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In vitro skin penetration and pharmacodynamic evaluation of prostaglandin E_1 ethyl ester, a vasoactive prodrug of prostaglandin E_1, formulated into alcoholic hydrogels

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Alcoholic hydrogels containing prostaglandin E_1 ethyl ester (PGE_1-EE), a prodrug of PGE_1 as a therapeutic agent for erectile dysfunction, were formulated. The prodrug was stable against chemical hydrolysis in aqueous solution (pH 7.4), devoid of esterase activities, but was hydrolyzed to the parent drug in rat skin homogenates within 240 min. In the rat skin penetration study for 24 h, the steady-state flux values ($\mu g/cm^2/h$) of PGE_1-EE and PGE_1 from alcoholic hydrogels having 20% ethanol content were 7.6 and 1.8, respectively. PGE_1-EE was superior to PGE_1 from a skin penetration point of view due to its increased lipophilicity. The fastest skin penetration rate was obtained for PGE_1-EE in 20% alcoholic hydrogel together with limonene or cineole. These formulations increased the flux of PGE_1-EE up to about 4-fold compared to control hydrogel in the absence of penetration enhancers. In the pharmacodynamic study using a cat, alcoholic hydrogel with limonene or cineole showed a significant effect in terms of increasing intracavernosal pressure compared to control hydrogel. Therefore, the transdermal alcoholic hydrogel formulation of PGE_1-EE with limonene or cineole can be a promising transdermal delivery system to overcome inconvenience associated with frequent intracavernosal injections for the treatment of erectile dysfunction.

1. Introduction

Current treatments for erectile dysfunction include oral medication, vascular surgery, penile prostheses, and intracavernosal injection of vasoactive substances. Among these treatments, the intracavernosal injection has been the most common treatment. However, approximately 50% of men treated with intracavernous injection therapy eventually discontinue the treatment largely due to severe pain upon injection and lack of confidence in self-administration (Maggi et al. 2000). Recently, alternative routes for the administration of vasoactive substances have widely been investigated including topical application (Kim and Mcvary 1995). Up to now, Prostaglandin E_1 (PGE_1) has been known as one of the most potent compounds used for a single-dose pharmacotherapy of erectile dysfunction (Maggi et al. 2000) and is generally administered by intracavernosal injection (e.g., Caverject®). PGE_1 increases the intracellular concentration of 3′,5′-cyclic adenosine monophosphate (cAMP) by activation of specific membrane receptors that interact with stimulatory guanine nucleotide proteins to trigger adenylate cyclase and elevate intracellular cAMP concentration (Palmer et al. 1994). Increasing the intracellular cAMP concentration induces the relaxation of arterial and trabecular smooth muscle in the corpus cavernosum and thereby causes human penile erection (Maggi et al. 2000).

Transdermal delivery of PGE_1 in the treatment of peripheral arterial occlusive diseases has been limited since PGE_1 is chemically labile and penetration through the skin is not rapid enough to be effective. It has been reported that percutaneous penetration of PGE_1 for 48 h was only less than 5% of initial dose. In addition, even in the presence of a mixture of enhancers, the penetration was still less than 10% (Wendel and Braun 1995). Thus, an essential prerequisite for the development of a transdermal delivery system for PGE_1 is that the drug is capable of penetrating the skin with a rate sufficiently high to obtain the desired pharmacologic activity (Rautio et al. 1998). Recently, the use of PGE_1 ethyl ester (PGE_1-EE), a prodrug of PGE_1, has been reported to be delivered transdermally (Wendel and Braun 1995; Frolich and Bippi 1993).

There have been numerous reports on an increase in percutaneous drug absorption primarily achieved by penetration enhancers. Interestingly, prodrug-enhancer combinations offered a significantly effective way to increase transdermal absorption of a wide range of poorly permeable drugs, such as acyclovir (Bando et al. 1996). Therefore, it is anticipated that the use of an ethyl ester derivative of PGE_1 in combination with penetration enhancers would considerably increase the skin penetration and maximise the vasoactive effect of PGE_1. In this study, we for-

mulated alcoholic hydrogels for the transdermal delivery of PGE_1-EE in which various penetration enhancers were incorporated and report *in vitro* characteristics such as hydrolysis of PGE_1-EE in rat skin homogenates and percutaneous transport profiles of PGE_1-EE. In addition, an *in vivo* pharmacodynamic study investigating penile erection in cats was also carried out.

2. Investigations, results and discussion

In the present study, enzymatic hydrolysis of PGE_1-EE in rat skin homogenates and chemical hydrolysis or degradation of PGE_1-EE in aqueous buffer solution were first investigated. This method has previously been applied in assessing bioconversion of propranolol prodrugs for dermal delivery (Kasting et al. 1992; Ahmed et al. 1995).
Sheu et al. (1998) suggested that assessing the bioconversion of a prodrug could be made by detection of a parent drug in the receiver solution during *in vitro* skin penetration studies; the formation of PGE_1 from PGE_1-EE during passage through the skin might occur by either enzymatic or chemical hydrolysis. In this study, however, as PGE_1-EE showed substantially higher chemical stability in aqueous buffer solution of pH 7.4 for 5 h (unpublished results), the conversion of PGE_1-EE to PGE_1 by skin esterases was considered to be the predominant degradation pathway of PGE_1-EE in the skin. For exact quantification, it was necessary to simultaneously analyse levels of PGE_1-EE and PGE_1 in the rat skin homogenates. HPLC showed that the separation of PGE_1 and PGE_1-EE from matrix constituents of the homogenates was sufficient for reliable quantification (Fig. 1). The retention times for PGE_1 and PGE_1-EE were 4.6 and 13.2 min, respectively. The hydrolysis was monitored by quantification of residual amounts of PGE_1-EE and produced amount of PGE_1. It was demonstrated by the HPLC chromatograms that only PGE_1-EE existed at 0 min and only PGE_1 was detectable after 240 min as PGE_1-EE was fully converted to PGE_1 after 240 min. At 30 min, an unknown peak was detected at a retention time of 9.5 min (Fig. 1). Fig. 2 shows a degradation profile of PGE_1-EE as a function of time and the concurrent formation of PGE_1 in rat skin homogenates. Unexpectedly, during the conversion of PGE_1-EE, the total amount of PGE_1-EE and PGE_1 was not consistent with the initial amount of PGE_1-EE added to the skin homogenates. This means the fact that even though the degradation of PGE_1-EE was completed within 100 min, the generation of PGE_1 was

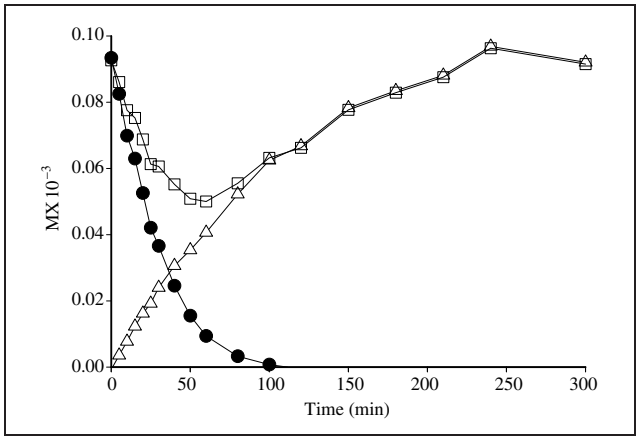

Fig. 2: Time-course profiles of concentration change in PGE_1 ethyl ester and PGE_1 during hydrolysis of PGE_1 ethyl ester in rat skin homogenates at 37 °C (●; PGE_1 ethyl ester, △; PGE_1, □; PGE_1 ethyl ester + PGE_1)

finished after 240 min. This is probably attributed to the fact that the formation rate of the parent drug is much slower than the degradation rate of PGE_1-EE in rat skin homogenate solution. This slower formation of PGE_1 might be responsible for the appearance of an intermediate metabolite detected in HPLC chromatograms at a retention time of 9.5 min.
In an *in vitro* rat skin penetration study for 24 h, only PGE_1 was detected in the receptor medium, indicating a total conversion of PGE_1-EE to PGE_1 during the skin penetration within 24 h. The skin penetration period (24 h) was long enough to allow bioconversion of the prodrug to the parent drug when considering complete hydrolysis of PGE_1-EE within 240 min. Since saturated solubility of PGE_1-EE is 330 μg/ml in 10% ethanol solution (i.e. receptor solution) as measured by HPLC, an 11 ml of 10% ethanol solution was enough to dissolve PGE_1-EE and it could maintain perfect sink conditions. Fig. 3 (A) shows the skin penetration profiles of PGE_1-EE from alcoholic hydrogels with various ethanol contents in rats. The steady-state fluxes (μg/cm^2/h) of PGE_1-EE from alcoholic hydrogels containing 0, 20, 30 and 40% ethanol were 3.72, 7.60, 7.69 and 6.38, respectively. The alcoholic hydrogels containing 20% or 30% ethanol showed a twofold higher flux value than that of ethanol-free hydrogel. The penetration promoting activity of ethanol was maximised at about 20–30% ethanol contents for PGE_1-EE. This might

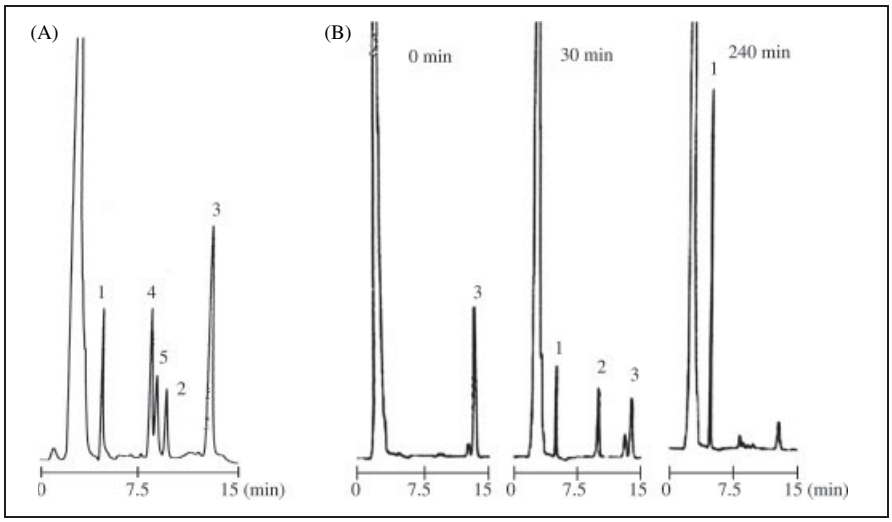

Fig. 1:
HPLC chromatograms (absorbance vs. retention time) for (A); standard solutions of (1) PGE_1 (retention time; 4.6 min), (2) intermediate metabolite (9.5 min), (3) PGE_1 ethyl ester (13.2 min), (4) PGA_1 (8.2 min) and (5) PGB_1 (8.7 min), respectively, (B); (1) PGE_1, (2) intermediate metabolite (9.5 min) and (3) PGE_1 ethyl ester (13.2 min) found in rat skin homogenates as time elapses (0 min, 30 min, and 240 min)

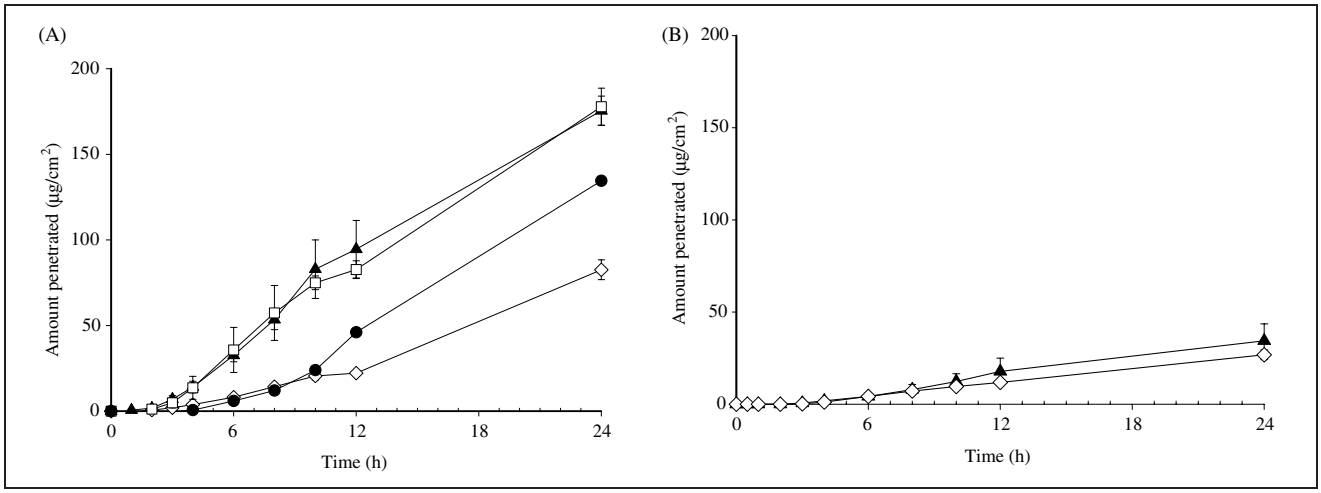

Fig. 3: Penetration profiles of PGE_1 ethyl ester (A) and PGE_1 (B) through rat skin from alcoholic hydrogels with various ethanol contents (◇; 0%, ▲; 20%, □; 30%, ●; 40%). Mean ± SD, n = 7

demonstrate an optimal composition required to maximise its thermodynamic activity by solubilising the drug. The ethanol can increase the solubility of PGE_1-EE in the alcoholic hydrogels so that the release of PGE_1-EE may also increase, probably leading to an increase in the penetration rate of PGE_1-EE. Moreover, the ethanol released from alcoholic hydrogels may interact with the lipid components of the skin causing an increase in the lipid fluidity, resulting in the improved permeability of the skin by reducing its resistance to a permeant (Puglia et al. 2001). The mechanism by which ethanol induces microstructural changes and increased diffusivity is not fully understood. However, lipid extraction and osmotic expansions might have been the most acceptable explanations (Magnusson et al. 1997).

To test the advantages of PGE_1-EE over PGE_1 in terms of skin penetration rate (Fig. 3 and Table 1), both were formulated to alcoholic hydrogels with 20% ethanol, which was considered the optimal ethanol content for maximal penetration rate. The steady-state flux (μg/cm²/h) of PGE_1 was calculated to be 1.8, which is about 4 times lower than that of PGE_1-EE (i.e. 7.60 μg/cm²/h). This result demonstrates that PGE_1-EE was superior to PGE_1 with regard to skin penetration ability due to its increased lipophilicity.

Although alcoholic hydrogel formulations increased the skin penetration rate of PGE_1-EE, there existed lag times of about 2–4 h for all formulations tested. To further increase the penetration rate, skin penetration enhancers such as isopropyl myristate, polyethylene glycol 400, oleic acid, cineole, and limonene were added at levels 5% to the 20%

Table 1: Steady-state flux and lag time of PGE_1 ethyl ester and PGE_1 from 20% alcoholic hydrogel and control gel (0% ethanol) through rat skin

Preparation	Flux (μg/cm²/h)	Lag time (h)
Alcohol-free hydrogel (0% ethanol, control)		
PGE_1 ethyl ester	3.7 ± 0.3[a]	3.4 ± 0.2
PGE_1	1.2 ± 0.1	2.8 ± 0.1
20% alcoholic hydrogel		
PGE_1 ethyl ester	7.6 ± 0.6[a, b]	2.0 ± 0.0
PGE_1	1.8 ± 0.1	3.1 ± 0.1

Mean ± SD, n = 7
[a] Significantly different from PGE_1 at $P < 0.05$
[b] Significantly different from PGE_1 ethyl ester control at $P < 0.05$

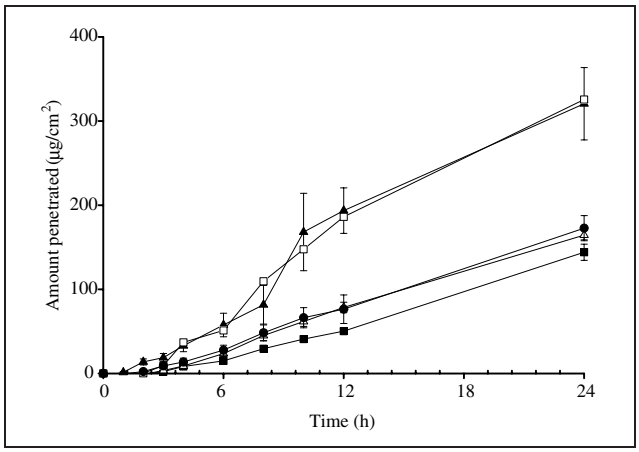

Fig. 4: Rat skin penetration profiles of PGE_1 ethyl ester from 20% alcoholic hydrogels with or without penetration enhancers (●; enhancer-free, ■; polyethylene glycol 400, △; isopropyl myristate, ▲; limonene, □; cineole). Level of penetration enhancers was 5%. Mean ± SD, n = 7

ethanolic hydrogel formulations as shown in Fig. 4. Based on the flux values obtained (Table 2), all the penetration enhancers tested in this study did not produce a significant increase in the percutaneous fluxes of PGE_1-EE. The best penetration enhancement effect for PGE_1-EE was obtained upon coadministration of limonene or cineole with 20% alcoholic hydrogels. The steady-state flux of PGE_1-EE increased about 4-fold compared to the control gel without

Table 2: Steady-state flux and enhancement ratio (ER) of PGE_1-EE from alcoholic hydrogel with various enhancers in rat skin penetration

Enhancer	Flux (μg/cm²/h)	Lag time (h)	ER*	ER**
Limonene	13.9 ± 1.6[a]	1.1 ± 0.1[a]	3.74	11.58
Cineole	14.1 ± 1.3[a]	1.5 ± 0.1[a]	3.79	11.75
Isopropyl myristate	7.8 ± 0.6[a]	2.5 ± 0.1	2.09	6.50
Polyethylene glycol	6.8 ± 0.5[a]	3.4 ± 0.2	1.82	3.74
Control (PGE_1-EE)	3.7 ± 0.3	3.4 ± 0.2	1	–
Control (PGE_1)	1.2 ± 0.1	2.8 ± 0.1	–	1

The control group did not contain ethanol (i.e. 0% ethanol). Mean ± SD, n = 7.
* Flux of PGE1-EE at steady state in the presence of enhancers/Flux of PGE_1-EE control.
* Flux of PGE1-EE at steady state in the presence of enhancers/Flux of PGE_1 control.
[a] Significantly different from PGE_1-EE control (i.e. no ethanol and penetration enhancer) at $P < 0.05$

penetration enhancers. Compared to PGE_1 control gel, the flux increased about 12-fold and the lag time was also reduced to some extent (Table 1). These results demonstrate that terpenes such as limonene and cineole are the most effective penetratation enhancers for PGE_1-EE in alcoholic hydrogels. In general, penetration enhancers are known to act by one or more of three possible penetration enhancing mechanisms: disruption of the highly ordered lipid structures of the stratum corneum, interaction with intracellular proteins, and improvement in partitioning of a drug, coenhancer, or cosolvent into the stratum corneum (Magnusson et al. 1997). For terpenes, it was considered that terpenes (limonene and cineole) act as penetration enhancers probably by disrupting the lipid structures of the stratum corneum, thereby causing easy pass of PGE_1-EE across the lipid domain of the skin. This can be backed by that D-limonene creates highly permeable micro-pores in intracellular lipid bilayers through which more drugs are able to pass (Cornwell et al. 1996).

Since the results obtained by *in vitro* studies were quite promising, we thought it was necessary to further investigate the pharmacodynamic activity of PGE_1-EE in alcoholic hydrogels containing limonene or cineole. There were several reports that intracavernous pressures (ICP, mmHg) can be measured in cats to investigate the effects of intracavernous injections of vasoactive agents on penile erectile responses (Wang et al. 1993; Champion et al. 1997).

Fig. 5 shows ICP measurements after topical application of PGE_1-EE in alcoholic hydrogels in the absence and presence of limonene or cineole on the cat penis. Alcoholic hydrogels with limonene or cineole demonstrated a significant increase in ICP values as 102 ± 3, 103 ± 5 mmHg, and the lag times of 20 and 30 min, respectively. ICP values of PGE_1-EE in alcoholic hydrogels with limonene or cineole were equivalent to that of PGE_1 intracavernosal injection (Kim et al. 2000). Unexpectedly, the control hydrogel without limonene or cineole showed no effect at all in terms of increasing ICP. Thus, our results suggest that topically applied PGE_1-EE alcoholic hydrogels in combination with limonene or cineole were as effective as PGE_1 intracavernosal injection in terms of increasing ICP in cats.

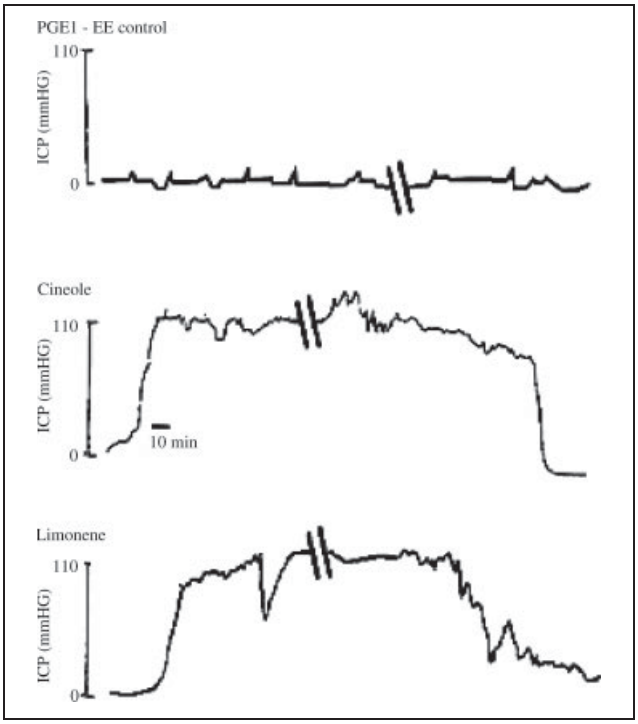

Fig. 5: Measurements of intracavernosal pressure (mmHg) in cats with Statham pressure transducer after topical administration of PGE_1 ethyl ester alcoholic hydrogels. The onset times obtained with PGE_1 ethyl ester alcoholic hydrogels having cineole and limonene were 20 and 30 min, respectively. PGE_1 ethyl ester alcoholic hydrogels having cineole and limonene resulted in ICP values of 102 ± 3 and 103 ± 5 mmHg and these values were maintained for about 4 h

In conclusion, PGE_1-EE, a prodrug of PGE_1, has several advantages to be developed to a topical preparation for the treatment of erectile dysfunction. Firstly, PGE_1-EE alcoholic hydrogel formulations can greatly increase skin penetration rate due to increased lipophilic property of PGE_1-EE, in combination with suitable enhancers. Secondly, topical application of PGE_1-EE can also improve patient compliance in comparison to the conventional preparations including intracavernosal injection and intraurethral injection. Finally, PGE_1-EE alcoholic hydrogel formulations show an effectiveness similar to that shown by PGE_1 intracavernosal injection in terms of increasing ICP even though there were lag times before displaying *in vivo* pharmacological response. Thus, it is expected that PGE_1-EE alcoholic hydrogels might be a promising topical delivery system of PGE_1 for the treatment of erectile dysfunction.

3. Experimental

3.1. Materials

PGE_1 ethyl ester (ethyl (11α,13*E*,15(*S*))-11,15-dihydroxy-9-oxoprosta-13-en-1-oate) and PGE_1 (11α,13*E*,15(*S*))-11,15-dihydroxy-9-oxoprosta-13-en-1-oic acid) were provided by Cascade Biochem Ltd. (Cork, Ireland). (*R*)-(+)-Limonene and cineole were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Other chemicals and solvents were of reagent grade and used as received.

3.2. Hydrolysis of PGE_1 ethyl ester in rat skin homogenates

All surgical operations to prepare rat skin homogenates (Ahmed et al. 1995) were carried out at 4 °C. After sacrificing rats by decapitation the rat dorsal skin was carefully shaved and excised. The fat and muscular tissues as well as capillaries adhering to the dermis were thoroughly removed. The skin was minced, mixed with five volumes of cold tris-buffer (pH 7.4) containing 0.15 M KCl, and subjected to a tissue homogenizer (Ultra Turrax T25, Ika Labortechnik, Germany) at 19,000 rpm. The homogenates were centrifuged at 10,000 × g for 20 min at 0 °C. The supernatant was further centrifuged at 100,000 g for 1 h with a Centrikon T-1170 ultracentrifuge (Kontron, Milano, Italy). The resulting supernatant was stored at −80 °C until used in the hydrolysis experiments.

The hydrolysis of PGE_1-EE in rat skin homogenates was carried out at 37 °C. It was initiated by addition of stock solution of PGE_1-EE in tris-buffer with 2 ml of rat skin homogenates, which had been pre-incubated at 37 °C for 10 min. At appropriate time intervals, an aliquot (50 µl) was withdrawn and added to 100 µl of methanol on ice bath to prevent further hydrolysis. After centrifugation the clear supernatant was analyzed for PGE_1 by RP-HPLC.

3.3. Preparation of alcoholic hydrogel formulations

Alcoholic hydrogels were prepared by adding aqueous sodium- carboxymethylcellulose solution (3%w/v) to an ethanol solution of PGE_1 or PGE_1-EE with constant stirring. Penetration enhancers including isopropyl myristate, polyethylene glycol 400, oleic acid, cineole and limonene were dissolved in ethanol (5%w/v) and the ethanol solution was used when preparing the alcoholic hydrogels for the *in vitro* skin penetration study. The resulting hydrogels were stored in a fridge for 24 h prior to use.

3.4. In vitro skin penetration study

Sprague Dawley rats (7–8 weeks old, 120 ± 10 g) were supplied by Han Lim Laboratory (Suwon, Korea). The rats were sacrificed by cervical dislocation before experiments and then the dorsal skin was carefully shaved and excised. The penetration study was performed using Franz-type diffusion cells having a receptor volume of 11 ml and a donor compartment

with an effective diffusion area of 2 cm^2, mounted with the excised rat skin. The receptor compartment was filled with a mixture of ethanol/water (10 : 90 v/v) to ensure perfect sink conditions, maintained at 37 °C by a circulating water jacket. The alcoholic hydrogels (1 g) were placed on a skin surface and the donor compartment was sealed with a silicone-greased cover glass to prevent vapourisation of water and ethanol. Samples from the receiver compartment (100 µl) were withdrawn at appropriate time intervals and analyzed for PGE_1 and PGE_1-EE by RP-HPLC.

3.5. HPLC assay

The HPLC system (Hitachi, Tokyo, Japan) consisted of L-7100 pump, L-4200H UV-Vis detector and D-2500 Chromato-Integrator. The separation was performed on Capcell Pak C_{18} column (5 µm, 4.6 mm I.D. × 250 mm). The mobile phase used was 50% acetonitrile in 20 mM phosphate buffer (pH 3.3) with a flow rate of 1.0 ml/min and the detector was set at 202 nm.

3.6. In vivo pharmacodynamic study

Mature male cats (Samyook Laboratory Animals, Taenoung, Korea) weighing 2.5–4.0 kg were used. A 23 gauge syringe was inserted into the right and left side of corpus cavernosum. After external application of alcoholic hydrogels containing PGE_1-EE at a dose of 1.2 mg as PGE_1, intracavernosal pressure (ICP; mmHg) was measured with Statham pressure transducer (P23 ID) connected to Grass Model 7 polygraph (Grass Instrument Co., Quincy, MA, USA). Penile length (mm) was also measured with a ruler.

3.7. Statistical analysis

The results were statistically analysed using ANOVA and P values less than 0.05 were considered significant except where stated otherwise.

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