 45.6 and 47.9°C and 42.4, 44.7 and 46.5 °C, respectively, for 3, 2 and 1 mm-pedestal. Decreasing the distance from the skin surface and moxa increased the skin temperature, as explained in Chapter 1. These temperatures were returned to the control value (without or before moxibustion) within 10 to 15 min.

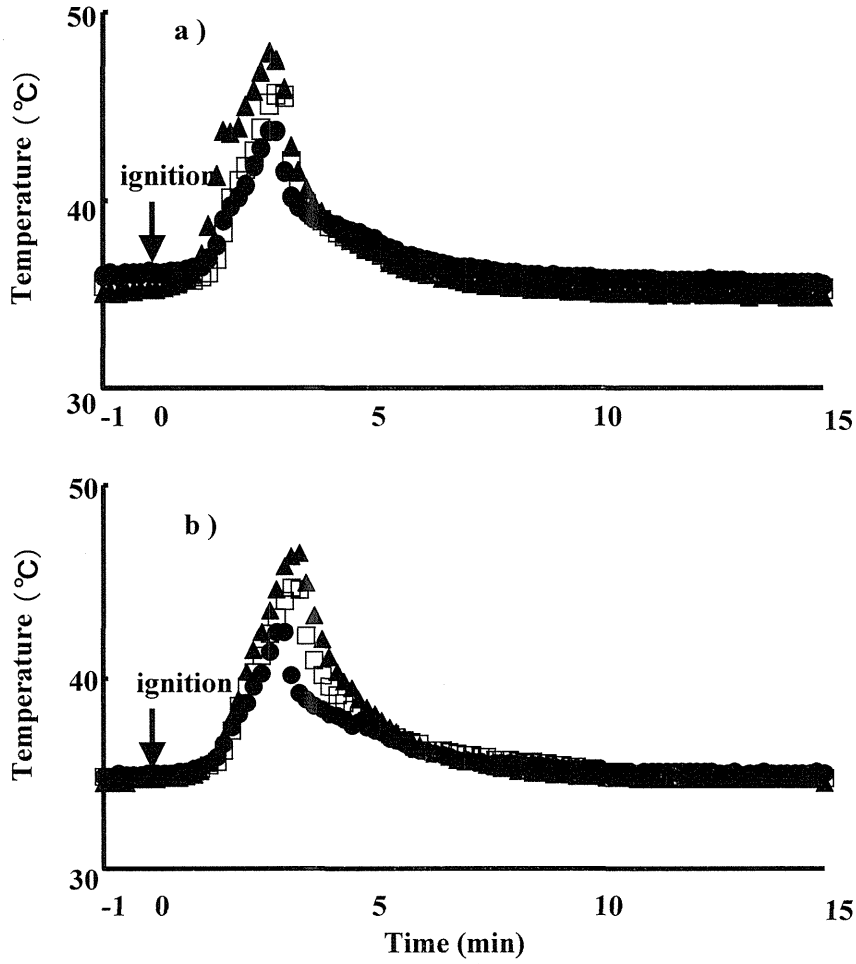


Fig. 16 Effect of moxibustion treatment on the temperature change on the skin surface (a) and in the subcutis (b).

●: 3 mm thickness pedestal, □: 2 mm thickness pedestal, ▲: 1 mm thickness pedestal.

Each data point represents the mean value.

2.3.2. In vitro skin permeation

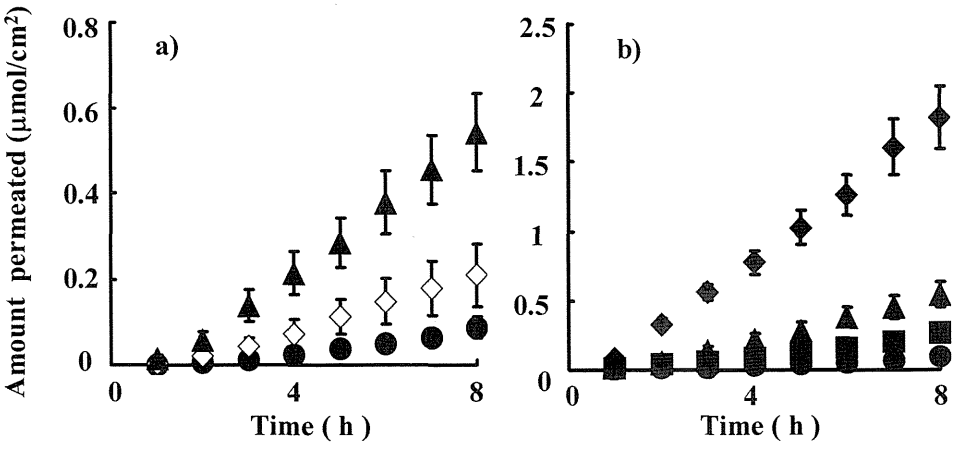


Fig. 17 Effect of number of moxibustion cylinder (a) and the distance between the cylinder tip and skin surface (b) on the in vitro skin permeation of salicylate

n = 5 – 7. ●: Control, ◇: Moxa 1 with 2 mm, ■: Moxa 3 with 3 mm, ▲: Moxa 3 with 2 mm, ◆: Moxa 3 with 1 mm.

Each data point represents the mean ± S.E., n = 5 – 7.

Figure 17 shows the effect of moxibustion pretreatment on the time course of the cumulative amount of SA that permeated through hairless rat skin. Table 5 summarizes the permeability coefficient of SA under each moxibustion treatment. The cumulative amount of SA at 8 h after permeation experiment was 2.4-, 3.1-, 6.2- and 21-fold higher than the control experiment (without moxibustion) for one moxibustion with 2 mm-pedestal and three moxa cylinder with 3, 2 and 1 mm-pedestal, respectively. Increasing the number of moxa and decreasing the pedestal thickness increased the skin permeation of SA, which is the same finding as explained in Chapter 1.

Table 5 Permeability of SA through excised hairless rat skin

Treatment	Permeability coefficient (cm/s)
Control	$(2.33\pm0.007)\times10^{-8}$
Moxa 1 with 2 mm	$(4.53\pm1.47)\times10^{-8}$
Moxa 3 with 3 mm	$(7.56\pm0.008)\times10^{-8}$
Moxa 3 with 2 mm	$(1.25\pm0.002)\times10^{-7}$
Moxa 3 with 1 mm	$(3.71\pm0.005)\times10^{-7}$

Each value represents the mean ± S.E., n = 5-7.

2.3.3. Intravenous injection

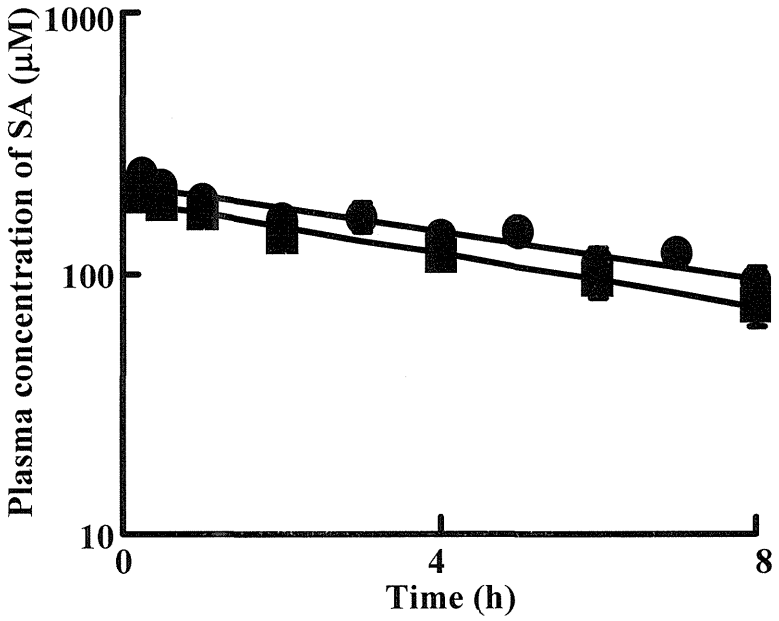


Fig. 18 Effect of moxibustion treatment on the in vivo plasma concentration of SA following *i.v.* injection

●: Control, ■: Moxa 3 with 1 mm.

Each point represents the mean ± S.E., n = 3 – 4.

First, SA-Na (5 mg/kg) was intravenously injected into rats, in order to evaluate the elimination kinetics and skin and muscle disposition of topically applied SA. Time course of plasma concentration and obtained pharmacokinetic parameters are shown in Fig.18 and Table 6, respectively. No significant difference was observed with and without moxibustion in the elimination pharmacokinetics of SA, suggesting that moxibustion did not affect to the elimination kinetics from the systemic circulation.

Table 6 Elimination PK parameters of SA

	$k_e l$	V_d **(ml)**
Control	**(1.83 ± 0.18) × 10-3**	**31.49 ± 1.98**
Moxibustion	**2.26 ± 0.17) × 10-3**	**29.7 ± 2.06**

Each value represents the mean ± S.E., n = 3.

Table 7 and Figure 19 show the amount of SA in the skin and muscle and the amount ratio of the skin and muscle/plasma amount (s/p and m/p ratio) of SA, respectively, where the plasma amount was calculated from the plasma concentration and distribution volume of SA. The s/p and m/p ratio with moxibustion was about 4 and 2, respectively, compared with those without moxibustion. These results suggest that moxibustion increased the skin and muscle concentration under the site of moxibustion as well as the skin permeation of the drug.

Table 7 SA amount in the skin and muscle after *i.v.* injection at 8 h

	Skin (nmol)	Muscle (nmol)
Control	5.42 ± 0.84	8.68 ± 2.08
Moxibustion	15.7 ± 5.45*	17.1 ± 1.71*

Each value represents the mean ± S.E., n = 3.

*$p < 0.05$ Mann-Whitney – U- test, compared with control

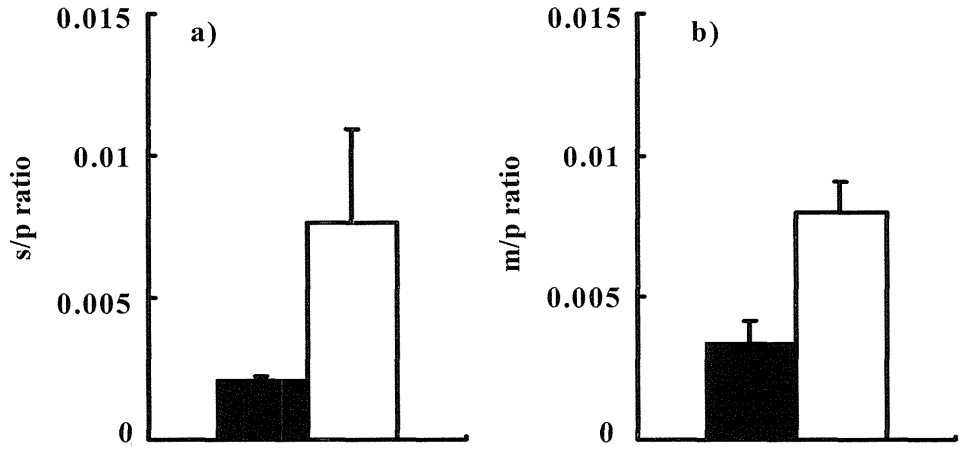


Fig. 19 Effects of moxibustion treatment on the amount ratio of SA-Na in the skin (a) and muscle (b) against plasma amount 8 h after *i.v.* injection

■: Control, □: Moxa 3 cylinder with 1 mm.

Each column represents the mean ± S.E., n = 3.

2.3.4. In vivo skin application

Figure 20 shows the time course of plasma concentration of SA after topical application. The skin permeation of the drug with moxibustion was about 5 times compared to that without moxibustion. The effect in the *in vivo* experiment, however, was lower than that the effect on the *in vitro* skin permeation experiment.

Table 8 and Figure 21 show the effect of moxibustion on the s/p and m/p ratio of SA after topical application. The s/p and m/p ratio with moxibustion were about 3 and 15, respectively. Especially, the high m/p ratio was observed by moxibustion. Since the plasma concentration itself was increased by moxibustion, the skin and muscle concentration were calculated to be 15 and 70 times compared to those without moxibustion.

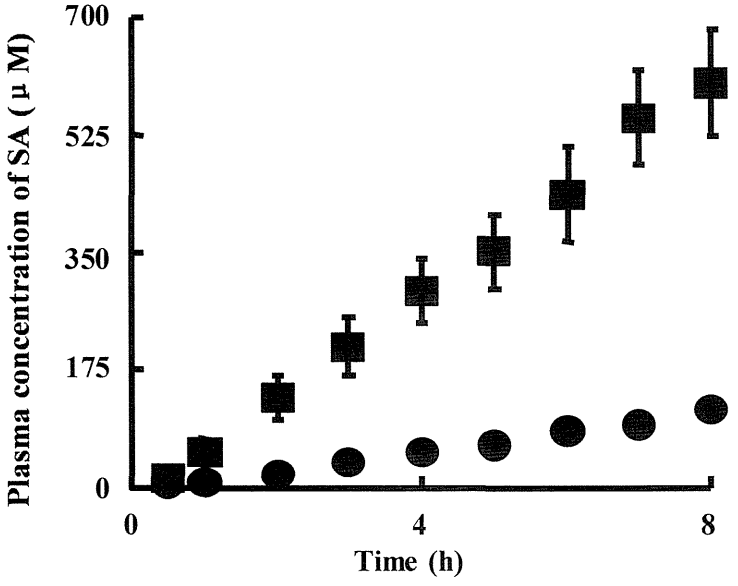


Fig. 20 Effect of moxibustion treatment on in vivo plasma concentration of SA following topical application

●: Control, ■: moxa 3 with 1 mm.

Each point represents the mean ± S.E., n = 3.

Table 8 SA amount in the skin and the muscle after transdermal application at 8 h

	Skin (μmol)	Muscle (μmol)
Control	1.61 ± 0.44	0.064 ± 0.014*
Moxibustion	21.1 ± 1.84*	4.89 ± 0.59*

Each value represents the mean ± S.E., n = 3.

*$p < 0.05$ Mann – Whitney – U- test, compared with control

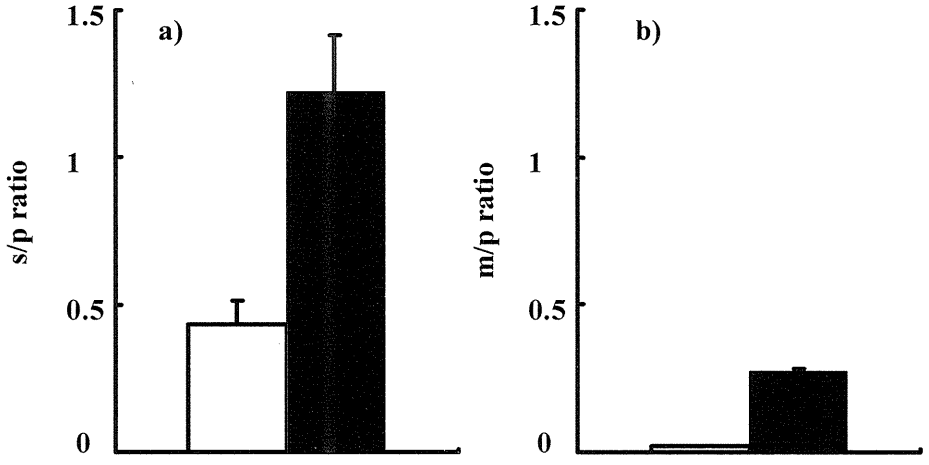


Fig. 21 Effects of moxibustion treatment on the ratio of SA amount in the skin (a) and muscle (b) against plasma amount 8 h after topical application

□: Control, ■: moxa 3 with 1 mm.

Each column represents the mean ± S.E., n = 3.

2.3.5. Tissue distribution

Figure 22 shows the effect of moxibustion treatment on the tissue distribution of Evans blue 25 min after intravenous injection. The skin color clearly became blue at the site of moxibustion due to Evans blue, suggesting that Evans blue easily distributed especially into the site of moxibustion after intravenous injection. By the way, the yellow color in Fig. 20 was due to resin from moxa.

A component in moxa, such as 1.8-cineole, may be related to the penetration enhancement effect by moxibustion. Increase in skin and muscle concentration of salicylate in the site of moxibustion was observed, however, even after intravenous injection. These phenomena are related to the effect of moxibustion on the epithelial membranes in the viable epidermis/dermis and muscle. Igarashi *et al.* reported that intravenously injected lipid microspheres containing an anti-rheumatoid drug are easily leaked from the epithelial membranes in the inflammatory focus, and these phenomena are closely related to the high targeting ability of the anti-rheumatoid drug. The present results, leakage of Evans blue from the cutaneous and muscle blood capillaries, must be the same kind of phenomena as the results by Igarashi *et al.* In addition, increase in the drug leakage from the cutaneous and muscle vascular must be one of the primary reasons for the increase in the skin and muscle concentration by moxibustion.

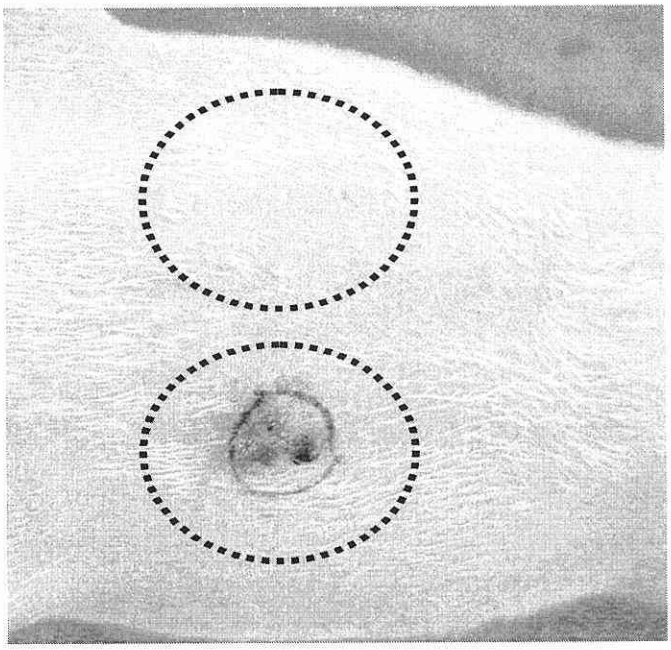


Fig. 22 Effect of moxibustion treatment on the tissue distribution of EB 25 min following *i.v.* injection

Photograph of hairless rat abdomen after intravenous injection of Evans blue.

2.4. Chapter Conclusion

In vivo skin permeation was enhanced by the moxibustion pretreatment as well as the *in vitro* skin permeation. However, the *in vitro* effect by moxibustion was greater than that by *in vivo*. Moxibustion pretreatment increased the skin permeation of drugs. The pretreatment also increased the skin and muscle concentration of drugs.

In conclusion, moxibustion can be utilized as a method to increase the skin and muscle concentration as well as the skin permeation of topically applied drugs.

Conclusion

The present work is a feasibility study to increase the skin permeation and distribution of drugs by moxibustion pretreatment. The obtained data after moxibustion pretreatment based on the safety condition suggests that moxibustion treatment is a unique and useful technique to increase the skin permeation and skin concentration of topically applied drug.

Acknowledgements

I am deeply indebted to my advisor, Professor Kenji Sugibayashi, not only for his mentorship but also for allowing me to explore different ideas and incorporate different techniques into my work other than those of his primary specialty. I would like to express my gratitude to my doctoral advisory committee of Professors Kazuhiko Juni and Yoshiaki Shirataki as well as Professor Sugibayashi. My special thanks go out to Dr. Hiroaki Todo and Mr. Hiroshi Ishii for providing me with ample pieces of advice on how to succeed as an independent scientist. I cannot thank enough all of my colleagues who gave the support and collaboration necessary to make through this onerous process: Mr. Tomoyuki Mitoma, Mr. Hirotaka Yasuno, Mr. Koichi Honda, Mr. Murayama Kazuhiro, Ms. Satoko Sugasawa, Ms. Kaori Yamaguchi. I also like to thank to Dr. Toshihiko Kitamura for his indispensable assistance with the various diffusion experiments. I thank to Ms. Y. Tazawa and Ms. N. Hayashi is thanked for their hosting technical assistance and helpful discussion. Foreign Student Office is thanked for awarding me a scholarship.

Finally, over the past three years, I came to associate and interact with many individuals and in the event that someone thinks that their name should have been explicitly mentioned in this section and it was not, I sincerely apologize for the omission.

References

Bareille P., MacSwiney M., Albanese A., De Vile C., Stahope R. 1997. Growth hormone treatment without a needle using the Preci-Jet 50 transjector. Arch. Dis. Child. 76: 65-67.

Bommannan D., Okuyama H., Stauffer P., Guy R., Sonophoresis 1992. The use of high-frequency ultrasound to enhance transdermal drug delivery. Pharm. Res. 9: 559-564.

Bouwstra J.A., Gooris G.S., van der Spek J.A., Lavrijsen S., Bras W. 1994. The lipid and protein structure of mouse stratum corneum: a wide and small angle diffraction study. Biochim Biophys Acta. 1212: 183-92.

Bouwstra J.A., Gooris G.S., Weerheim A., Kempenaar J., Ponec M. 1995. Characterization of stratum corneum structure in reconstructed epidermis by X-ray diffraction. J Lipid Res. 36: 496-504.

Chiba A., Nakanishi H., Chichibu S. 1997. Effect of indirect moxibustion on mouse skin. Am. J. Chin. Med. 25:143-151.

Doliwa A., Santoyo S., Yagartua P. 2001. Effect of passive and iontophoretic skin pretreatments with terpenes on the in vitro skin transport of piroxicam. Int. J. Pharm. 229: 37-44.

Elias P.M. 2005. Stratum corneum defensive functions: an integrated view. J. Invest. Dermatol. 125: 183-200.

Gallo S.A., Sen A., Hensen M.L., Hui S.W. 2002. Temperature-dependent electrical and ultrastructural characterizations of porcine skin upon electroporation. Biophys. J. 82: 109-119.

Goates C.Y., Knutson K. 1994. Enhanced permeation of polar compounds through human epidermis. I. Permeability and membrane structural changes in the presence of short chain alcohols. Biochim Biophys Acta. 1195:169-79.

Herndon C.M. 2007. Iontophoretic drug delivery system: focus on fentanyl. Pharmacother. 27: 745-754.

Igarashi R., Takenaga M., Takeuchi J., Kitagawa A., Matsumoto K., Mizushima Y. 2001. Marked hypotensive and blood flow-increasing effects of a new lipo-PGE1 (lipo-AS013) due to vascular wall targeting. J. Control. Release, 71: 157-164 (2001).

Inoue N., Kobayashi D., Kimura M., Toyama M., Sugawara I., Itoyama S., Ogihara M., Sugibayashi K., Morimoto Y. 1996. Fundamental investigation of a novel drug delivery system, A transdermal delivery system with jet injection. Int. J. Pharm. 137: 75-84.

Jacques S.L., McAuliff D.J., Blank I.H., Parrish J.A. 1987. Controlled removal of human stratum corneum by pulsed laser. J. Invest. Dermatol, 88: 88-93.

Jain M., Murrayb A. S., Bøtter-Jensena L. 2003. Characterisation of blue-light stimulated luminescence components in different quartz samples: implications for dose measurement. Radiation Measurements 37: 441-449.

Kobayashi K. 1988. Organic components of moxa. Am. J. Chin. Med. 16:179-185.

Koichi ITO. 2006. "Basic physics and engineering for hyperthermia," 4th Asian Congress of Hyperthermic Oncology (ACHO) & 23rd Annual Meeting of the Japanese Society of Hyperthermic Oncology p.27.

Lindmayer I., Menassa K., Lambert J., Moghrabi A., Legendre L., Legault C., Letendre M., Halle J.P 1986. Development of new jet injector for insulin therapy. Diabetes Care 9: 294-297.

Liu H.L., Wang L.P. 2006. Randomized controlled study on ginger-salt-partitioned moxibustion at shenque (CV 8) on urination disorders poststroke. Zhongguo Zhen Jiu. 26:621-624 (in Chinese).

Martanto W., Moore J.S., Kashlan O., Kamath R., Wang P.M., O'Neal J.M., Prausnitz M.R. 2006. Microinfusion using hollow microneedles. Pharm. Res. 23: 104-113.

McAllister D.V., Henry S., Allen M.G., Prausnitz M.R. 1998. Microfabricated microneedles: A novel approach to transdermal drug delivery. Proceed. Int'l. Symp. Control. Release Bioact. Mater. 25: 30-31.

Mitragotri S., Kost J. 2004. Low-frequency sonophoresis: a review. Adv Drug Deliv Rev. 56: 589-601.

Mori K., Hasegawa T., Sato S., Sugibayashi K., 2003. Effect of electric field on the enhanced skin permeation of drugs by electroporation. J. Control. Release 90: 171-179.

Morimoto Y., Hatanaka T., Sugibayashi K., Omiya H. 1992. Prediction of skin permeability of drugs: comparison of human and hairless rat skin. J. Pharm. Pharmacol. 44: 634-9

Ogiso T., Fuji H., Yoshimoto M., Iwaki M., Tanino T. 2000. The effect of heating and the combination of heating and d-limonene on the percutaneous permeation of some drugs. J. Pharm. Sci. Technol. Jpn. 60: 207-214.

Okazaki M., Aizawa S., Yamauchi M., Oguchi K. 1990. Effect of single moxibustion on cutaneous blood vessel and microvascular permeability in mice. Am. J. Chin. Med. 18:121-130.

Okumura M., Sugibayashi K., Ogawa K., Morimoto Y. 1989. Skin permeability of water-soluble drugs. Chem. Pharm. Bull. 37:1404-1406.

Publication of patent application (A) 1991. p. 109-119.

Publication of patent application (A) 1991. p. 147-149.

Purdon C. H., Azzi C.G., Zhang J., Smith E.W., Maibach H.I., 2004. Penetration enhancement of transdermal delivery – current permutations and limitations. Crit. Rev. Ther. Drug Carr. Syst. 21: 97-132.

Riegelman S. 1974. Pharmacokinetic factors affecting epidermal penetration and percultaneous absorption. Clin Pharmacol Ther. 16: 873–883.

Sage B.H., Riviere J.E. 1992. Model systems iontophoresis-transport efficacy. Advanced Drug Delivery Reviews 9: 265-287

Sloan K.B., Prodrug 1992. Topical and Ocular Delivery, Marcel Dekker, Inc. New York.

Smith E.W., Maibach H.I. 1995. Percutaneous Penetration Enhancers, CRC Press, Inc. Boca Raton.

Sugibayashi K., Morimoto Y. 1998. Transdermal therapeutic system. Japanese clinic 56: 619-627.

Thewalt J., Kitson N., Araujo C., MacKay A., Bloom M. 1992. Models of stratum corneum intercellular membranes: the sphingolipid headgroup is a determinant of phase behavior in mixed lipid dispersions. Biochem. Biophys. Res. Commun. 188: 1247-1252.

Tohya K., Urabe S., Igarashi J., Tomura T., Take A., Kimura M. 2000. Appearance of peculiar vessels with immunohistoligical features of high endothelial venules in the dermis of moxibustion-stimulated rat skin. Am. J. Chin. Med. 28: 425-433.

Tojo K., Chien Y. W., Sun Y., Ghannam M. 1987. Membrane transport of drug under nonisothermal conditions. J. Chem. Eng. Jpn. 20: 626-9.

Tokudome Y., Sugibayashi K. 2004. Mechanism of the synergic effects of calcium chloride and electroporation on the in vitro enhanced skin permeation of drugs. J Control Release

95: 267-274.

Tokumoto S., Higo N., Sugibayashi K. 2006. Effect of electroporation and pH on the iontophoretic transdermal delivery of human insulin. Int. J. Pharm. 326:13-19.

Uchida S.A., Kagitani F., Nakjima K., Aikawa Y. 2003. Effect of moxibustion stimulation of various skin areas on cortical cerebral blood flow in anesthetized rats. Am. J. Chin. Med. 31:611-621.

Weaver J.C., Chizmadzhev Y.A. 1996. Theory of electroporation: A review. Bioelectrochem. Bioenger. 41: 135-160.

Wu X.M., Todo H., Sugibayashi K. 2006. Effects of pretreatment of needle puncture and sandpaper abrasion on the in vitro skin permeation of fluorescein isothiocyanate (FITC)-dextran. Int. J. Pharm. 316:102-108.

Wu X.M., Todo H., Sugibayashi K. 2007. Enhancement of skin permeation of high molecular compounds by a combination of microneedle pretreatment and iontophoresis. J. Control. Release 118: 189-195.

Xiaoxiang Z. 2006. Jinger moxibustion for treatment of cervical vertigo --a report of 40 cases. J. Tradit. Chin. Med. 26: 17-18.

Zhou W. 2003. Acute lymphangitis treated by moxibustion with garlic in 118 cases. J. Tradit. Chin. Med. 23:198.