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一、中文摘要

此研究計劃以 indomethacin 為模式藥物進一步對經皮輸藥系統促進穿透速率方法從事研究及探討。今擬以經皮吸收研究上之物理促進方法—超音波促進輸藥法 (sonophoresis; phonophoresis) 並配合 capsaicin 及 nonivamide 為經皮吸收促進劑進行經皮輸藥系統之研究並闡明其機轉。於體外試驗方面，主要是以 cellulose 人工膜、裸鼠離體皮膚及人體上表皮層皮膚為滲透障壁，以 Franz vertical diffusion cell 為測試儀以檢測不同藥物穿透滲透障壁之穿透速率 (flux)。施藥端充填定量之不同藥物處方，而促進劑及超音波之給予方式除了直接加入或施放於處方中以外，另外也以前處理方式先使皮膚產生結構性改變再投與含藥處方已避免促進劑對處方之 thermodynamic activity 產生變化的影響，而受藥端則充填 pH 7.4 之緩衝液以模擬實際體內情況。以 capsaicin 之促進效果較佳。因此預測 capsaicin 及其衍生物會比 Azone 更容易穿透皮膚到達受藥端中，併合超音波後亦有協同作用產生，因此辣椒素及超音波皆可作為 indomethacin 之新穎促進方法。

關鍵詞：辣椒素、超音波、經皮輸藥系統

Abstract

The study was conducted in vitro to investigate the changes of indomethacin transdermal permeation pretreated by capsaicin and nonivamide, two compounds chemically similar to Azone. The combined effect of low frequency ultrasound (20 kHz) and enhancers on the indomethacin across nude mouse skin. Enhancement effects of both analogues were very similar and depended predominantly on the concentration tested. Histological examination coupled with visual scores indicated the safety of capsaicin and nonivamide on skin structure. Simultaneous application of ultrasound and enhancers significantly increased skin permeation of indomethacin compared with either ultrasound or enhancers alone. Better effect was obtained by the combination with capsaicin than nonivamide.

Keywords: capsaicin, low-frequency ultrasound,

transdermal delivery system

二、緣由與目的

Many investigators have examined the possibility of the percutaneous delivery of drugs, but a major difficulty is the impermeability of stratum corneum. An effective method employed to reduce this effect of diffusional barrier is to use permeation enhancers. One of the most promising permeation enhancers is Azone (larrocacpram, 1-dodecylazacycloheptan-2-one). It is non-irritant to human skin and hardly absorbed through skin. Azone consists of a seven-membered ring and a long hydrocarbon side chain which appears to act by inserting itself into the structured lipids located in the intercellular routes. This fluidity of microenvironment of lipids is reduced and the permeation of drugs enhanced. Capsaicin (8-methyl N-vanillyl-6-nonenamide; $C_{18}H_{27}NO_3$), the pungent principle of red pepper, has a variety of therapeutic advantages such as antinociceptive, hypotension and hypolipidemia activities. Besides the pharmacological actions, capsaicin itself can act as a skin permeation enhancer for naproxen because of the similarity of chemical structure to Azone. Nonivamide (N-nonanoyl; $C_{17}H_{27}NO_3$) is one of the synthetic analogues of capsaicin which has the similar chemical structure and pharmacological profiles to those of capsaicin. The main difference in structures of capsaicin and nonivamide is in the long alkyl chain. A side chain of $-CH_3$ and a double bond in alkyl chain is observed in capsaicin but not in nonivamide. It can be a substitute of capsaicin because of the lower cost than capsaicin extracted from natural products. In our previous study, the incorporation of nonivamide could increase the in vivo transdermal permeation of sodium nonivamide acetate.

The aim of this study is to investigate the effect of capsaicin and nonivamide on the in vitro transdermal permeation of indomethacin. The present study also quantifies the skin irritation and damage produced by application of these enhancers on skin. The developed method presented in this study is able to distinguish between epidermal and dermal changes in histological structure, thereby allowing differentiation between mild and moderate irritants. This study also examines the combined effect of low frequency ultrasound (20 kHz) application together

with capsaicin or nonivamide.

三、結果與討論

The diffusion cell used in present in vitro study was Franz vertical diffusion assembly. The dorsal skin of female nude mouse (10-12 weeks old) was used as the barrier membrane. One milliliter of enhancer with different concentrations (1, 3 and 5 %) in ethanol/water or PG/water (1:1, v/v) vehicle was applied to the skin surface for 2h by the conclusion dressing technique. The applied area was then gently swabbed clean with cotton and rinsed with distilled water. One milliliter of indomethacin (1%, w/v) was then applied to the treated skin. The donor compartment was covered with parafilm. The receptor medium (10 ml) was composed of 50% ethanol and 50% pH 7.4 buffer. The receptor compartment was kept at a constant temperature of 37°C and stirred by a magnetic stirrer at 600 rpm. Samples (0.3 ml) were withdrawn from the receptor solution as added. Samples were analyzed using HPLC system described previously.

The enhancing effect of 3% Azone, capsaicin and nonivamide on the permeation of indomethacin as the cumulative amount-time profiles. The slopes of the resulting plot from 0 to 12 h was computed and the flux ($\mu\text{g}/\text{cm}^2/\text{h}$) was calculated from the slope. The skin flux of indomethacin pretreated by Azone, capsaicin and nonivamide in ethanol/water (1:1, v/v) vehicle was 69.32 ± 11.35 , 23.24 ± 2.47 and 23.74 ± 4.35 $\mu\text{g}/\text{cm}^2/\text{h}$ individually, which was significantly higher (*t*-test, $P < 0.05$) than that of control group (15.47 ± 4.13 $\mu\text{g}/\text{cm}^2/\text{h}$). The cumulative amounts of capsaicin and nonivamide in the receptor compartment after pretreatment was performed. It indicated that capsaicin and nonivamide greatly permeated across the skin. The flux of capsaicin (56.54 ± 3.33 $\mu\text{g}/\text{cm}^2/\text{h}$), which was slightly higher than that of nonivamide (50.41 ± 4.73 $\mu\text{g}/\text{cm}^2/\text{h}$) which was similar to our previous report.

The permeation of indomethacin after pretreatment by Azone, capsaicin and nonivamide in PG/water (1:1, v/v) vehicle was examined. It was demonstrated that capsaicin and nonivamide exhibit no enhancing effect on indomethacin permeation when using PG/water (1:1, v/v) was used as the pretreatment vehicle. It also shows that the permeation of capsaicin and nonivamide in PG/water is significantly lower (*t*-test, $P < 0.05$) than that in ethanol/water.

The flux of indomethacin pretreated by ethanol/water vehicle in the presence of 1, 3 or 5% (w/v) capsaicin or nonivamide was calculated. The permeation of indomethacin increased as the concentration of the enhancers increased. The flux of capsaicin and nonivamide across skin after pretreatment at different concentrations was also performed. There was an ascendant trend for capsaicin or nonivamide to increase its flux from 3 to

5% was observed in the anatomical structure of skin after treatment as compared with control group.

To measure the influence of enhancers on the thermodynamic activity and permeability of drug across skin, transdermal permeation of indomethacin was determined by incorporating 3% capsaicin or nonivamide in the donor of in vitro diffusion cell. The indomethacin flux was significantly enhanced after incorporating capsaicin or nonivamide into the donor cell as compared to the control group. However, no significant difference (*t*-test, $P > 0.05$) was detected between the permeation flux enhanced by capsaicin and nonivamide. It shows the effect of pretreating the skin with enhancer and 0.2 W/cm² ultrasound followed by application of the indomethacin-containing vehicle. The result demonstrated that the low frequency ultrasound alone was effective to enhance the permeation of indomethacin, however, significant difference (*t*-test, $P > 0.05$) was observed. Ultrasound also increased the indomethacin permeation when combined with all the enhancer formulations examined. As shown in Fig. 7, both of the flux of capsaicin and nonivamide was increased after simultaneous application of ultrasound.

The enhancing effect of Azone, capsaicin and nonivamide on the permeation of indomethacin is shown in Fig. 1 as the cumulative amount-time profiles. The result demonstrated that the three compounds were capable of enhancing the permeation of indomethacin across nude mouse skin. Because of the structural likeness in molecular size, it has been suggested that the enhancers may share with a similar mechanism. It was thought that Azone might intercalate into the ceramide matrices and create disruption in their stacking. It consequently decreased the diffusion resistance of the stratum corneum. Lower enhancing effect of capsaicin and nonivamide maybe due to the different partition behavior with Azone. Even though with similar chemical structures, the *n*-octanol / water partition coefficient of Azone was significantly higher than that of capsaicin and nonivamide. Thus it was deduced that the amount of Azone retained in the stratum corneum might be far more than that of capsaicin and nonivamide, resulting in a greater disruption of lipid matrices. Previous studies had demonstrated that Azone did not permeate deeply into the dermis. The data in current study showed that capsaicin and nonivamide greatly permeated across the skin.

The enhancing effects of capsaicin and nonivamide on indomethacin permeation are comparable. It is because the similarity of their chemical structures. The same result was observed in the permeation of capsaicin and nonivamide after pretreatment, although the flux of capsaicin was slightly higher than that of nonivamide. The contents of pretreatment vehicles used in this present study, water, ethanol and PG, have been reported and demonstrated partial effect on skin permeation. The

indomethacin fluxes in this study showed no significant difference (ANOVA test, $P > 0.05$) with or without pretreatment with vehicles, indicating water, ethanol and PG did not exert the enhancing effect. The permeation of capsaicin and nonivamide when using PG/water (1:1, v/v) as the pretreatment vehicle was significantly lower (t -test, $P < 0.05$) than that from ethanol / water. This result may verify the inactivity of capsaicin and nonivamide in PG /water on permeation of indomethacin. It may be due to the lower solubility of capsaicin and nonivamide in PG/water water vehicle (suspension type) than that in ethanol / water vehicle (solution type), resulting in the poorer efficiency of capsaicin and nonivamide partitioned from PG/water vehicle into the skin. The permeation of indomethacin increased with increasing the concentration of enhancers. After calculating the correlation coefficient (r) between enhancer concentration and indomethacin flux, a linear relationship ($r = 0.98$ for capsaicin; $r = 0.99$ for nonivamide) was observed. It can be concluded that the concentration of capsaicin and nonivamide used is extremely important in enhancing indomethacin permeation.

The degree of skin irritation caused by the topical application of capsaicin and nonivamide was evaluated in this study. Initial histological examination coupled with visual observation was performed to preliminarily assess the safety of enhancers. The histology scores provide a means of comparing the irritant effects of the different enhancers. Scores from 0 to 10 were regarded as not causing undue reactions in skin. Scores from 11 to 20 caused skin reactions, which alone are not sufficiently extensive to exclude their potential use. Preparations scored above 21 were considered to cause unacceptably severe damage. The histology scores of skin after pretreated with 1, 3 and 5% capsaicin were 0, 1.3360.58 and 1.6760.58, respectively. The scores of 1, 3 and 5% nonivamide concentrations were 3.6760.58, 3.3361.15 and 3.6760.58, respectively, which were slightly higher than those of capsaicin. The data were significantly lower than those of Azone-treated nude mouse skin as previously reported. Although no skin damage occurred, the clinical value of capsaicin and nonivamide might be limited by the heat sensitization following in vivo topical application of these two analogues. In our previous report, the healthy volunteers could undergo pungent sensation when applied capsaicin and nonivamide topically. On the other hand, the volunteers could not distinguish the degrees of heat sensation of various concentrations. Capsaicin and nonivamide also cause marked vasodilation and ascendant skin temperature. It would be expected that the skin permeation-enhancing effect of these two analogues is more significant in the in vivo status because of increased blood flow and temperature in skin surface.

The indomethacin flux was significantly enhanced

after incorporation of capsaicin or nonivamide in donor compared to control group, but no significant difference (t -test, $P > 0.05$) between the permeation of the two enhancers. This indicated that both analogues showed similar enhancing effect either by the pretreatment method or by the partitioning into drug vehicle. The flux of capsaicin or nonivamide itself was also determined. A consideration of the physicochemical properties suggests that capsaicin and nonivamide will permeate the skin more readily than Azone. Since both analogues act as enhancers on indomethacin permeation and also have antinociceptive activities, it may be possible to create topical analgesic formulations in which there are two active ingredients. The formulations with enhancer attain greater therapeutic effects after calculation of the therapeutic index. The therapeutic index was determined by the result of the steady-state flux multiplied by the potency ratio. The result suggests a synergistic effect was achieved and the formulation may be created which is more effective than indomethacin or enhancer alone.

Sonophoresis (phonophoresis), the movement of drug molecules through skin in coupling medium under influence of ultrasound, has been extensively studied for over 30 years, and well documented to practically increase skin permeation of indomethacin. Since physical ultrasound and chemical enhancers individually can increase transdermal drug transport, it is hypothesized that the combination of ultrasound and enhancers may result in a greater degree of enhancement than that resulting from each enhancement method alone. The flux of indomethacin was significantly strengthened by the combined method, indicating that the combination of both methods is more effective than either method alone on the skin permeation of indomethacin.

Various mechanisms have been suggested to explain the enhancement of drug permeation via ultrasound. They include: radiation pressure, temperature increase and cavitation. Simonin has ruled out the involvement of radiation pressure after using mathematical calculations recently. Little increase in skin temperature (58°C) was measured under the present experimental condition which is the same with our previous investigation. The heating effect on skin can be discounted as a mechanism of increasing indomethacin permeation. It also illustrates that heat alone has no effect on the transdermal absorption of indomethacin.

Cavitation consists of creation and subsequent collapse of microbubbles from dissolved gas. Application of low frequency ultrasound has been suggested to enhance skin permeability due to cavitation near the keratinocyte-lipid bilayer interfaces which induces structural disorder of the stratum corneum lipid bilayers. This cavitation may further reduce the barrier properties of enhancer-pretreated skin, resulting in the synergistic effect on enhancing drug permeation. Ultrasound may drive enhancers into the skin over time

This would increase the capsaicin or nonivamide levels in the stratum corneum, which would likely result in increased bilayer disorder relative to the passive case. Without stir in the donor of in vitro Franz diffusion cell in this present study, percutaneous ultrasound induced cavitation may produce significant agitation in aqueous systems. Ultrasonic disruption of stagnant aqueous diffusion layers in poorly stirred diffusional systems would reduce the boundary layer thickness and decrease the total barrier resistance, leading to the higher amount of permeant or enhancer in skin

四、計畫成果自評

In conclusion, the experimental data in this present study have suggested that both capsaicin and nonivamide improved the permeation of indomethacin across skin in a similar degree. Nonivamide caused milder skin erythema than capsaicin in human. Synthetic nonivamide may be a good substitute for natural extracted capsaicin due to lower skin pungent sensation and cheaper price to capsaicin. The microscopic observation and histology scores showed that there was no disruption in skin structure after pretreatment of capsaicin and nonivamide.

The combination of low frequency ultrasound and enhancer pretreatment increased transdermal indomethacin transport more than each enhancement method. Capsaicin and nonivamide might permeate across the skin much better than Azone, resulting in eliciting the pharmacological effect sufficiently. It is possible to create topical analgesic formulation with indomethacin and capsaicin/nonivamide which is more effective than indomethacin or capsaicin /nonivamide alone, thus reducing the individual applied doses.

Further investigation is needed and in progress to study the in vivo drug permeation enhanced by capsaicin and nonivamide.

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