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EXPERIMENTAL STUDY

Correlation between the transdermal characteristics of pseudoephedrine and amygdalin in majiepingchuan *in vitro*

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Abstract

OBJECTIVE: To analyze the transdermal profile of pseudoephedrine and amygdalin in the Traditional Chinese Medicine majiepingchuan in rat skin and to reveal their interaction.

METHODS: A Franz diffusion cell was used in vitro to evaluate the transdermal parameters of cumulative transdermal flux (Q_{tot}), cumulative transmission (T_{tot}), and mean penetration rate (Kp) of pseudoephedrine and amygdalin in majiepingchuan. Linear regression analyses of Q_{tot} over time of pseudoephedrine vs amygdalin and their ratios was adopted for correlation evaluation.

RESULTS: At 1, 2, 4, 6, and 8 h, the Q_{tot}, T_{tot} and Kp of pseudoephedrine showed a good correlation with that of amygdalin.

CONCLUSION: There was a small difference in the ratios of Q_{tot}, T_{tot} and Kp between pseudoephedrine and amygdalin, and a correlation between them.

Key words: Pseudoephedrine; Amygdalin; Majiepingchuan; Transdermal flux; Correlation

INTRODUCTION

Majiepingchuan comprises Mahuang (*Herba Ephedra Sinica*), Kuxingren (*Semen Armeniacae Amarum*), Baijiezi (*Semen Sinapis*), Yanhusuo (*Rhizoma Corydalis Yanhusuo*), and Shengjiang (*Rhizoma Zingiberis Recens*). It is a topical transdermal formulation employed to treat acute and chronic asthma, and has been used in China for more than 300 years. Effects of majiepingchuan have been noted in clinical studies for asthma treatment.[1]

It has been reported that amygdalin[2] and ephedrine[3] can penetrate the skin *in vitro*. Pseudoephedrine (an isomer of ephedrine) can also be absorbed through the skin.

Traditional Chinese Medicine (TCM) compounds have been used as single[4] or combined components[5] in skin-penetration studies in vitro to show transdermal behavior.[6] The relationship among multiple components (small-molecule compounds) has not been reported. However, this relationship could help to reveal the pharmacodynamics of multiple components of TCM compounds.

Transdermal experiments using extracts from TCM compounds have revealed the main parameters of the principal components of such extracts: cumulative transdermal flux (Q_{tot}),[7] cumulative transmission (T_{tot}),[8] and mean penetration rate (Kp).[9] Linear regression analyses[10,11] and different analytical methods[12] have been adopted for correlation studies. Positive correlations between transdermal behaviors of the TCM ingredients have been documented.

We investigated the transdermal behavior of two ingredients of majiepingchuan, pseudoephedrine and amygdalin, to study their interaction.

MATERIALS AND METHODS

Instruments

A 1260 series liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, diode array detector, autosampler, and thermostatically controlled column apartment, BT 125D electronic balance (Sartorius Scientific Instruments, Goettingen, Germany), and a modified Franz diffusion cell (Shanghai Kai Kai Technology, Shanghai, China) were employed.

Medicines and reagents

Mahuang (*Herba Ephedra Sinica*), Kuxingren (*Semen Armeniacae Amarum*), Baijiezi (*Semen Sinapis*), and Yanhusuo (*Rhizoma Corydalis Yanhusuo*) were purchased from Beijing Tongrentang Herbal Medicines (Beijing, China). Standards of pseudoephedrine (batch No. 171237-200304) and amygdalin (110820-201004) were purchased from the National Institutes for Food and Drug Control (Beijing, China). All other reagents were available commercially, including reagents for chromatography.

Animals

Animal studies were undertaken according to the Guidelines for the Care and Use of the Laboratory Animals (National Institutes of Health, Bethesda, MD, USA). The study protocol was approved by the Animal Experimentation Ethics Committee of the Beijing University of Chinese Medicine (Beijing, China).

Male Sprague-Dawley rats (6-8 weeks; 18-22 g) were obtained from Beijing Vital River Laboratory Animal Technology (Beijing, China).

Sample preparation

Weighed quantities of ephedra, mustard seed, bitter almond, and corydalis tuber were mixed according to prescription proportions, followed by addition of 6 × volumes of ethanol at different concentrations.[13 - 16] The mixture was extracted in different times under nine conditions,[17] and nine extractions were obtained. Ethanol was recovered through a vacuum at 60 ℃ and the remainder was plaster. The plaster was dried at 60 ℃ by dry-box decompression (− 0.08 MPa). The dry plaster was smashed to a fine powder and named "majiepingchuan extractions" before being stored in a dryer. Ginger was squeezed to obtain its juice, which was then centrifuged at 8000 × *g*. for 5 min at room temperature. The supernatant was stored at 4 ℃. This powder was mixed with the corresponding amount of ginger juice and stirred to obtain paste samples, which were the transdermal test preparations.

Skin preparations

Rats were killed and their back skin excised immediately. Skin was fixed along the edge of a smooth foam board, with the hair facing outwards, and dipped in 8% sodium sulfide solution.[18] Once the hair color became yellow, the hair was removed with gauze along the same direction. The skin surface was washed with water and subcutaneous fat removed with a scalpel. Samples were stored in physiologic (0.9%) saline at 4 ℃ but used < 7 days of preparation.

Transdermal experiments

The modified Franz diffusion cell[19] was fixed with a volume in the accepting pool of 6.5 mL and an effective contact area in the receiving pool of 2.8 cm^2. Then, 30% ethanolic saline[20] was added to the accepting pool. The dermal side of isolated rat skin faced the accepting pool (thereby ensuring the absence of bubbles) while excess skin was excised. Paste samples were applied to the cutting side of isolated rat skin, and were clamped. The receptor compartment was filled with receiver solution, which was stirred by an electromagnetic stirring bar (200 rpm) and maintained at 32 ± 1 ℃[21] by a circulating water bath. Aliquots of the receiving phase (50 mL) were withdrawn at 1, 2, 4, 6, and 8 h, and were replaced immediately with an equal volume of fresh solution to the pool. The sample was filtered with a microporous membrane (size, 0.45 μm) and was injected into the high-performance liquid chromatography (HPLC) system. The peak area, reference drug concentration, and cumulative transdermal flux at each time point (Qn) were calculated using the following formula:[22]

$$Qn = \mathbf{YCn} \times \mathbf{Vo} + \sum_{i=0}^{n\mathbf{l}_1} \frac{[Ci \times V)}{A}]$$

where C_n is the measured concentration at n sampling points, C_i is the measured concentration at the first sampling point, V_0 is the accepted pool volume, V is the sampling volume at each time, A is the skin diffusion area, and Qn (μg·cm^{-2}) is the cumulative transdermal flux at each time point.

Linear regression of Qn *vs* $t^{1/2}$ was the Higuchi equation and the slope of the line was Kp (μg·cm^{-2}·$h^{-1/2}$).

RESULTS

Donor paste

Quantity of pseudoephedrine and amygdalin from nine majiepingchuan extractions were analyzed by HPLC (Table 1). Ratios of the quantities of pseudoephedrine and amygdalin in different samples were equal to those of the donor (Table 2).

Receiver solution

For transdermal experiments, each paste sample was tested in replicate. The receiver solution was withdrawn according to preset time points, and injected into the HPLC system. Characteristics of the penetration