行政院國家科學委員會補助專題研究計畫成果報告

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※ 計畫名稱： ※

※ 利用電穿孔輸藥法促進Nalbuphine ※

※ 前驅藥經皮吸收之研究 ※

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計畫類別：☑個別型計畫 □整合型計畫

計畫編號：NSC－89－2320－B－041－013

執行期間：89年08月01日至90年07月31日

計畫主持人：宋國峻

共同主持人：

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□國際合作研究計畫國外研究報告書一份

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行政院國家科學委員會專題研究計畫成果報告

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執行機構及單位名稱：嘉南藥理科技大學藥學系

Abstract

The aim of this study was to assess the effects of electroporation on transdermal permeation of nalbuphine (NA) and its prodrugs. The various electrical factors as well as skin barriers on permeation characteristics were investigated to elucidate the mechanisms involved in transdermal delivery of NA and its prodrugs by skin electroporation.

The prodrugs were fully converted to parent drug after skin permeation. Application of electroporation significantly increased transdermal delivery of NA and its prodrugs; the enhancement effects were highest for NA and decreased as the prodrug lipophilicity increased. The permeation amounts of NA and its prodrugs may be increased by application of higher pulse voltage, pulse duration as well as pulse number. Various kinetics and mechanisms were observed for the permeation of NA and nalbuphine enanthate (NEN) through different skin barriers by applying electroporation.

Keywords: nalbuphine; prodrug; transdermal; electroporation

Introduction

The delivery of drugs via skin routes has been studied extensively in the pharmaceutical field. Drug delivery across skin offers the advantages of accessibility, noninvasiveness, compliance, safety and effectiveness. Nevertheless, its clinical application has been limited due to barrier properties of skin, especially the stratum corneum (SC). Consequently, several chemical as well as physical approaches for enhancing drug permeation across skin have been explored, including enhancer approach, prodrug approach, sonophoresis and electrically-assissted approaches. Iontophoresis and electroporation are two of the electrically-assisted methods that have been demonstrated as effective means to enhance transdermal delivery of drugs. Electroporation technique involves the use of high voltage and short electric field pulse to create transient elevation in the permeation of lipid bilayer membranes (1). It is believed that new permeation pathways are created across lipid bilayers of skin, and thus increases drug transport across skins (1,2). Several variables have been shown to affect transdermal drug delivery by applying skin electroporation, including the physicochemical properties of drug, the vehicle compositions, electrical factors and the skin's barrier properties (1-4). Since the delivery rates required for various pharmaceuticals are different; in order to obtain optimum plasma drug concentration profile as well as the therapeutic effectiveness, it is imperative to assess the effects of those variables on skin permeation characteristics while applying electroporation.

The aim of this study was to assess the effects of electroporation on transdermal delivery of NA and its prodrugs. In the first part of this study, the transdermal permeation of NA and its prodrugs were evaluated under vapplication of electroporation. The second part of the study utilized various skin membranes as permeation barriers, including intact hairless mouse skin, stratum corneum (SC)-stripped skin, delipidized skin and Wistar rat skin, to explore the permeation kinetics as well as mechanisms of NA and NEN through skins by applying electroporation.

Results and discussion

Fig.1 shows the total amount of drug (nmol/cm^2) permeated across skin after 6.5 h via passive diffusion for NA and its various prodrugs. All of the prodrugs were more lipophilic than their parent drug as evaluated by the logarithm of Octanol/H_2O partition coefficient (log P). The total amount of drug permeated increased with drug lipophilicity, in the order of NDE>NEN> NPI>NPR. This observation suggests that, as the lipophilicity of prodrug increased, more drug molecules partition into skin membrane, resulting in a higher skin membrane-water partition and thus higher skin permeability. The total amounts of NA and its prodrugs permeated across skins after 6.5 h by electroporation were also shown in Fig. 1. The electroporation treatment was twenty 300 V exponentially decaying pulses and each pulse with a duration of 200 ms. Comparing to the passive permeation, the application of high voltage pulses significantly increased the skin permeation of all drugs. The enhancement ratio (ER), defined as ratio of the amount of drug permeated by skin electroporation to the amount of drug permeated by passive diffusion, increased as the drug hydrophilicity increased. The results suggest that the application of electroporation has a more pronounced enhancement effect on the permeation of more hydrophilic drugs.

In order to elucidate the mechanisms involved in the transdermal delivery of NA and its prodrugs by electroporation, permeation studies of NA and NEN were performed using various skins. Table 1 shows that the total amount of NA permeated through SC-stripped hairless mouse skin via passive diffusion is 7.40 fold higher than that through intact mouse skin, indicating SC was the principal barrier in its passive permeation. The amount of NA permeated by electroporation through SC-stripped skin was 2.8 fold higher than that through intack skin; moreover, the amounts of NA permeated through SC-stripped skin by passive diffusion and by electroporation (Table 1) were similar (ER=1.15); those two observations suggest that the electric pulsing may have effects on SC to increase permeation of NA through intact skin.

For the passive permeation of NEN through intact skin and SC-stripped skin, a slightly higher amount of NEN (1.77 fold) permeated through SC-stripped skin was observed (Table 2). The results suggest that the passive permeation of NEN may be hindered by SC as well as by epidermis/dermis layer. The application of electroporation on permeation of NEN through SC-stripped skin shows a significant higher permeation amount comparing to that through intact skin (Table 2), indicating SC was an important permeation barrier for NEN permeation by skin electroporation. Furthermore, the total amount of NEN permeated through SC-stripped skin by applying electroporation was much higher than that by passive diffusion, suggesting that the permeation of NEN through epidermis/dermis layer may also be enhanced by skin electroporation.

The total amounts of NA as well as NEN permeated across delipid skin and SC-stripped skin via passive diffusion were also shown in Table 1 and Table 2. The similar amounts of drug permeated across delipid and SC-stripped skin suggests the predominant route for the passive permeation of NA and NEN across SC layer was the intercellular pathway. The total amount of NA permeated across delipid skin by applying electroporation was similar to that by passive diffusion (ER=0.82, Table 1), suggesting the enhancement effects of electroporation on permeation of NA through SC was majorly via intercellular matrix. The amount of NEN permeated across delipid skin by applying electroporation was higher than that by passive diffusion (ER=1.61); this indicates that electroporation may have enhancing effects on permeation of NEN through intracellular pathways. This inference may be confirmed by comparing the permeation amounts of NEN through SC-stripped skin (~998.79 nmole/cm^2) and through delipid skin (~213.54 nmole/cm^2), suggesting that the enhancement effect of electroporation through intracellular pathways was substantial. The results were also in accordance with previous studies that the application of high voltage pulses affect both

intercellular and intracellular pathways (1, 5, 6)

The total amounts of NA permeated across furry Wistar rat skin by passive diffusion and by electroporation were also shown in Table 1. The application of electroporation increased NA permeation from 0 up to 126.16±17.97 nmol/cm^2 (Table 1). This enhancement effect on NEN was less pronounced, with the amount of 32.60±8.30 nmol/cm^2 permeated via passive diffusion and 105.03±6.89 nmol/ cm^2 via applying electroporation, respectively (Table 2). These results suggest the application of electroporation may enhance the permeation of NA as well as NEN through the transfollicular pathway and this enhancement effect was more significant for NA than NEN. The observation is similar to previous findings that transfollicular route constitutes an more important pathway for iontophoretic delivery of hydrophilic molecules (9). The present results again demonstrate that the enhancement effects of electrophoretic/ iontophoretic movement for charged molecules are more important for hydrophilic molecules (such as NA) than lipophilic molecules (such as NEN).

The permeation of NA and NEN through cellulose membrane was also studied to validate the excised skin membranes as well as to explore the permeation mechanisms (7). Much higher permeation amounts were observed for permeation of NA and NEN through cellulose membrane, demonstrating the various skin membranes indeed provide barrier characteristics in the permeation studies. The permeation amount of NA through cellulose membrane by electroporation was slightly but significantly higher (t-test, $p<0.05$) than that by diffusion, indicating the electrophoresis/iontophoresis contributed to the enhancing effects of NA permeation. However, the similar permeation amounts for NEN by diffusion and by electroporation demonstrate that electrophoresis/iontophoresis had no effect in the permeation of NEN. The results also confirmed that the enhancing effects of electrophoresis/iontophoresis in drug permeation are more pronounced for hydrophilic molecules (9, 20).

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Table 1. The in vitro permeation data of nalbuphine across various skins by passive diffusion and by electroporation

Skin types	Total amount permeated[a] by passive diffusion (nmol/cm^2)	Total amount permeated[a] by electroporation (nmol/cm^2)	ER[b]
Intact skin	51.16±5.50	164.13±25.31	3.21
SC-stripped skin	378.46±10.01	435.30±113.61	1.15
Delipid skin	365.38±20.95	301.90±35.82	0.82
Wistar rat skin	0	126.16±17.97	—
Cellulose membrane	1892.50±72.76	2179.72±109.27	1.15

[a]Total amount permeated is obtained from the cumulative amount of drug in receptor at 6.5 h.

[b]ER: Enhancement ratio=total amount permeated by electroporation/ total amount permeated by passive diffusion. Each value represents the mean±S.D. (n=4).

Table 2. The in vitro permeation data of nalbuphine enanthate across various skins by passive diffusion and by electroporation

Skin types	Total amount permeated[a] by passive diffusion (nmol/cm^2)	Total amount permeated[a] by electroporation (nmol/cm^2)	ER[b]
Intact skin	100.99±6..05	173.53±14.43	1.72
SC-stripped skin	178.77±23.10	998.79±117.02	5.59
Delipid skin	132.81±22.62	213.54±19.78	1.61
Wistar rat skin	32.60±8.30	105.03±6.89	3.22
Cellulose membrane	1693.45±82.28	1603.37±300.47	0.95

[a]Total amount permeated is obtained from the cumulative amount of drug in receptor at 6.5 h.

[b]ER: Enhancement ratio=total amount permeated by electroporation/ total amount permeated by passive diffusion. Each value represents the mean±S.D. (n=4).