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Research paper

Effect of *N*-methyl-2-pyrrolidone on skin permeation of estradiol

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Abstract

N-Methyl-2-pyrrolidone (NMP) increased the skin permeation of estradiol (E2) in Yucatan micropig epidermis using a modified Franz-type diffusion cell. The addition of NMP significantly increased the fluxes of E2 from water and soybean oil. The flux and skin concentration of E2 were higher from soybean oil than from water and increased with increasing NMP concentrations in soybean oil. Correlation was observed with E2 flux and skin concentration ($R^2 = 0.804$). NMP enhanced E2 skin permeation because NMP made E2 skin concentration higher. Thus, NMP (10%) was added to the oily gel made by isocetyl isostearate and hydrogenated phospholipid. E2 permeation from the gel without NMP was the same as that from soybean oil suspension. The flux of E2 from the gel with NMP was 0.6 μg/h per cm^2 and might be sufficient for estrogen replacement therapy.

Keywords: *N*-Methyl-2-pyrrolidone; Estradiol; Skin permeation; Oily gel; Yucatan micropig skin; In vitro

1. Introduction

Estradiol (E2) is prescribed in estrogen replacement therapy to prevent bone loss and relieve climacteric symptoms of menopause. E2 is metabolized almost completely after oral administration because of the first pass effect. Transdermal drug delivery, which is a well-accepted delivery system for minimizing the first pass effect, is an attractive approach for the long-term treatment of postmenopausal symptoms [1,2].

E2 is almost insoluble in water. Drugs that penetrate the skin are dissolved in the vehicle and partition from the vehicle to the stratum corneum [3]. In the case of insoluble drugs, such as E2, low solubility of drugs in the stratum corneum causes the low flux of the drugs. Changes in the vehicle can affect solubility. For example, an increase in ethanol concentration in the vehicle increased solubility of some drugs such as E2 and ethanol concentrations were also directly related to the drugs' fluxes through the skin of hairless rats [4]. Unfortunately, ethanol often causes skin problems in humans [5].

N-Methyl-2-pyrrolidone (NMP) is known as a good solvent for sparingly soluble drugs in water. The solubility parameter of NMP (23.0 $MPa^{1/2}$) is similar to those of ethanol (26.7 $MPa^{1/2}$) and dimethyl sulfoxide (DMSO) (26.5 $MPa^{1/2}$). In view of partial solubility parameters, NMP is similar to DMSO, with δ_D, δ_P and δ_H of NMP at 18.0, 12.3 and 7.2 $MPa^{1/2}$, respectively, and those of DMSO at 18.4, 16.4 and 10.2 $MPa^{1/2}$, respectively [6]. As for NMP, it has been reported that skin irritation is low for pyrrolidone derivatives [7]. In addition, NMP increased transdermal absorption of some drugs such as phenolsulfonphthalein, ibuprofen and flurbiprofen [8,9].

In this investigation, we examined the effect of NMP on E2 permeation using Yucatan micropig (YMP) epidermis, which is similar to human skin with regards to the permeation rate of drugs [10]. Also, E2 and NMP concentrations in skin were measured to clarify mechanisms of enhancement. In addition, the permeation of E2 from an oily gel, a kind of ointment consisting of isocetyl isostearate (ICIS) and hydrogenated soybean phospholipid (HSL), with or without NMP was studied.

2. Materials and methods

2.1. Materials

E2 was obtained from Sigma Chemical (St Louis, USA). NMP (Pharmasolve™, Japanese Pharmaceutical Excipient

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grade) was a gift from ISP Japan (Tokyo). Soybean oil was provided from Nikka Yushi (Tokyo). ICIS and HSL were gifts from Nikko Chemicals (Tokyo). All other chemicals were reagent grade and were used without further purification.

2.2. Measurement of solubility

The solubility of E2 was measured in various NMP and ethanol concentrations. E2 was mixed with NMP or ethanol; water or soybean oil was added to each solution, and they were equilibrated at 37 °C for 24 h. Samples were centrifuged for 15 min at 3000 rev./min and E2 was quantified using HPLC.

2.3. Preparation of samples for skin permeation study

All formulations contained 2% of E2. E2 was dissolved in NMP and added to water or soybean oil or directly added to water or soybean oil. E2 was present as a suspension in water or soybean oil and a solution in soybean oil when NMP was added at 10–30%. The prepared suspension and solution were kept at 37 °C overnight.

The oily gel was prepared by the method reported previously [11]. Briefly, E2, HSL and NMP were added to ICIS in a flask and capped tightly, then heated at 95 °C with stirring until a homogeneous solution was obtained. The solution was packed into ointment tubes and cooled to 20 °C. Then they were maintained at 37 °C for 3 days.

2.4. Skin permeation study

Yucatan micropig skin (YMP skin set, Charles River Japan, Yokohama), after removal of the fat and sub-dermal tissue, was immersed in water at 60 °C for 45 s and the epidermal membranes were teased off the underlying dermis [12]. The epidermis was placed on a cellulose acetate membrane filter with a pore size 0.8 μm (Toyo Roshi Kaisya, Tokyo) and positioned on a modified Franz-type diffusion cell with an area of 1.1 cm^2. The receptor phase, which consisted of 16 ml of isotonic phosphate buffer solution (pH 7.1) containing 0.1% γ-cyclodextrin and 0.01% kanamycin, was kept at 37 °C with stirring at 600 rev./min. γ-Cyclodextrin was added to maintain the E2 sink condition in the receptor phase [13]. The sample (solution and suspension, 0.2 ml; oily gel, 0.1 g) was applied to the donor phase. A glass ball was used to occlude the upper portion of the donor phase. At predetermined intervals, 0.2 ml of the receptor phase was withdrawn and an equivalent volume of fresh solution was added. The E2 and NMP concentrations in the receptor phase were determined by HPLC. After the permeation studies, skin samples were washed with purified water followed by 75% methanol in water and then homogenized. Homogenates were centrifuged and concentrations of E2 and NMP in the supernatants were determined using HPLC.

2.5. Release study

Release of E2 from the oily gel was determined by a procedure similar to the skin permeation study except that only a membrane filter and not the epidermal membrane was used.

2.6. Determination of E2 and NMP by HPLC

The concentrations of E2 and NMP were determined using HPLC. The analytical system included a pump (LC-10A, Shimadzu, Kyoto), a UV detector (SPD-10AD, Shimadzu) operated at 212 nm for E2, and a refractive index detector (RID-10A, Shimadzu) for NMP. Samples were injected using an autoinjector (SIL-10AD, Shimadzu). The column (Wakosil-II-5C18 HG, Wako Pure Chemical, Osaka) was eluted at ambient temperature with mobile phases of acetonitrile: 0.1% phosphoric acid (6:4) for E2 and methanol:water (1:9) for NMP at a flow rate of 1.0 ml/min.

2.7. Data analysis

The cumulative amounts of permeated E2 or NMP were plotted as a function of time. The flux was calculated from the slope of the linear portion of the profiles. The lag time was the *x*-intercept of the above linear fit of the permeation profile. For statistical analysis, the data were subjected to analysis of variance (ANOVA) followed by the Fisher's PLSD test. Differences of $P < 0.05$ were considered significant.

3. Results and discussion

3.1. Effect of NMP on E2 solubility

The solubility of E2, which was about 1.0 μg/ml in water, significantly increased after adding NMP or ethanol (Fig. 1). For example, E2 solubility increased to 850 μg/ml in 40% NMP and 350 μg/ml in 40% ethanol. E2 solubility was higher in soybean oil (1.2 mg/ml) than in water. Ethanol could not mix with soybean oil homogeneously, but NMP was well mixed with soybean oil and increased E2 solubility. When 10% NMP was added to soybean oil, E2 solubility was more than 20 mg/ml. Thus, NMP is a more effective solvent than ethanol in improvement of E2 solubility.

3.2. Effect of NMP on the skin permeation of E2

The permeation of E2 from both water and soybean oil without NMP was extremely low at about 2 μg/cm^2 after 24 h application (Fig. 2). The addition of NMP significantly increased the permeation of E2. The fluxes of E2 from 30%

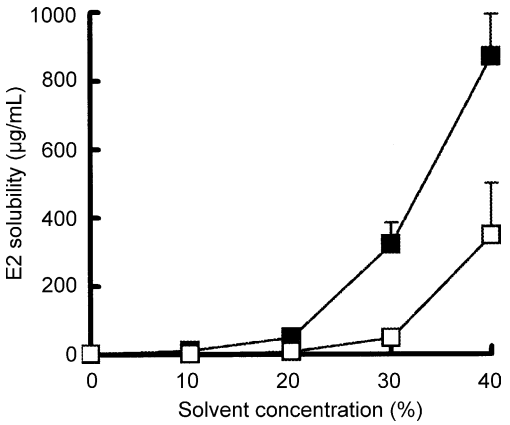

Fig. 1. Solubility of E2 in the NMP/water and EtOH/water mixture at 37 °C. ■, NMP; □, EtOH. Each value represents the mean ± SD of at least three experiments.

NMP in water and oil were 0.2 and 1.8 μg/cm^2 per h, respectively.

E2 skin concentrations for the aqueous and soybean oil formulations without NMP were 0.4 and 1.8 mg/cm^3, respectively (Fig. 3). These values increased to 3.3 and 44 mg/cm^3, respectively, with NMP. The fluxes of NMP itself from water and soybean oil were 0.45 and 1.11 mg/cm^2 per h, respectively, and its lag time was shorter than that of E2 (Fig. 4a). The corresponding concentration of NMP in the skins were 280 and 490 mg/cm^3, respectively (Fig. 4b).

E2 flux was higher from the NMP soybean oil formulation. Thus, we next examined E2 permeation as a function of NMP concentration in soybean oil. The skin concentrations of E2 and NMP as well as E2 fluxes increased with increasing NMP concentrations, however, E2 lag times were not affected by NMP concentrations (Table 1).

There have been some reports about skin permeation enhancement by NMP and related pyrrolidone derivatives,

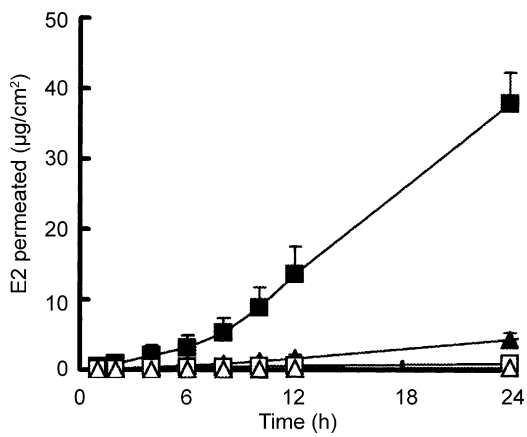

Fig. 2. Effect of formulations on E2 permeation using water or soybean oil as vehicle, with or without NMP. ■, NMP 30% soybean oil formulation; ▲, NMP 30% water formulation; □, soybean oil; △, water. Each value represents the mean ± SD of three experiments.

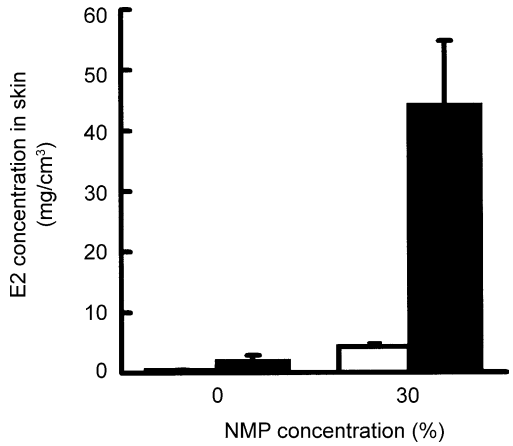

Fig. 3. Effect of formulations on E2 skin concentration using water or soybean oil as vehicle, with or without NMP. □, NMP water formulation; ■, NMP soybean oil formulation. Each value represents the mean ± SD of at least three experiments.

and the mechanism of enhancement effect was reported differently. Akhter and Barry reported that NMP enhanced the skin permeation of flurbiprofen and ibuprofen applied as solid drug film because the dissolution step was removed, and thermodynamic activity of the drug remaining in the solution increased after rapid permeation of NMP and NMP changed the diffusional resistance of the skin [9]. Bennett et al. reported that skin retention of betamethasone 17-benzoate was improved by application as an NMP solution [14]. Sasaki et al. reported the enhancement effect of pyrrolidone derivatives, showing that the skin permeation of NMP itself and enhancement effect on drug permeation were lower from an aqueous vehicle than from a lipophilic vehicle (isopropyl myristate) and demonstrated that a reduction of NMP penetration decreased the enhancement effect [8,15]. Yoneto et al. reported that *N*-alkyl-pyrrolidone derivatives, although NMP was not included, increased drug solubility in skin and also fluidized lipids in stratum corneum [16,17]. Seki et al. reported that the zidovudine permeation rate was reduced with time due to the lower permeation rate of NMP, because NMP might act as a carrier for zidovudine [18]. All the mechanisms of skin permeation enhancement were suggested in these papers. In summary, the possible mechanisms of enhancement effect of NMP on skin permeation are (1) solubilization of drug in vehicle; (2) increasing thermodynamic activity in vehicle; (3) fluidization of lipid in stratum corneum, reducing the diffusional resistance; (4) co-permeation of NMP and drug; and (5) increasing drug solubility in skin.

In our case, E2 was applied as a suspension or solution. The solubility of E2 in soybean oil was sufficient for the permeation amount. Increasing the concentration of NMP in soybean oil reduced the thermodynamic activity of E2 in vehicle, however, the permeation of E2 increased with NMP concentration. These findings indicate that factors (1) and (2) are not considerable in our case. The lag time did not change with NMP, which suggested no effect of NMP on lipid

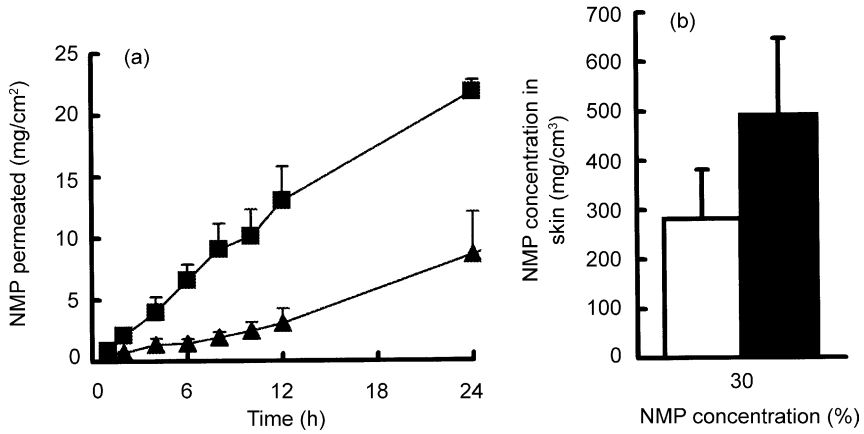

Fig. 4. The effect of formulations on (a) NMP permeation and (b) concentration of NMP in the skin using water or soybean oil as vehicle, and with 30% or without NMP. ■, NMP 30% soybean oil formulation; ▲, NMP 30% water formulation, □, NMP water formulation mixture; ■, NMP soybean oil formulation mixture. Each value represents the mean ± SD of at least three experiments.

fluidity. Increasing chain length of the alkyl group in pyrrolidone derivatives systematically increase the enhancement effect [16,17], therefore NMP, which has a short alkyl chain, may not affect lipid fluidity much. Thus, factor (3) may also be negligible.

E2 flux increased with increasing NMP flux, E2 skin concentration and concentration of NMP in the skin. To define the main factor of permeation enhancement, the relationships between them were examined (Fig. 5). The correlation between NMP flux and E2 flux was highest among them. This suggests that E2 co-permeated with NMP according to the concentration gradient of NMP between donor phase and receptor phase (factor (4)). Accordingly, if the concentration gradient of NMP (NMP fluxes) decreased, then the flux of E2 should also have decreased. Thus, 30% NMP in PBS was used as receptor phase and E2 suspension in 30% NMP in water was applied to the donor phase, so there was no concentration gradient of NMP between donor and receptor phases. The concentration of NMP in the skin was about two times higher when the receptor phase contained NMP. This was because there was no gradient of NMP concentration in the skin. The fluxes of E2, E2 skin concentration and lag time were not changed when the receptor phase was either PBS or 30% NMP in PBS (Table 2). This suggests that E2 co-permeation with NMP, factor (4), was not the main reason for enhancement.

Concentration of NMP in the skin and E2 skin concentration had the same relationship, as did E2 skin concentration and E2 flux. It was considered that NMP partitioned into the skin, further increased E2 solubility in the skin, and consequently, the permeation of E2 was improved, although the correlation factor was lower than that between NMP flux and E2 flux. One of the reasons for the lower correlation is that the fluxes of E2 were calculated from the permeation profiles during 12 h, whereas the skin concentrations were determined after 24 h, thus the values, in particular the NMP concentration applied with soybean oil, might be lower than those after 12 h.

NMP enhanced the skin permeation of E2 because NMP partitioned in the skin and improved the solubility of E2 in the skin, that is to say factor (5) is the main mechanism of enhancement of NMP on the permeation of E2. The effect was greater when NMP was added to soybean oil than to water because NMP partitioning into skin was higher from soybean oil than from water.

3.3. Permeation of E2 from the oily gel

NMP increased E2 partition in the skin and increased E2 permeation through skin. NMP is a candidate for an effective permeation enhancer for transdermal delivery systems of E2. Thus, efficacy of NMP in a dosage form was confirmed. NMP is more effective when it is added to a lipophilic vehicle, hence an ointment was chosen as a dosage form. Oily gel was used for the vehicle because of good combination with NMP. Various kinds of fatty acid ester can be used as a base of oily gel, and ICIS was selected because of its high lipophilicity [11].

Table 1
Effect of the NMP concentrations in soybean oil on E2 fluxes, E2 lag time and skin concentration of E2 and NMP

NMP concentration (%)	E2 flux (μg/cm² per h)	E2 lag time (h)	Skin concentration of E2 (mg/cm³)	Skin concentration of NMP (mg/cm³)
0	ND	ND	1.8 ± 1.1	–
10	0.78 ± 0.20	3.2 ± 0.4	3.0 ± 1.4	138 ± 53
20	1.50 ± 0.07	3.3 ± 1.9	13.0 ± 3.4	173 ± 42
30	1.80 ± 0.51	4.8 ± 0.6	44.0 ± 10.7	490 ± 155

Each value represents the mean ± SD of at least three experiments. ND, not determined.

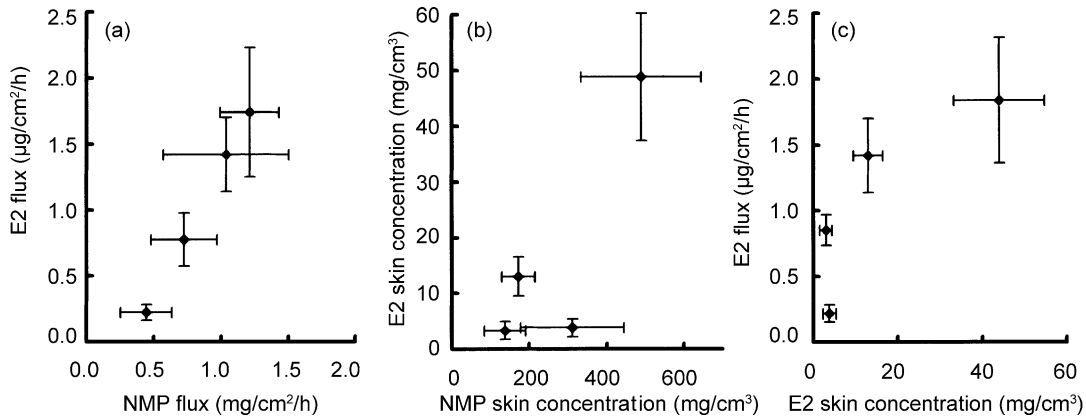

Fig. 5. The relationships between NMP flux and E2 flux (a), concentration of NMP in the skin and E2 skin concentration (b) and E2 skin concentration and E2 flux (c). (a) $R^2 = 0.968$ ($P < 0.01$), (b) $R^2 = 0.779$ ($P < 0.05$), (c) $R^2 = 0.804$ ($P < 0.05$).

Sometimes the release from the dosage form limits the permeation of drugs, thus, the release of E2 from the oily gel was determined. Fig. 6 shows the release profiles of E2 from the oily gel with or without NMP. The release from the gel with 10% NMP was about 3 times that from the gel without NMP. This was probably because E2 solubility in the vehicle became high with NMP. The amount released from the gel without NMP within 2 h was 15 μg/cm^2, and it was expected that the limiting step might be skin permeation, not release from the vehicle.

Table 2
Effect of the receptor phase on E2 fluxes, E2 lag time and skin concentration of E2 and NMP from NMP 30% water mixture

Receptor phase	E2 flux (μg/cm^2 per h)	E2 lag time (h)	Skin concentration of E2 (mg/cm^3)	Skin concentration of NMP (mg/cm^3)
PBS	0.53 ± 0.18	3.5 ± 1.2	3.18 ± 0.84	636 ± 377
NMP 30% PBS	0.45 ± 0.19	5.2 ± 1.6	3.93 ± 1.63	1460 ± 255

Each value represents the mean ± SD of at least three experiments.

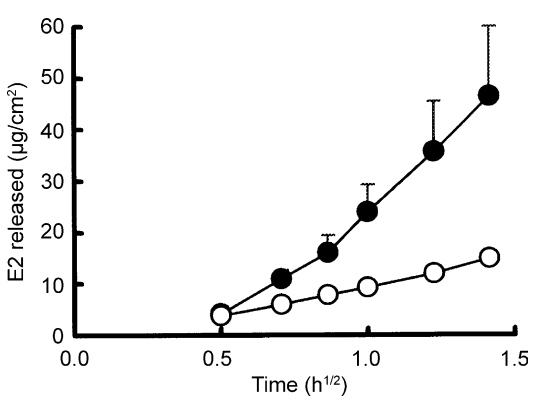

Fig. 6. The release profiles of E2 from the oily gel with or without NMP. ●, with 10% NMP; ○, without NMP.

The permeation profiles of E2 from various formulations are shown in Fig. 7. Some fatty acid esters have an enhancement effect on skin permeation [19], but ICIS showed no effect and E2 permeation from ICIS with 10% NMP showed no significant difference from soybean oil with 10% NMP. The permeation of E2 from the gel without NMP was as low as that from an oil or water suspension, at 1.5 μg/cm^2 after 24 h application. When 10% of NMP was added to the oily gel, the permeation of E2 was as high as that from NMP 10% soybean oil formulation and NMP 10% ICIS formulation. The skin concentration of E2 with or without NMP was 8.5 and 0.2 mg/cm^3, respectively. It suggests that the mechanism of enhancement might be high skin solubility of E2, which is the same as that in the case of soybean oil or water with NMP.

Roux reported that application of 1.5 mg/day as gel or 50 μg/day as patch is effective for preventing the bone loss of postmenopausal condition [20]. Rohr et al. reported that delivery of 25 μg/day is needed for therapy for postmenopausal women [21]. The permeation rate from the oily gel

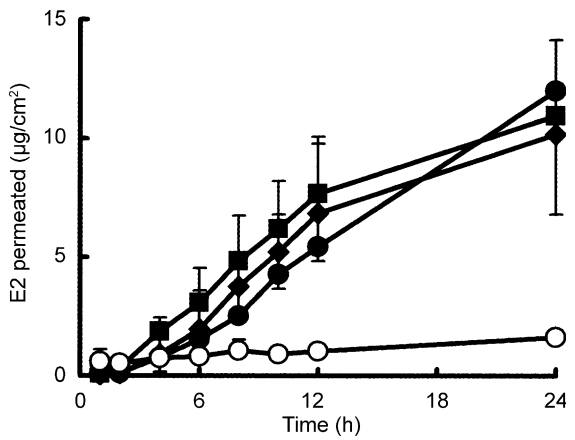

Fig. 7. The permeation profiles of E2 from the oily gel and related formulations. ○, oily gel without NMP; ●, oily gel with 10% NMP; ■, NMP 10% soybean oil formulation; ◆, NMP 10% ICIS formulation.

with 10% NMP was 0.62 μg/cm^2 per h, namely 15 μg/cm^2 per day, thus, sufficient permeation of E2 is expected.

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