การขนส่งและการเกิดเมตาบอลิสมของ prodrug ในผิวหนังคนไทย

TRANSPORTATION AND METABOLISM OF PRODRUGS IN THAI HUMAN SKIN

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บทคัดย่อ :

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของคุณสมบัติทางเคมีและกายภาพของ สารนิโคตินิก แอซิด (NA) และอนุพันธ์ในรูปเอสเธอร์อีก 3 ชนิด คือ เมธิลนิโคติเนต (MN) เอธิลนิโคติเนต (EN) และ บิวธิลนิโคติเนต (BN) ต่อการขนส่งและการเกิดเมตาบอลิสมในผิวหนังคนไทย โดยใช้ผิวหนังส่วนที่ดีของก้อนมะเร็งที่ตัดจากเต้านมผู้ป่วยของโรงพยาบาลศรีนครินทร์ การศึกษาการขนส่งหรือดูดซึมยาผ่านผิวหนังนั้นใช้อุปกรณ์ diffusion cell แบบแนวระนาบ ส่วนผิวหนังบดปั่นละเอียดถูกนำมาศึกษาการเกิดเมตาบอลิสมที่อุณหภูมิ 37 องศา ความเข้มข้นของสารวิเคราะห์โดยใช้เครื่อง HPLC พบว่าอัตราการซึมผ่านผิวหนังสูงสุด (J_{ss}) ของ MN EN BN และ NA มีค่า =10.3493, 2.2962, 0.3604 และ 0.0552 μmol/cm^2/hr ตามลำดับ และค่าสัมประสิทธิ์การซึมผ่าน (permeability coefficients ; k_p) ของ EN BN MN และ NA มีค่า = 2.6141×10^{-6} 9.6539×10^{-7} 5.7496×10^{-7} และ 5.3093×10^{-8} cm/sec ตามลำดับ จากการทดลองนี้สรุปได้ว่าสารที่มีค่าความชอบไขมันมากกว่าจะซึมผ่านผิวหนังได้มากกว่า ส่วนการเกิดเมตาบอลิสมของยาในผิวหนังนั้นสอดคล้องกับกฎของ Michaelis-Menten และพบว่า BN มีความจำเพาะต่อเอนไซม์ในผิวหนังมากกว่าอนุพันธ์ในรูปเอสเธอร์อื่นที่นำมาศึกษา

คำสำคัญ การดูดซึมยา ผิวหนัง เมตาบอลิสม นิโคตินิก แอซิด

Abstract

This study was to investigate the effect of physicochemical properties of nicotinic acid (NA) and its alkyl ester derivatives on both permeation and metabolism characteristics through Thai human skin. The horizontal (side by side) diffusion cell was used for all experiments in this study. Three alkyl ester derivatives of NA, methyl nicotinate (MN), ethyl nicotinate (EN) and butyl nicotinate (BN), were selected and used as the model prodrugs. Thai human skins were obtained from Srinagarind university hospital. The experiment of skin metabolism was conducted using skin homogenate at 37 °C. The concentrations of NA and its alkyl ester derivatives were determined by HPLC. Permeation profiles were plotted between cumulative permeated amount against time. The maximum steady state flux (J_{ss}) was found as follows; MN (J_{ss}=10.3493 mmol/cm^2/hr), EN (J_{ss} = 2.2962 mmol/cm^2/hr), BN (J_{ss} = 0.3604 mmol/cm^2/hr) and NA (J_{ss} =0.0552 mmol/cm^2/hr), respectively. While the permeability coefficients (k_p) were as follows; EN (k_p =2.6141$\times10^{-6}$ cm/sec), BN (k_p =9.6539$\times10^{-7}$ cm/sec), MN (k_p =5.7496$\times10^{-7}$cm /sec) and NA (k_p = 5.3093$\times10^{-8}$ cm/sec), respectively. The more lipophilic ester prodrug showed higher permeability through human skin. However, the k_p value of BN was lower than EN. Compared with EN, BN remained in the skin due to its higher partition coefficient. In this study, it was shown that the enzyme reaction of skin homogenates followed Michaelis-Menten's rule and BN showed high affinity to skin enzyme.

Keywords: Skin metabolism, Nicotinic acid

Introduction

Simultaneous transport and metabolism through skin of many model drugs and chemicals were investigated using animal skin and cell culture (Cheing and Po, 1985; Das et al., 1986; Lilienblum 1986; Ghosh and Mitra , 1990; Wanders and Van Roermund 1993; Lamb et al., 1994). Some of such researches were carried out in human skin (Rohatagi et al., 1997; Rittirod et al., 1999). Our previous study, clearly shown that significant different in skin permeability and metabolism of prodrugs were found among 3 species, rats, mice and human (Female Japaneses) (Rittirod et al., 1999). The effect of race on skin permeation of methyl nicotinate was shown that Balck < Asian < Caocasian < Hispanic (Michel and Kenneth, 1998). However, the study of transport and metabolism of any prodrugs in Thai human skin was not established yet. To obtain such skin permeation and metabolism data of model prodrugs, especially from Thai human skins, thus Thai human skins were used through out this study. Nicotinic acid (NA), a drug with small molecule (MW. 123) was selected as parent compound. Three alkyl esters derivatives of

NA as Methyl nicotinate (MN), Ethyl nicotinate (EN) and Butyl nicotinate (BN) with different solubility and partition coefficient were used as model prodrugs. It was already known that NA, only one metabolite of those prodrugs was found after skin metabolism. In this study, the effect of physicochemical properties of NA and its alkyl ester derivatives on both permeation and metabolism characteristics were also investigated.

Materials and Methods

Materials

Nicotinic acid (NA) and its three alkyl ester prodrugs; methyl nicotinate (MN), ethyl nicotinate (EN) and butyl nicotinate (BN) were purchased from Tokyo Chemical Industries (Tokyo, Japan). All other chemicals were of analytical grade.

Skin Preparation

The specimens of Thai human skin were obtained from 13 operated breasts of cancer patients of Srinagarind university hospital in Khon Kaen province. The average age of patients was 56.84 years (50-68 years). The skins were kept in at -20 °C and then were used in skin permeation and hydrolysis experiment after 1-3 hrs immersed in PBS at room temperature. Using such the specimens of human skin were declared by ethical committee of the university.

Skin Permeation Study

After removed the subcutaneous fat then the split thickness skins (0.6-0.7 mm) were obtained as described by Morimoto et al., 1991 and Rohatagi et al., 1997. Each skin specimen was attached between two half diffusion cells which was connected to the water bath set at 37 $\pm$ 1.0 °C. The specific diffusion surface area and volume of each cell were 0.95 cm^2 and 3.0 ml, respectively. To obtained fully hydrated skin and activate skin enzymes, the donor and receiver compartments were filled with physiological phosphate buffer saline, pH 7.4 (PBS) for one hour. Then the receiver fluid was replaced with fresh PBS while the donor solution was replaced with saturated solutions of NA, MN, EN and BN in PBS solution. The concentration of NA, MN, EN and BN in PBS solution were 2.888×10^{2}, 5.000×10^{3}, 2.440×10^{2}, 1.037×10 mM respectively.

Hydrolysis of Alkyl Nicotinate in Skin Homogenates

Human skin homogenates (25 %w/w) were made with split-thickness skin and PBS solution using a tissue homogenizer. The homogenates were centrifuged for 10 min at 9000xg and 4 °C. The supernatant and various concentrations of MN, EN and BN in PBS were incubated for 15 min, mixed together to make 5 % w/w homogenate and maintained at 37 °C. The samples were then withdrawn at predetermined time. The concentrations of those substrates were determined by high performance liquid chromatography (HPLC).

Analytical Methods

The concentrations of NA, MN, EN and BN were determined by HPLC as described by Rittirod

et al.,1999. The HPLC system consisted of a pump (200LC, Perkin, USA.) in a column oven set at 35 °C, a 4.6 mmX150 mm reverse phase stainless steel column, an auto injector, a UV detector 785A/ CORAD, USA, and an intergrator (PE Nelson model 1022). The mobile phase were methanol (MeOH) : 0.1% (v/v) phosphoric acid (20:80) containing 5 mM of sodium 1-heptane sulfonate for NA, MeOH : 0.1% (v/v) phosphoric acid (35:65) for MN and EN, and acetonitrile : water (35:65) for BN. The internal standard were p-hydroxybenzoic acid for NA, methyl papraben for MN and EN and propyl paraben for BN.

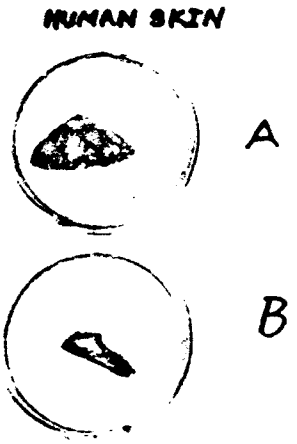


Fig. 1 Fresh human skin.

Results and Discussions

Permeation Study

Skin permeation and metabolism experiments were carried out using female Thai human skin. Due to the limitation of skin samples, only one concentration, maximum concentration or saturated solution of NA, MN, EN and BN were used in this permeation experiment. The permeation profile through human skin of NA and MN saturated solution is shown in Fig. 2 (a) and (b) respectively. In Fig. 2 (a) it was found that NA slowly penetrated through human skin due to the ionization of NA, a weak acid in PBS. While MN and NA were found in receiver solution (Fig 2 (b)). It was indicated that MN was hydrolyzed during skin permeation process. The patterns of permeation profiles of the saturated solution of EN and BN were similar to those of MN. The steady state fluxes of 4 chemicals were obtained within 8 hrs. The cumulative permeated amount of NA from 3 ester prodrugs increased continuously, which suggested that the viability of human skin and metabolic reaction were maintained under 37 °C conditioned. The total steady state flux of those prodrug was the sum of flux of ester product and metabolit (NA flux). In table I, the maximum steady state flux (J_{ss}) was found as follows; MN (10.3493 μmol/cm^2/hr). EN (2.2962 μmol/ cm^2/hr), BN (0.3604 μmol/cm^2/hr) and NA (0.0552 μmol/cm^2/hr), respectively. While the appearance permeability coefficients (k_p) were as follows; EN (2.6141×10^{-6} cm/sec), BN (9.6539×10^{-7} cm/sec), MN ($5.7496\times10^{-7}\times10^{-6}$cm /sec) and NA ($5.3093\times10^{-8}$ cm/sec), respectively. These results corresponded to our previous study (Rittirod et al., 1999).

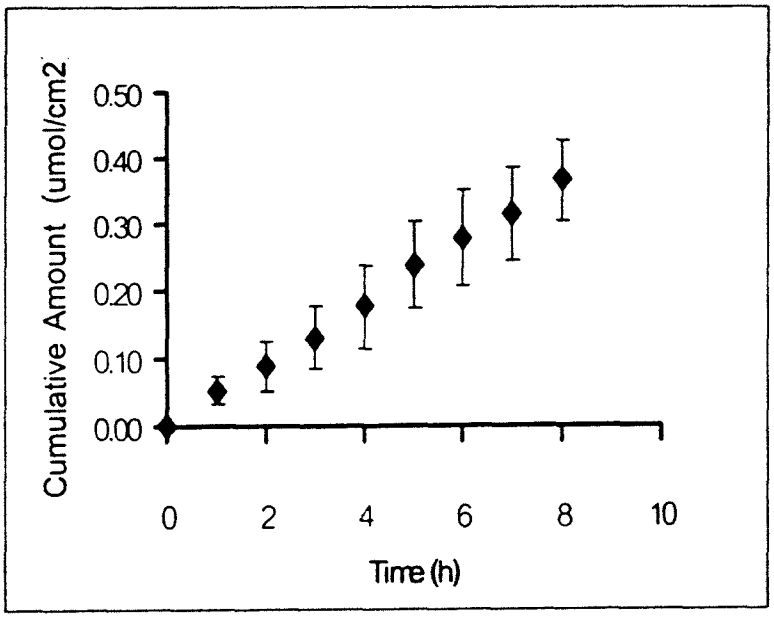
Hydrolysis of Alkyl Nicotinates

Hydrolysis of 3 alkyl nicotinates; MN, EN and BN were performed with human skin homogenates at 37 °C. The hydrolysis rate of MN sharply increased at the lower concentration and reached a plateau at higher concentration (Fig.3). The similar relationships between hydrolysis rate and ester concentrations were

obtained for EN and BN. The kinetic parameters of all model prodrugs were estimated and listed in Table II. It was shown that the value of V_{max} of all esters were in the same range, while the lowest K_m of BN showed high affinity to skin enzymes.

Fig. 2 Permeation profiles through human skin fromsaturated solution of NA (a) and MN (b).

(a)



(b)

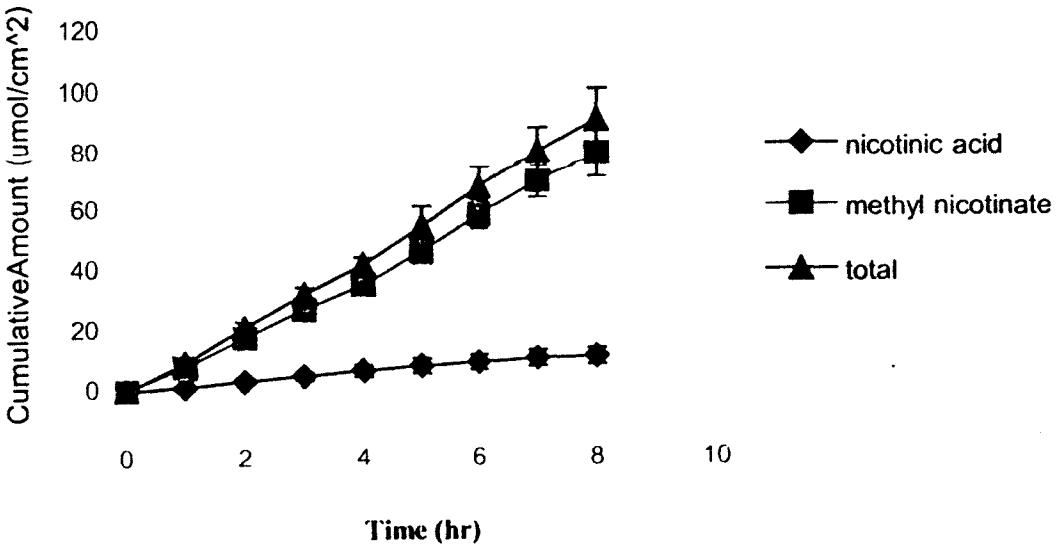


Table I Steady state flux from saturated solution (Max. conc.) of NA and its ester derivatives

Solution	Max. Conc. (mM)	Flux (μmol/cm²/hr)		
		NA	Ester	Total
NA	2.888×10^2	0.0552	-	0.0552
MN	5.000×10^3	1.0376	9.3117	10.3493
EN	2.440×10^2	0.6692	1.6270	2.2962
BN	1.037×10	0.2955	0.0649	0.3604

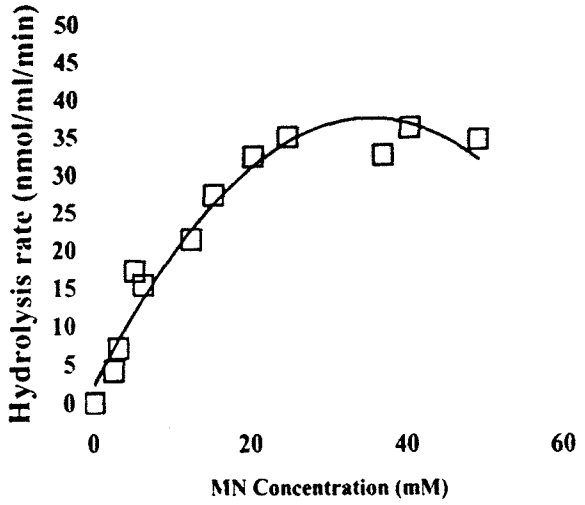


Fig. 3 Relationship between MN concentration and hydrolysis rate

Table II Kinetic parameters of three alkyl nicotinates

Compounds	V_{max} nmol/min/mg protein	K_m (mM)
MN	20.3 ± 4.6	19.2 ± 2.2
EN	14.2 ± 2.7	5.61 ± 1.4
BN	15.1 ± 1.4	1.31 ± 0.46

Conclusion

In this study, simultaneous permeation and metabolism of three alkyl nicotinates were investigated. The more lipophilic ester prodrug (BN and EN), the higher permeability coefficient was obtained. NA was found in all receiver chamber which indicated that after apply such 3 ester prodrugs (MN, EN and BN), simultaneous penetration and metabolism was occurred. For skin metabolism, it was indicated that the enzymatic reaction of skin homogenate followed Michaelis-Menten's rule and BN showed higher affinity to skin enzyme than EN and MN. The effect of physicochemical properties of prodrugs on permeation and metabolism through Thai human skin was also confirmed. This study provided primary data of skin permeation and skin metabolism of Thai human skin.

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