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A statistical approach to the development of a transdermal delivery system for ondansetron

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ABSTRACT

Transdermal delivery of drugs has gained attention as an alternative to intravenous and oral methods of delivery. However, the skin permeation of drugs is generally poor. To overcome this problem, many permeation enhancers have been developed. In this study, ondansetron hydrogels were prepared, and their skin permeation and pharmacological effects were evaluated in mice. To prepare the hydrogels, a Box–Behnken design was introduced. Fifteen formulations of ondansetron hydrogels composed of hydroxyethylcellulose and hydroxypropylcellulose as gel bases, *l*-menthol as a penetration enhancer and isopropanol (IPA), *N*-methyl-2-pyrrolidone (NMP) and water as a solvent were prepared. The quantities of IPA (X_1), *l*-menthol (X_2) and NMP (X_3) were selected as causal factors. We performed an *in vitro* skin permeation study and an *in vivo* skin irritation study with the test hydrogels. The flux and the total irritation score were selected as response variables. The optimal formulation, one that has an appropriate penetration and an acceptable skin irritation score, was estimated using a nonlinear response surface method incorporating thin-plate spline interpolation. The optimal formulation also delivered the desired pharmacological activity. These results indicated the feasibility of delivering ondansetron transdermally.

1. Introduction

Transdermal drug delivery has many advantages: it is convenient, it bypasses first-pass metabolism, and it provides a steady-state plasma concentration of the drug and long-term therapy in a single dose. These advantages lead to improved patient compliance. However, the skin permeation of clinically useful drugs is generally poor with some exception (it has small molecular weight (<300 Da) and lipophilic nature) because the stratum corneum functions as a barrier against foreign substances (Ranade, 1991). To overcome this problem, many penetration enhancers that temporarily increase the permeability of the skin have been examined (Barry, 1987; Sinha and Pal Kaur, 2000). Transdermal delivery does have shortcomings, too. One potential shortcoming, the long lag time before absorption begins, has been overcome in the case of the turobuterol delivery system. Another shortcoming, low absorption levels, could be advantageous in some cases by preventing a sudden increase in the plasma concentration of drugs immediately after administration. Especially in case of drugs that act on the brain, slow and sustained absorption of drugs via the skin and the maintenance of low plasma concentrations might be desirable.

To develop a new delivery system, we selected a drug that is often used clinically but that has no available option for transdermal delivery. Ondansetron, a serotonin (5-hydroxytryptamine) subtype 3 (5-HT_3) receptor antagonist, is used to treat nausea and vomiting associated with cancer chemotherapy, radiotherapy, anesthesia and surgery (Gregory and Ettinger, 1998). However, intravenous and oral administration are not appropriate for children and patients with various side effects. Moreover, ondansetron undergoes extensive hepatic metabolism by the cytochrome P450 enzyme system, and the elimination half-life of ondansetron is short (3–3.5 h) (Gwak et al., 2004). Therefore, the development of a transdermal delivery system for ondansetron has been desired and expected by clinicians.

In this study, the possibility of developing transdermal hydrogels containing ondansetron was evaluated. In developing transdermal preparations, it is important to design an optimized pharmaceutical formulation that has appropriate penetration with concomitant acceptable skin irritation levels. For this purpose, it is considered important to discover the optimized formulation of ondansetron hydrogels by employing a nonlinear response surface method incorporating thin-plate spline interpolation (RSM-S). Using RSM-S, we can easily understand nonlinear relationships between causal factors and response variables and obtain a stable and reproducible simultaneous optimal solution (Takayama et al., 2004). A bootstrap (BS) resampling method was used to evaluate

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Table 1
Experimental design and model formulations of ondansetron hydrogels.

Formulation	X_1	IPA (%)	X_2	*l*-Menthol (%)	X_3	NMP (%)
1	−1	0	−1	0	0	5
2	−1	0	0	1	−1	0
3	−1	0	0	1	1	10
4	−1	0	1	2	0	5
5	0	10	−1	0	−1	0
6	0	10	−1	0	1	10
7	0	10	0	1	0	5
8	0	10	0	1	0	5
9	0	10	0	1	0	5
10	0	10	1	2	−1	0
11	0	10	1	2	1	10
12	1	20	−1	0	0	5
13	1	20	0	1	−1	0
14	1	20	0	1	1	10
15	1	20	1	2	0	5

The amounts of ondansetron, HEC and HPC were fixed at 1, 1 and 1%, respectively. Total amount of each hydrogel was adjusted to 100% by the addition of water.

the reliability of the optimal solution estimated by RSM-S. The BS method is a simulation technique based on the empirical distribution of the observed sample (Arai et al., 2007; Onuki et al., 2008). Those established statistical approaches are helpful in fabricating an appropriate transdermal delivery system for ondansetron. Moreover, a Kohonen self-organizing map (SOM) analysis was applied to gain a mechanistic understanding of the relationships between causal factors and response variables.

2. Materials and methods

2.1. Materials

Ondansetron was generously supplied by Nippon Zoki Pharmaceutical Co., Ltd. (Osaka, Japan). *l*-Menthol was purchased from Tokyo Kasei Chemical Industries (Tokyo, Japan). Hydroxyethylcellulose (HEC) was purchased from Sumitomo Seika Chemicals Co., Ltd. (Osaka, Japan). Hydroxypropylcellulose (HPC) and cis-diamminedichloroplatinum (II) (cisplatin) purchased from SIGMA-ALDRICH Corp. SIGMA-ALDRICH Japan K.K. (Tokyo, Japan). All other chemicals and solvents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Hydrogel preparation

Hydrogels were prepared according to formulations obtained from a Box-Behnken experimental design (Miyazaki et al., 2008). As shown in Table 1, 15 kinds of ondansetron hydrogels composed of HEC and HPC as gel bases, *l*-menthol as a penetration enhancer and isopropanol (IPA), *N*-methyl-2-pyrrolidone (NMP) and water as a solvent were prepared. Previous studies indicated that *l*-menthol enhanced the absorption of many drugs (Obata et al., 1990). The mechanism of *l*-menthol is to increase drug partition and diffusion parameters (Okusa et al., 1997). The quantities of IPA (X_1), *l*-menthol (X_2) and NMP (X_3) were selected as causal factors. The quantities of ondansetron, HEC and HPC were fixed at 1, 1 and 1%, respectively. The total amount of each hydrogel was adjusted to 100% by the addition of water. The gel bases, HEC and HPC, were dissolved in water. Separately, ondansetron, NMP and *l*-menthol were dissolved in IPA. The components were then mixed thoroughly, and the resulting hydrogels were stored in airtight containers at room temperature prior to use. In case of Formulation 2 (without solvent), ondancetron and *l*-menthol was directly added to gel base and stirred overnight.

2.3. In vitro skin permeation study

The excised skin of hairless mice (Laboskin®, HOS: HR-1 Male, 7 weeks, Sankyo Labo Service Corporation, INC., Tokyo, Japan) was used as a permeation membrane for the *in vitro* study. A Franz diffusion cell with an available diffusion area of 2.01 cm^2 was employed. The receiver side was filled with 16 mL of phosphate-buffered saline (pH 7.4, 37 °C) and the donor side was filled with the test hydrogel (1.0 g) under occlusive conditions. At appropriate times, an aliquot of the receiver fluid was withdrawn and the same volume of fresh buffer solution was supplied to the receiver side. The concentration of drugs in the aliquot was analyzed using an HPLC.

2.4. Determination of ondansetron concentration

The HPLC system consisted of a Model L-2200 auto sampler, a Model L-2130 pump and a Model L-2400 UV detector (Hitachi High-Technologies Corporation, Tokyo, Japan). The analytical column was YMC-Pack ODS-A (150 mm × 4.6 mm i.d., S-5 μm) (YMC Co. Ltd., Kyoto, Japan) and the mobile phase consisted of 0.05% acetic acid/acetonitrile (75:25) set at flow rate of 0.5 mL/min for all separations (Hussain et al., 2000).

2.5. Skin irritation study

Male ddY mice (7 weeks) were anesthetized with a pentobarbital sodium (50 mg/kg intraperitoneally). Their dorsal hair was gently removed with an electric clipper. A glass cell with an inner diameter (i.d.) of 16 mm and a height of 10 mm was attached to the shaved dorsal skin with a cyanoacrylate-type adhesive (Aron Alpha A, Sankyo Company Ltd., Tokyo, Japan) and filled with the test hydrogel (1.0 g) under occlusive conditions. Eight hours later, the application site for each formulation was excised under anesthetization and the excised tissue was fixed with formaldehyde solution (10 w/w%). Tissues were divided into small pieces and stained with hematoxylin and eosin. All sections were examined by light microscopy. The microscopic findings were graded along a five-point irritation scale, from no change to a marked change that included liquefaction of the epidermis, edema of the subepidermis, collagen fiber swelling and inflammatory cell infiltration in both the dermis and hypodermis, and degeneration of skin appendages (Quan et al., 1991). The total irritation score (TIS) was obtained summing all irritation scores and was used as an index of skin damage caused by the application of the ondansetron hydrogel.

2.6. Seeking an optimal formulation

A formulation optimization study based on RSM-S was performed with the data set obtained for the model formulations. The details of simultaneous optimization methods incorporating RSM-S were fully described previously (Takayama et al., 2004; Lucian and Neamtu, 2003). The optimal formulation was defined as an ondansetron hydrogel with sufficient flux and TIS. Namely, the best formulation would have the maximum value of flux and the minimum value of TIS. Once the RSM-S-estimated optimal solution was obtained, we evaluated its reliability using BS resampling. The BS technique was fully described previously (Ueda and Nakano, 1995; Dupret and Koda, 2001; Zhang, 1999). The number of BS replications was fixed at 1000. In this study, we used the dataNESIA software developed by Yamatake Corporation (Tokyo, Japan) to generate the RSM-S and the bootstrap (BS) resampling; this software consists of a multidimensional interpolating program and a nonlinear optimization program.

SOM was used to clarify the relationship between causal factors and response variables. SOM maps multidimensional data onto a two-dimensional surface (Yasuda et al., in press). To make the

Table 2
Formulation for dermal transfer study of *l*-menthol.

Formulation	IPA (%)	*l*-Menthol (%)	NMP (%)
A	10	1	5
B	10	1	10
C	20	1	5

The amounts of ondansetron, HEC and HPC were fixed at 1, 1 and 1%, respectively. Total amount of each hydrogel was adjusted to 100% by the addition of water.

maps, the response variables (flux and TIS) were assigned a weight value of 1, but no weight was assigned to causal factors (X_1–X_3). The number of nodes in the output was set at 45. We used the Viscovery version 4.0 software developed by Eudaptics Software Gmbh (Vienna, Austria) and based on SOM.

2.7. Dermal transfer study of l-menthol

The hydrogels were prepared according to the formulations shown in Table 2. Each hydrogel (1.0 g) was applied a 2.01 cm^2 area of excised hairless mouse skin (Laboskin®, HOS: HR-1 Male, 7 weeks, Sankyo Labo Service Corporation, Inc., Tokyo, Japan). Eight hours later, the hydrogel was removed from the skin surface. The skin was extracted with 2 mL chloroform/methanol solution (2:1). After extraction, the solvent was evaporated under nitrogen gas stream. The residue was redissolved in 2 mL of methanol for gas chromatography injection.

2.8. Determination of l-menthol concentrations

l-Menthol was analyzed by gas chromatography using GC-17A with a flame-ionization detector (Shimadzu Corporation., Kyoto, Japan). The detector temperature was 250 °C. The initial oven temperature was 70 °C, increasing gradually 10 °C/min up to 120 °C. The analytical column was a "fused silica" capillary column 15 m × 0.25 mm i.d. with a 0.25 μm film thickness SAC^{TM}-5 (Supelco, Bellefonte, USA). Concentrations of *l*-menthol in the samples were calculated using the internal standard method. *d*-Limonene (10 μg/mL) was chosen as internal standard because it shares some structural similarity with *l*-menthol (monocyclic monoterpene).

2.9. Evaluation of therapeutic effects

Male ddY mice (7 weeks) were placed in cages for a 3-day habituation period before drugs were administered. The cages were in a temperature and humidity controlled room with 9 h of light beginning at 9:00 a.m. and 15 h of darkness beginning at 6 p.m. On the day before the start of the experiment, the hydrogel administration areas on the backs of the mice were shaved without damaging the skin. A glass cell with an effective diffusion area of 2.01 cm^2 was attached to the hair-free dorsal region using an adhesive (Daiichi Sankyo Co. Ltd., Japan). On the day of experiment, the cell was filled with hydrogel (1 g) and capped with a plastic lid and Parafilm (Pechiney Plastic Packaging Inc., Canada). The previous day's food consumption was recorded. Between 9:00 and 10:00 a.m., the mice were given either an ondansetron hydrogel (optimal formulation) or no ondansetron hydrogel (control), followed later by an injection of cisplatin (6 mg/kg intraperitoneally). The cisplatin was dissolved in saline (sonicated for about 1 min). Food consumption was recorded for 1-day post-drug administration.

2.10. Statistical analysis

Statistical analysis (Student's *t*-test) was performed using STATISTICA software (StatSoft Inc., Tulsa, OK, USA).

2.11. Ethics in animal study

This animal study was performed at Hoshi University and complied with the regulations of the Committee on Ethics in the Care and Use of Laboratory Animals.

3. Results and discussion

3.1. Feasibility of skin permeation of ondansetron

Predicting the skin permeability coefficient of drugs is important in selecting drugs for which to fabricate a transdermal delivery system. However, the prediction is difficult because of the nonlinear relationship between the physicochemical parameters of drugs and permeability properties. The database of human skin permeability coefficients appeared in a previous study (Flynn, 1990). Using those data, the skin permeation of ondansetron was predicted by employing an ensemble neural network methodology recently developed and utilized in the pharmaceutical research field (Takagaki et al., 2010). With the molecular weight (293.4) and apparent partition coefficient ($\log K = 1.87$) of ondansetron determined by SCIGRESS software, the log value of the permeability coefficient of ondansetron was estimated as −6.82. The estimated value of skin permeability of ondansetron was similar to that of turobuterol (−6.26). Thus, the feasibility of transdermal delivery system of ondansetron was positively evaluated.

3.2. Identification of the response surface by RSM-S

The skin permeability of model formulations was evaluated with an *in vitro* skin permeation study. The steady-state flux, used to examine skin permeability, was defined as the slope of the accumulated amount of permeated ondansetron along a time curve.

Fig. 1 shows the response surfaces of flux and TIS estimated by RSM-S. The accuracy of the response surfaces was determined with a leave-one-out cross-validation (LOOCV) (Bourquin et al., 1998). The results are shown in Fig. 2. The correlation coefficients of estimated value and observed value for flux and TIS were sufficiently high (0.936 and 0.903, respectively) to suggest that RSM-S successfully estimated the relationships between the causal factors and response variables.

In the response surfaces for flux, the flux values increased as the quantity of IPA, *l*-menthol or NMP increased. Depending on the degree of increase of IPA and NMP, the amount of *l*-menthol dissolved into the hydrogel might be increased. Therefore, the effect of *l*-menthol was considered to be increased. On the other hand, response surfaces for TIS suggested that the addition of NMP resulted in a slight decrease in TIS. It has been reported that NMP reduced skin irritation in several formulations (Sasaki et al., 1990), and NMP is known as a prominent solvent. The solubility parameter of NMP is similar to those of ethanol and dimethylsulfoxide (DMSO) (Hansen and Just, 2001). NMP decreases the thermodynamic activity of *l*-menthol, suggesting that NMP reduces the skin irritative effect of *l*-menthol. Contribution indexes (CI) to flux and TIS were calculated and are shown in Fig. 3 (Wu et al., 2001). IPA contributed most to the prediction of flux and *l*-menthol contributed most to the prediction of TIS.

3.3. Clustering of response variables with SOM

SOM was used to visualize the relationships between causal factors and response variables more clearly. The SOM feature maps of response variables and formulation factors are shown in Fig. 4. Each feature map shows the values of one variable in each map unit. The map of flux and the map of TIS were clearly distinguished. In node *a* (comparatively high flux and high TIS), the quantities of IPA and *l*-

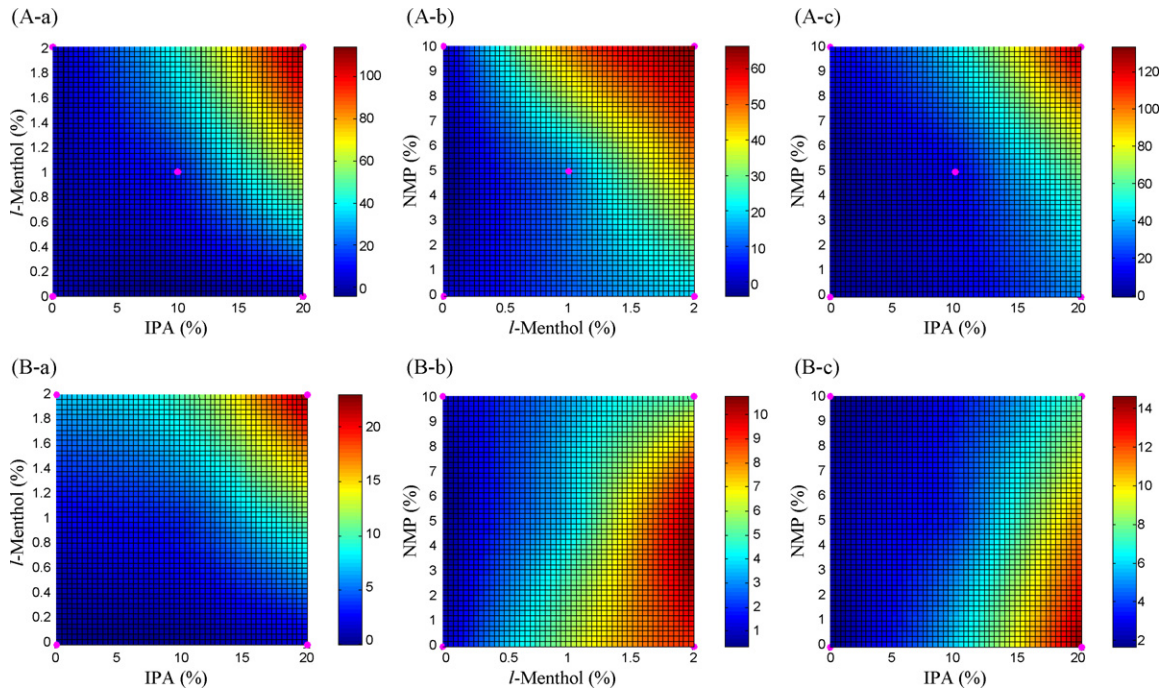

Fig. 1. Response surfaces of (A) flux and (B) TIS. Each figure shows the function of (a) IPA and *l*-menthol at NMP = 5%, (b) *l*-menthol and NMP at IPA = 10% and (c) IPA and NMP at *l*-menthol = 1%.

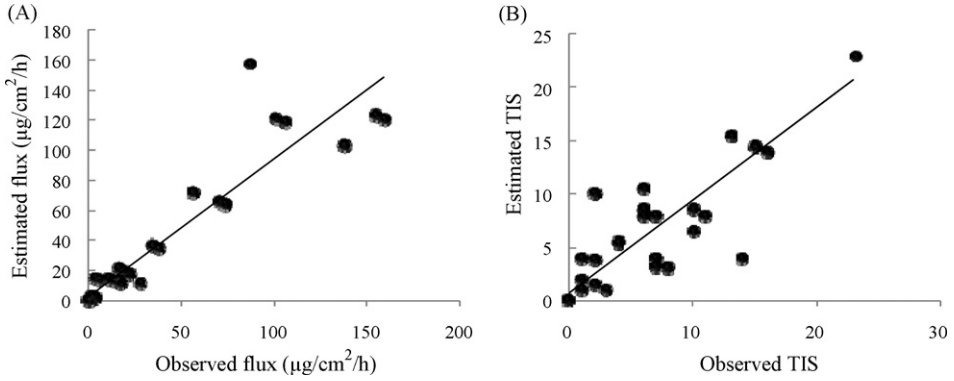

Fig. 2. Leave-one-out cross-validation estimated accuracy of the RSM-S model for (A) flux and (B) TIS.

menthol were high, but the medium quantity of NMP was included. In node *b* (high flux and medium TIS), IPA and NMP quantities were high, but the medium quantity of *l*-menthol was included. In node *c* (low flux and low TIS), IPA and *l*-menthol quantities were low, but the medium quantity of NMP was included. Therefore, to obtain a formulation that has appropriate penetration with concomitant acceptable skin irritation, the addition of NMP might be important.

3.4. Partition of l-menthol to skin

The results of response surfaces and SOM maps indicated that flux values increased and TIS values decreased depending on NMP concentration. This suggests that the partition of *l*-menthol to skin decreased in relation to the increase in NMP. To validate this suggestion, the dermal transfer amount of *l*-menthol was determined

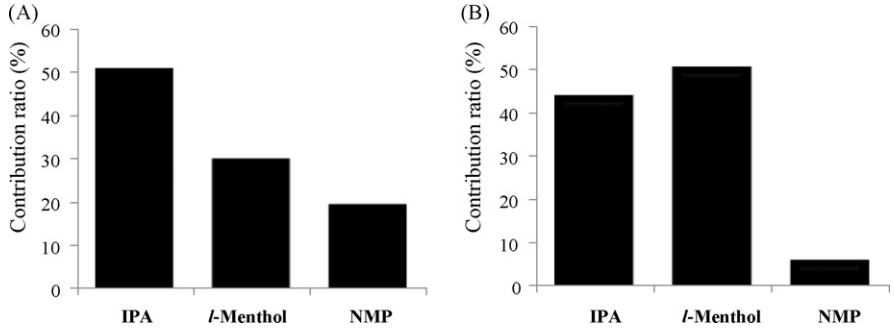

Fig. 3. Contribution indexes of each formulation factor for (A) flux and (B) TIS estimated by RSM-S.

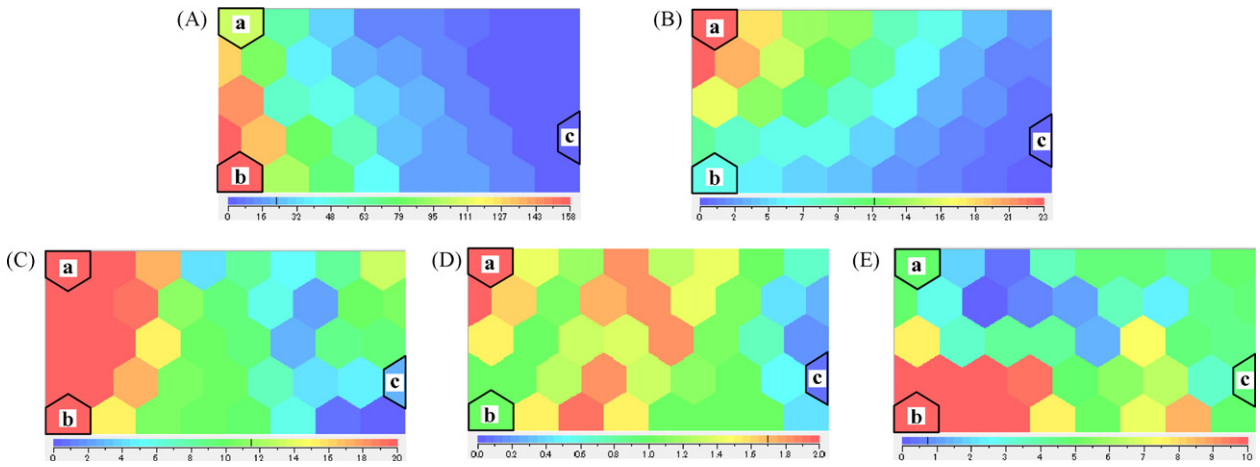

Fig. 4. SOM feature maps of response variables (A and B) and causal factors (C–E). SOM was constructed from two response variables. The two upper plots depict the distribution of response variables (A) flux and (B) TIS. The three lower plots depict the distribution of causal factors (C) IPA, (D) *l*-menthol and (E) NMP. Feature nodes were marked as node *a* (flux = 104.9, TIS = 23, IPA = 20, *l*-menthol = 2, NMP = 5), node *b* (flux = 158.5, TIS = 6.5, IPA = 20, *l*-menthol = 1, NMP = 10) and node *c* (flux = 0.3, TIS = 0.3, IPA = 2.6, *l*-menthol = 0.1, NMP = 4.7).

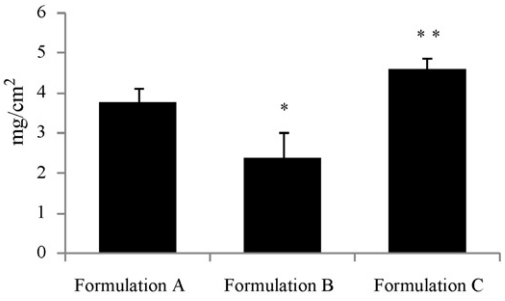

Fig. 5. Dermal transfer of *l*-menthol in hairless mouse skin after application of hydrogel (formulations A–C). Each column represents the mean ± SD ($n = 4$). *: $p < 0.05$ vs. formulation A, $p < 0.01$ vs. formulation B.

and the result is shown in Fig. 5. As the data indicate, the dermal transfer amount of *l*-menthol decreased in formulation B (which contained the large quantity of NMP) compared with formulation A (which contained an intermediate quantity of NMP). In contrast, dermal transfer of *l*-menthol was greater in formulation C (which contained a large quantity of IPA) than in formulation A (which contained an intermediate quantity of IPA). These results suggest that as the quantity of IPA increased, the amount of *l*-menthol dissolved into hydrogel increased and dermal transfer of *l*-menthol increased. On the other hand, as the quantity of NMP (which has a higher lytic potential than IPA) increased, the affinity of *l*-menthol for the gel base increased. Therefore, dermal transfer of *l*-menthol decreased and TIS decreased. However, the amount of *l*-menthol remained sufficient to obtain sufficient flux of ondansetron.

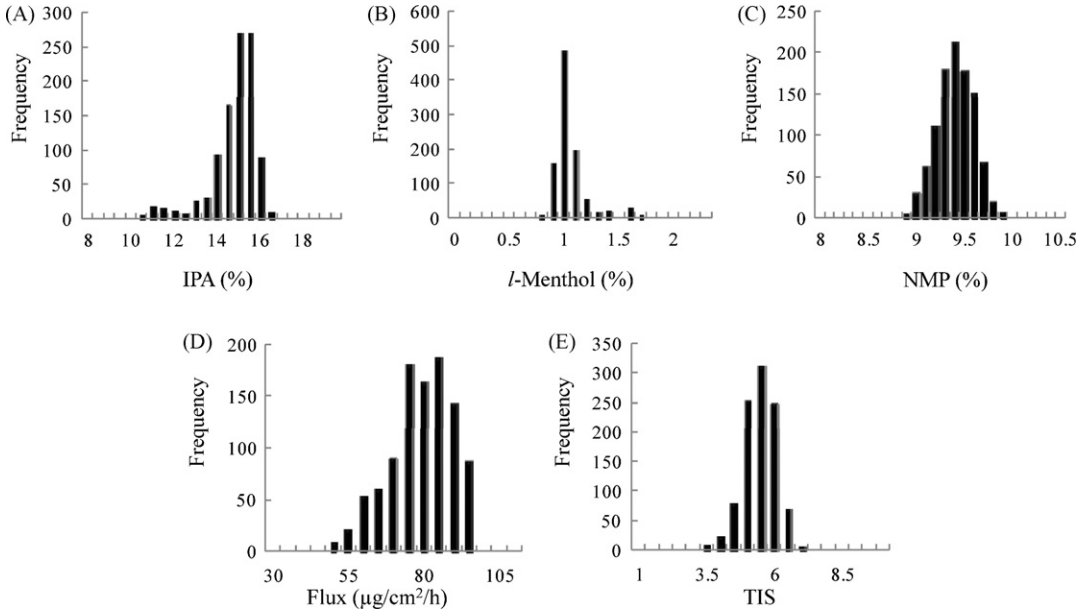

Fig. 6. Histograms of optimal causal factors (A–C) and response variables (D and E) estimated by bootstrap resampling. The three upper plots depict the distribution of causal factors (A) IPA, (B) *l*-menthol and (C) NMP. The two lower plots depict the distribution of response variables (D) flux and (E) TIS. Bootstrap resampling was repeated 1000 times. These histograms are composed of 1000 optimal solutions estimated from the bootstrap samples.

Table 3
Simultaneous optimal solution of ondansetron hydrogel estimated by RSM-S.

	IPA (%)	*l*-Menthol (%)	NMP (%)	Flux (μg/cm^2/h)	TIS
Estimated mean	14.98	0.96	9.33	78.40	5
95% Confidence intervals	11.16–15.77	0.83–1.56	9.00–9.72	53.87–92.66	4–6

This optimal solution was estimated from original sample that consists of 15 kinds of model formulation ($n = 3$). Bootstrap resampling frequency, approximately 1000.

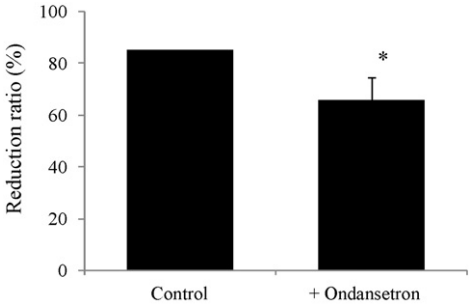

Fig. 7. Reduction rates of food intake. Each column represents the mean ± SD ($n = 5$). *: $p < 0.05$ vs. control.

3.5. Formulation optimization of the ondansetron hydrogels using RSM-S

Formulation optimization of the ondansetron hydrogels was performed based on the original data set using RSM-S. BS resampling was applied to evaluate the reliability of the optimal solution. Histograms of the causal factors X_1, X_2 and X_3 and of the response variables, flux and TIS, of the BS optimal solutions are shown in Fig. 6. The 95% confidence intervals for the optimal solutions were calculated using the percentile method (Higgins, 2003). The results are shown in Table 3. The ranges of the 95% confidence intervals were sufficiently narrow for the practical study of the formulations.

To evaluate the optimal solution by experiment, an *in vitro* skin permeation study and an *in vivo* skin irritation study were performed with the optimal formulation. The flux values predicted by the RSM-S coincided well with the experimental value (76.8 ± 1.5 μg/cm^2/h). In TIS, the predicted value and experimental value (8 ± 5) were slightly different. This may be because the degree of skin irritation may depend on individual variability.

3.6. The recovery effect of the optimal formulation on cisplatin-induced reduction in food intake

As reported previously, cisplatin markedly reduces food intake in mice (Liu et al., 2005; Malik et al., 2007). The reduction in food intake could serve as an index of nausea. Fig. 7 shows the reduction rate of food intake (pre- and postadministration). In the control group, the food intake reduction rate was about 85.4%. In the group treated with ondansetron (the optimized formulation), the food intake reduction rate was 65.8%. The optimal formulation of ondansetron had a significant effect on the recovery of the cisplatin-induced reduction in food intake compared to controls. This result indicates that the ondansetron released from the optimized hydrogel performed a systemic action via the skin.

4. Conclusions

The optimal formulation, defined as formulation with appropriate penetration of ondansetron and concomitant acceptable skin irritation levels, was estimated. Additionally, the therapeutic effect of the optimal formulation significantly improved the cisplatin-induced reduction in food intake in mice. Considering the flux value and the pharmacological activity of this formulation, the results obtained in this study we have succeeded to show the feasibility of transdermal delivery of ondansetron.

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