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Novel approach to improve permeation of ondansetron across shed snake skin as a model membrane

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Abstract

The purpose of this study was to investigate the feasibility of transdermal drug delivery of ondansetron, an antagonist of the 5-HT_3 receptor, used for the treatment of chemotherapy-induced emesis. The permeability of ondansetron from an aqueous suspension through shed snake skin as a model membrane was very low and in order to improve it, several enhancers were tested. Ethanol increased the flux at a concentration of 40% or more. The solubility of ondansetron also increased as the ethanol concentration increased. The permeability coefficient increased after pretreatment of the shed snake skin with Azone, oleic acid or lauryl alcohol. Further improvement of the permeability was observed when ethanol was combined with other enhancers and was maximum for the combination of ethanol and oleic acid. Oleic acid dramatically increased the partition of ondansetron to *n*-hexane and shed snake skin. Oleic acid may enhance the permeation of ondansetron in two ways: by a direct effect on the stratum corneum or via counterion formation of an ion-pair. The maximum flux obtained from the combination of ethanol and other enhancers seems to be high enough to obtain a therapeutic effect.

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Introduction

Ondansetron is a novel and specific antagonist of the 5-HT_3 receptor, indicated for chemotherapy-induced nausea and vomiting in cancer patients (Markham & Sorkin 1993). Intravenous and oral dosage forms of the drug are commercially available. The recommended oral dosing regimen of ondansetron for emeogenic neoplastic agents is 8 mg three times a day. Sustained-release formulations may enable the ondansetron dosing frequency to be reduced and therefore increase patient compliance. A less invasive administration route is also needed for use in specific patient populations. Rectal (Hsyu et al 1994) and nasal (Hussain et al 2000) absorption of ondansetron have been reported. However, there have been no reports on skin permeation of ondansetron.

The advantages of transdermal administration of drugs include by-passing the first-pass effect, minimizing inter- and intra-patient variation, providing the steady-state plasma concentration of the drug and long-term therapy from a single dose. The primary barrier to transdermal permeation of drugs is the stratum corneum, the outermost layer of the skin, which comprises keratin-rich cells embedded in multiple lipid bilayers (Barry 1987, 1991). A well known approach to reduce the barrier function of the stratum corneum involves the use of enhancers (Barry 1987, 1991; Williams & Barry 1992). To investigate the skin permeability of drugs, a variety of model membranes have been used. Although human skin is the best

model membrane, its use is limited. As a substitute, shed snake skin is feasible because of its similarity to the human stratum corneum in terms of composition. The permeability of compounds through shed snake skin has been found to be similar to, though often slightly less than that of human skin (Itoh et al 1990; Rigg & Barry 1990).

In this study, we evaluated the permeability of ondansetron through shed snake skin, and tested several enhancers in an effort to increase its permeability. We discuss the possibility of transdermal administration of ondansetron based on kinetic analysis with the permeation parameters observed in this study and the pharmacokinetic parameters reported for man (Hsyu et al 1994).

Materials and Methods

Chemicals

Ondansetron hydrochloride dihydrate was kindly supplied by Glaxo Wellcome (Research Triangle Park, NC). Azone was obtained from Whitby Research Inc. (Irvine, CA). All other reagents were of analytical grade and were used without further purification.

In-vitro penetration study

Shed snake skins of *Elaphe obsoleta* (black rat snake) were used as a model membrane. The skin was hydrated by immersing it in water at 40°C for 30 min before the experiments. The skin was mounted in a Franz-type diffusion cell. For the pretreatment study of enhancers, 15 μL of the enhancer was applied to the shed snake skin 2 h before the experiment. Delipidized skin was prepared by treatment with a chloroform–methanol mixture (2:1, v/v) for 24 h. Any remaining enhancer or solvent on the skin was blotted with a Kimwipe after pretreatment and the skin was mounted in the diffusion cell after hydration. In this study, 10 mL phosphate buffer (pH 6.0, 10 mM) was used as the receptor solution, except in the ethanol experiments, and 0.5 mL of the same buffer containing drug was placed on the donor side. For the ethanol experiments, the same concentrations (v/v) of ethanol in phosphate buffer (pH 6.0, 10 mM) were used as the receptor and donor solutions. The surface exposed to diffusion was 1.77 cm^2 (diam. 1.5 cm). The receptor solution was kept at 32°C and stirred with a magnetic stirrer.

Solubility measurements

An excess amount of ondansetron was stirred in 10 mL phosphate buffer (pH 6.0, 10 mM) or several concentrations of ethanol in the phosphate buffer (pH 6.0, 10 mM) at 32°C for 24 h. The solution was centrifuged for 5 min at 2000 rev min^{-1}, and the supernatant was filtered with a cellulose acetate membrane filter (0.45 μm pore size). The concentration of drug in the filtrate was determined by HPLC.

Determination of the partition coefficient

The partition coefficient of ondansetron between *n*-hexane and phosphate buffer (pH 6.0, 10 mM) was measured. *n*-Hexane (5 mL) was mixed with 5 mL phosphate buffer solution (pH 6.0) containing the drug (100 μM) for 24 h at 32°C. The mixture was then centrifuged and the drug concentration in buffer was measured by HPLC.

Determination of the partition to shed snake skin

Shed snake skin (4 cm^2) was weighed, and then 100 μL of the enhancer was applied to the skin 2 h before the experiment. Any enhancer remaining on the skin was blotted with a Kimwipe and the skin was incubated with 10 mL phosphate buffer (pH 6.0, 10 mM) containing 50 μM ondansetron at 32°C. After 24 h, the drug concentration in the buffer was measured by HPLC.

HPLC conditions

The HPLC system consisted of a pump (Series 410 LC Pump; Perkin-Elmer), and a detector (LC 90 UV; Perkin-Elmer), a column packed with ASI CN (Analytical Sciences Inc., Santa Clara, CA) and an integrator (CR601; Shimadzu, Kyoto, Japan). The mobile phase was phosphate buffer (10 mM, pH 5.0)–CH_3CN (65:35). The flow rate was 1.5 mL min^{-1}, with UV detection at 216 nm.

Data treatment

Steady-state fluxes for ondansetron (J, nmol cm^{-2} h) were calculated using linear regression analysis of the straight line portion of the cumulative drug penetration vs time plots. Data are presented as the mean $\pm$ s.d. Statistical evaluation of the data was carried out using the Student's *t*-test.

Results and Discussion

The permeability of ondansetron from the aqueous suspension was low and the permeability coefficient was 0.203 ± 0.066 nmol cm^{-2} h. One way to improve the permeation of a drug is to incorporate vehicles that can enhance the permeation rate across the skin. Such enhancers may act by affecting drug solubility or the intercellular lipids of the stratum corneum, or by increasing partitioning of the drug into the stratum corneum (Barry 1991; Sasaki et al 1991; Yamane et al 1995; Yokomizo & Sagitani 1996). One well known enhancer is ethanol, which can promote the penetration of several drugs across skin (Berner & Liu 1995). Ethanol has been incorporated into successful transdermal systems for estradiol (Good et al 1985), nitroglycerin (Gale & Berggren 1986, 1987), and fentanyl (Gale et al 1986). To improve the permeation of ondansetron, we first selected ethanol as an enhancer and found that ondansetron solubility increased as the ethanol concentration increased. No effect on permeation was observed up to a concentration of 20% ethanol. At a concentration of 40% or more ethanol, the flux increased (Figure 1; Table 1). In buffer without ethanol or with 20% ethanol, the drug suspension was used as a donor solution, and 30 mM ondansetron solution as a donor solution was used in an ethanol concentration of 40% or more. This suggested that the thermodynamic activity of the drug in the vehicle differed at ethanol concentrations above and below 40%.

We also selected Azone, oleic acid and lauryl alcohol as penetration enhancers. These compounds have been shown to enhance the permeation of many drugs through rat, human and shed snake skin (Allan 1995; Aungst 1995; Berner & Liu 1995). Figure 2 shows the

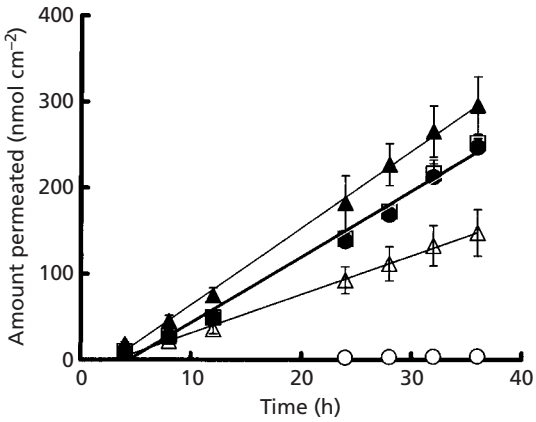

Figure 2 Effect of pretreatment with enhancers on the cumulative permeation of ondansetron through shed snake skin at 32°C. ○, Control; ●, Azone; □, oleic acid; △, lauryl alcohol; ▲, delipidization. Enhancer (15 μL) was applied to shed snake skin 2 h before the experiment. Each point represents the mean ± s.d., n = 3–5.

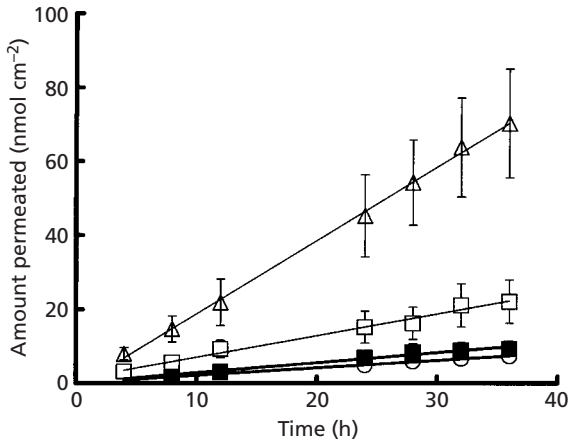

Figure 1 Effect of ethanol concentration on the cumulative permeation of ondansetron through shed snake skin at 32°C. ○, Control; ■, 20% ethanol; □, 40% ethanol; △, 60% ethanol. Each point represents the mean ± s.d., n = 3–5.

Table 1 Effect of ethanol concentration on the permeation parameters of ondansetron.

	Ethanol (v/v)			
	0%	20%	40%	60%
Ondansetron concn[a] (mM)	2.2	8.3	29.4	31.6
$J \times 10^3$ (mmol cm^{-2} h)	0.20 ± 0.07	0.19 ± 0.06	0.54 ± 0.17*	0.20 ± 0.04*
Lag time (h)	2.8 ± 2.7	0	0	0.4 ± 0.5
Solubility (mM)	2.2	8.3	43.3	130.5

[a]Initial donor concentration of ondansetron. Each value represents the mean ± s.d., n = 3–5.
*$P < 0.05$ compared with control.

Table 2 Effect of pretreatment with enhancer on the permeation parameters of ondansetron.

	Control	Azone	Oleic acid	Lauryl alcohol	Delipidization
$J \times 10^3$ (mmol cm^{-2} h)	0.20 ± 0.07	7.82 ± 0.36*	7.87 ± 0.36*	4.65 ± 0.85*	9.16 ± 1.00*
Lag time (h)	2.8 ± 2.7	5.5 ± 0.6	5.8 ± 0.6	3.9 ± 0.7	3.5 ± 0.5
Solubility (mM)	2.2	2.2	2.2	2.2	2.2

Enhancer (15 mL) was applied to shed snake skin 2 h before the experiment. A drug suspension in buffer was applied to the donor side. Each value represents the mean ± s.d., n = 3–5. *$P < 0.05$ compared with control.

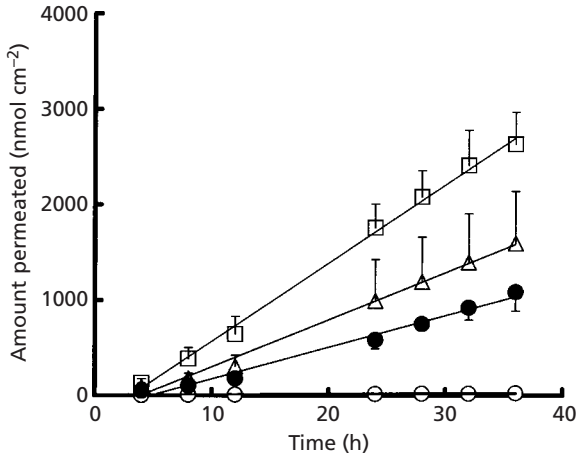

Figure 3 Combined effect of ethanol and pretreatment with enhancers on the cumulative permeation of ondansetron though shed snake skin at 32°C. ○, Control; ●, Azone; □, oleic acid; △, lauryl alcohol. Enhancer (15 μL) was applied to shed snake skin 2 h before the experiment. A 60% ethanol solution of ondansetron was applied to the donor side. Each point represents the mean ± s.d., n = 3–5.

cumulative amounts permeated through the shed snake skin pretreated with Azone, oleic acid or lauryl alcohol. Penetration was significantly increased by the pretreatment with these enhancers compared with untreated skin, the effects being in the following order: Azone = oleic acid > lauryl alcohol. Table 2 lists the permeation parameters and solubilities. We also showed the effect of delipidization of shed snake skin on the permeation of ondansetron. The flux significantly increased after pretreatment with these compounds, and the effects of Azone and oleic acid were about equal to that of the delipidization. These findings indicate that Azone and oleic acid may be useful penetration enhancers for ondansetron. Some reports have suggested the benefits of the combined effects of enhancers or enhancer and solvent combinations (Morimoto et al 1993; Zhao & Singh 1999). To further improve ondansetron penetration, we examined the effect of combining ethanol with other enhancers. After pretreatment with enhancer, 60% ethanol solution containing 30 mM ondansetron was applied at the donor side (Figure 3; Table 3). Further improvement of the flux was observed for the combination of ethanol and enhancer pretreatment, the effects being in the following order: oleic acid > lauryl alcohol > Azone.

Green & Hadgraft (1987) reported the facilitated transfer of cationic drugs across a lipoidal membrane by oleic acid and lauric acid. Ondansetron is a cationic compound and most of it may be ionized in the solution of the donor phase (pH 6). Also, hydrogen bonding

Table 3 Combined effect of ethanol on the permeation parameters of ondansetron after pretreatment with enhancer.

	Control	Azone	Oleic acid	Lauryl alcohol
Ondansetron concn.[a] (mM)	31.1	31.1	31.1	31.1
$J \times 10^3$ (mmol cm^{-2} h)	1.85 ± 0.44	34.9 ± 4.8*	84.1 ± 8.7*	52.0 ± 18.9*
Lag time (h)	1.2 ± 1.3	6.0 ± 1.4*	3.8 ± 1.4	5.3 ± 0.8*
Solubility (mM)	130.5	130.5	130.5	130.5

[a]Initial donor concentration of ondansetron. A 60% ethanol solution of ondansetron was applied to the donor side. Each value represents the mean ± s.d., n = 3–5. *$P < 0.05$ compared with control.

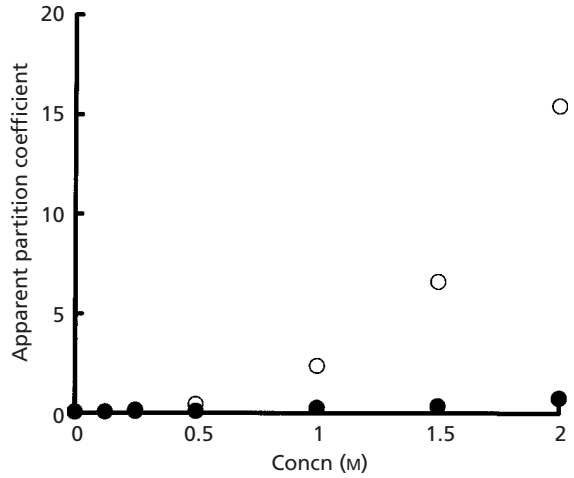

Figure 4 Effect of concentration of oleic acid or lauryl alcohol on the apparent partition coefficient of ondansetron between *n*-hexane and phosphate buffer (pH 6.0) at 32°C. The initial concentration of ondansetron was 100 μM. ○, Oleic acid; ●, lauryl alcohol.

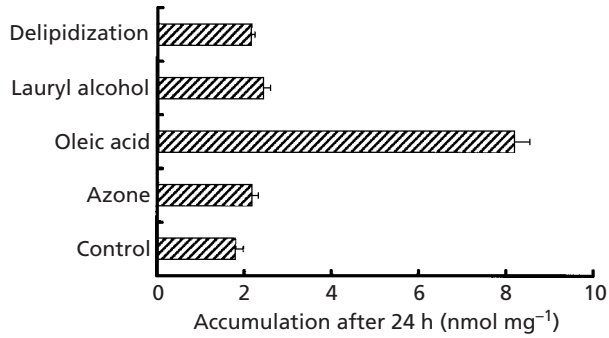

Figure 5 Accumulation of ondansetron in shed snake skin pretreated with enhancer at 32°C. Enhancer (100 μL) was applied to shed snake skin (4 cm^2) 2 h before the experiment. Each point represents the mean ± s.d., n = 3–5.

with drugs and fatty alcohol may affect the partition of drugs to the lipid phase. To investigate the effect of oleic acid or lauryl alcohol on the partition to the lipid phase, the partition coefficient of ondansetron between *n*-hexane and buffer solution was obtained. *n*-Hexane is well known as a non-hydrogen bonder. The partition coefficient increased as the concentration of oleic acid in *n*-hexane increased, and a slight increase was observed in lauryl alcohol (Figure 4). To further investigate the effects of enhancers, the accumulation of ondansetron in shed snake skin pretreated with enhancer was estimated. With lauryl alcohol and Azone, the accumulation of ondansetron increased slightly compared with the control (but was not significant). However, the increase was dramatic with oleic acid pretreatment. These results suggest two enhancement mechanisms of oleic acid: a direct effect on skin or via a counter ion of ondansetron forming an ion-pair (Figure 5).

There were no significant differences between the lag time observed for control or ethanol solutions and enhancers (Tables 1 and 2). However, longer lag times were observed for the combination of ethanol and Azone or lauryl alcohol compared with control (Table 3). A lag time is inversely proportional to the diffusivity of a drug in the skin. From these results, it is considered that Azone and lauryl alcohol may suppress the diffusivity of ondansetron in the skin. Further investigation may be need to clarify the detailed mechanism underlying the effects of a combination of ethanol and enhancer on the permeation of ondansetron.

The permeability of compounds through shed snake skin is similar to that through human skin (Itoh et al 1990; Rigg & Barry 1990). To determine the possibility of transdermal administration of ondansetron, kinetic analysis was done using the permeation parameters observed in this study. The recommended oral dosing regimen of ondansetron for emetogenic neoplastic agents is 8 mg three times a day. Hsyu et al (1994) reported the pharmacokinetic parameters of ondansetron in man. The total body clearance was 26 L h^{-1}, and the maximum plasma concentrations were 40 and 26 ng mL^{-1} after oral and rectal administration of 8 mg ondansetron, respectively. In the present study, 23.47 μg ondansetron penetrated every cm^2 of skin per hour. Using 25 cm^2 of skin, a plasma concentration of approximately 22 ng mL^{-1} can be expected.

In conclusion, the permeability of ondansetron through shed snake skin was very low. Ethanol enhanced the permeability in a concentration-dependent manner. Azone, oleic acid and lauryl alcohol also enhanced the permeability, and further enhancement was possible by combining ethanol and these enhancers. Oleic acid may act via two mechanisms: a direct effect on skin or via a counterion. The maximum flux observed in this study should be high enough for a therapeutic effect of ondansetron.

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