Transdermal patches for D-threo-methylphenidate (free base): Formulation aspects and in vivo pharmacokinetics

Chenghao Zhang, Huafei Luo, Guobei Lin, Zhuangzhi Zhu, Furong Zhang, Junyun Zhang, Yubo Wu, Jing Luo, Hao Wang

National Pharmaceutical Engineering Research Center, China State Institute of Pharmaceutical Industry, 1111 Ha Lei Road, Shanghai, 201203, China

A R T I C L E   I N F O

Article history:
Received 27 February 2016
Received in revised form 13 May 2016
Accepted 13 May 2016
Available online 15 June 2016

Keywords:
D-threo-methylphenidate
Transdermal patch
Drug-in-adhesive
Pharmacokinetics

A B S T R A C T

This study was designed to develop a drug-in-adhesive (DIA) type transdermal drug delivery system (TDDS) for D-threo-methylphenidate (D-threo-MP) that could be further developed for treating Attention-Deficit/Hyperactivity Disorder (ADHD) in children. During initial formulation optimization, three formulation factors, i.e., different adhesives, permeation enhancers and drug loading of D-threo-MP were screened using vertical Franz diffusion cells across newborn pig skin. A patch containing 15% (w/w) D-threo-MP in DURO-TAK® 87-4098 was considered as an optimum patch which was selected for in vivo studies. The optimum D-threo-MP patch pharmacokinetic parameters were determined in Sprague Dawley (SD) rats and bama miniature pigs (Sus scrofa domestica) after application of transdermal patches, intravenous (i.v.) injection or oral administration. The absolute bioavailability and the relative bioavailability of D-threo-MP DIA patch in the SD rats model were 53.4% and 218.8%, respectively, and the relative bioavailability was 83.4% in the bama miniature pigs model. In this study, we showed that the optimized D-threo-MP patches could be further developed for treating Attention-Deficit/Hyperactivity Disorder (ADHD) in children.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Attention-Deficit/Hyperactivity Disorder is a common neurobehavioral disorder with a prevalence around 6% [5,27]. Symptoms of ADHD lead to impairments in cognition and behavior such as learning, social, and family interactions. Moreover, ADHD may extend into adolescence and adulthood [9]. Stimulants are the most common medications used for the pharmacotherapy of ADHD, for which methylphenidate (D.L-threo-MPH) is widely prescribed in racemic form, i.e., a 50:50 mixture of D- and L-isomers (Fig. 1) [16].

D,L-threo-MPH is available in both immediate and extended release (ER) oral formulations. Although immediate-release (IR) formulations of D,L-threo-MPH provide a good therapeutic response, they have a short duration of action, ranging from 3 to 5 h after ingestion [27]. This likely contributes to the poor adherence to therapy reported in children with ADHD [2,27]. In response to such concerns, extended-release forms of once-daily oral D,L-threo-MPH have been developed and are useful for children with ADHD [18,24].

To expand the current extended-release stimulant treatment options, the D,L-threo-MP transdermal system (Daytrana®, Noven Pharmaceuticals, Inc., Miami, FL, U.S.A.; and Shire U.S., Inc., Wayne, PA, U.S.A.) was approved by the Food and Drug Administration (FDA) in 2006 for the treatment of ADHD in children aged 6–12 years [6,13].

D-threo-methylphenidate hydrochloride (D-threo-MPH) is the pharmacologically active D-threo enantiomer of racemic MPH. L-threo-methylphenidate hydrochloride (L-threo-MPH) does not appear to contribute to the clinical efficacy of D,L-threo-MPH, and demonstrates substantial differences in receptor binding from D-threo-MPH [19,28]. No in vivo chiral inversion of D-threo-MPH occurs after dosing, allowing similar efficacy to be achieved at a half dose of D,L-threo-MPH [24]. A preparation containing only D-threo-MPH could provide a better therapeutic index than a racemic MPH mixture and represents an advance in single-enantiomer technology. The extended-release D-threo-MPH-ER (Focalin® XR, Novartis Pharmaceuticals Corporation, U.S.A.) was approved by the FDA in June 2005 [17].

To combine the advantages of the transdermal drug delivery system and single-enantiomer technology, the D-threo-MP patch was designed as an attractive alternative to improve the treatment...
2. Materials and methods

2.1. Materials

L-threo-MPH, D-threo-MPH, D-threo-MP, and D,L-threo-ritalinic acid (D-threo-RA) with purity of 99.0% were supplied by China State Institute of Pharmaceutical Industry (Shanghai, China). Methylphenidate-D$_3$ HCl, D,L-threo-ritalinic acid-D$_{10}$ HCl, trifluoroacetic acid, and ammonium formate (for HPLC, ≥99.0%) were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). DuroTak® adhesives 87-2852, 87-2510, and 87-4098 were purchased from Henkel (Holthausen, Germany). Methanol and acetonitrile of high performance liquid chromatography (HPLC) grade were obtained from Merck (Darmstadt, Germany). Azone (AZO), 1,2-propanediol, oleic acid (OA), menthol, N-methyl-2-pyrrolidone (NMP) and poly (ethylene glycol) (PEG) 400 were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other chemicals and solvents of analytical reagent (AR) grade or HPLC grade were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Animals

Fresh newborn pig skin was obtained from a local slaughter house. SD rats (age: 7–8 weeks, weight: 200–250 g) and nude mouse (female, age: 6–7 weeks, weight: 15–20 g) were supplied by the Shanghai Super-B&K Laboratory Animal Co., Ltd. (Shanghai, China). Bama miniature pigs (female, age: 8–9 weeks, weight: 7–8 kg) were purchased from the Jia Gan Biotech Co., Ltd. (Shanghai, China). The animals were allowed to acclimate for 2 weeks before the experiment was initiated. During the experimental period, SD rats were housed under controlled conditions of 12:12 h light–dark cycle and 22 ± 2 °C, with food and water ad libitum. The bama miniature pigs were maintained on commercial concentrate piglet feed offered twice daily, with water ad libitum. All animal studies were performed in accordance with the Ethical Guidelines for Investigations in Laboratory Animals and were approved by the National Pharmaceutical Engineering and Research Center.

2.3. Fabrication of patches

DIA patches containing D-threo-MP, various PSAs and enhancers were prepared by solvent evaporation technique. A laboratory coating unit (Labcoater Trans-I, Xiandai Pharmaceutical Co., Ltd., Shanghai, China) was used to fabricate the D-threo-MP patches. An appropriate amount of D-threo-MP, enhancers and PSAs were dissolved in ethyl acetate and mixed homogenously with a mechanical stirrer. The formed mixture was coated onto a fluoropolymer released liner (ScotchPak® 1022, 3M, U.S.A.) with a thickness of 0.25 mm. After solvent evaporation, the products were laminated with a polyester backing film (ScotchPak® 9680, 3M, U.S.A.).

2.4. In vitro percutaneous permeation experiments

Vertical Franz diffusion cells with a nominal receptor compartment volume of 7 mL and diffusion area of 3.14 cm$^2$ were used to investigate the D-threo-MP release from the investigated formulations, as well as D-threo-MP delivery through nude mouse, SD rat, and newborn pig abdominal skin. SD rats’ abdominal hair was removed with an electric clipper and depilatory cream. After heating for 24 h, the abdominal skin was checked carefully in case of any skin breakage. Before in vitro experiments, nude mouse and hair removed SD rats were sacrificed by inhalation methods with carbon dioxide, and then the abdominal skin was cut off immediately. Fresh nude mouse, SD rat, and newborn pig abdominal skins were excised and subcutaneous fat and other extraneous tissues were carefully removed by forceps and scissors. Excised abdominal skin was mounted between the donor and receptor compartment of the Franz diffusion cell with the stratum corneum facing the donor compartment. 0.2 M phosphate buffer saline (PBS) with pH 4.5 was used as the receiver medium according to the stability study of D-threo-MP (data not shown). The receiver medium was constantly stirred with a magnetic stirrer at 200 rpm. Assembled diffusion cells were placed in a transdermal permeation diffusion instrument, and the temperature was maintained isothermally at 32 °C. Samples (0.3 mL) collected at different time intervals for HPLC analysis were replaced with an equal volume of receiver medium. All samples were centrifuged at 12,000× g for 3 min before HPLC analysis.

2.5. In vivo pharmacokinetic study

Fifteen female Sprague-Dawley rats (240 ± 20 g) were randomly assigned to three groups (five rats per group). One group of animals’ abdominal hair was removed with an electric clipper and depilatory cream. After heating for 24 h, the animals were checked carefully in case of any skin breakage. All rats were anesthetized with pentobarbitalum natricum (30 mg/kg). After confirming the induction of anesthesia, each rat was fixed in a supine position, and the femoral artery (for blood sampling) was cannulated with a PE-50 polyethylene tube (Clay Adams, Parsippany, NJ, U.S.A.) filled with 20 IU/ml heparinized saline to prevent blood clotting. After the rats recovered from anesthesia, the D-threo-MP patches (1 cm$^2$, 1.1 mg/cm$^2$) were applied to the shaven dorsal skin, and removed 9 h later. As the reference, D-threo-MPH dissolved in normal saline (0.1 mg/ml) was administrated intravenously via the tail vein at a dose of 1 mg/kg or orally at a dose of 2 mg/kg to the other two groups. The samples were collected from the femoral artery cannula at 0.083, 0.25, 0.5, 1, 2, 3, 6, 9 and 24 h following drug administration.

Four female bama miniature pigs (8–9 kg) were weighed before the experiment. A crossover trial was designed, which meant that four miniature pigs were used for the D-threo-MP patches (5 cm$^2$, 1.1 mg/cm$^2$) transdermal study, and then an oral dose (5 mg) of D-
three-MPH capsule after a washout period of two weeks. Before the patches were applied, hair on a certain area of abdominal skin was removed with an electric clipper. After healing for 24 h, the skin was checked carefully in case of any skin breakage. A single patch with an area of 5 cm² was applied to the shaved area after being cleaned with warm water. Blood samples of 0.5 mL were taken via the jugular vein into the tubes containing heparinized saline at 0.083, 0.166, 0.25, 0.5, 1, 2, 4, 6, 9, 24, 28 and 32 h after either transdermal or oral administration.

Plasma samples were obtained immediately by centrifugation (12,000 × g for 3 min) at 4 °C. The separated serum of 100 μL was stored at −80 °C immediately until UFLC-MS/MS analysis.

2.6. In vivo skin irritation study

In vivo skin irritation study was evaluated by the Draize patch test method using SD rat as the animal model. The SD rats (weighing 220 ± 20 g) were randomly divided into three groups, with three female and three male rats in each group. Three groups of animals' dorsal hair were removed with an electric clipper and depilatory cream. After healing for 24 h, the animals were checked carefully in case of any skin breakage. The tested group was treated with the optimized formulation (5 cm²) attached to the preshaved skin and occluded with adhesive tape (3M Transpare®, U.S.A.). The control group was treated with the blank patch (5 cm², without any drug) and occluded with adhesive tape. The blank group was only occluded with adhesive tape without drug treatment.

The SD rats were examined for signs of irritation after 2, 6 and 9 h application. Then, the skin reactions, such as erythema and edema, were scored in accordance with the Draize method[30]. After 9 h of application, the SD rats were sacrificed. The 5 cm² skin pieces were cut from the center of the treated area and then formalin fixed immediately. Formalin-fixed tissue samples were dehydrated using a Tissue-Tek VIP 5 (Sakura Finetek, Tokyo, Japan) and paraffin embedded using an embedding station (Tissue-Tek TEC 5, Sakura Finetek, Tokyo, Japan). Samples were sectioned (10 μm) using a sliding microtome (Sakura IVS-410, Sakura Finetek, Tokyo, Japan). Sections were hematoxylin- and eosin-stained according to previous reports [8,26]. The stained sections were visualized using an Olympus BX 53 microscope (Olympus, Tokyo, Japan).

2.7. Analytical methods

A HPLC system consisting of a system controller (SCL-20 ATVP, Shimadzu, Japan), a pump (LC-20 AT, Shimadzu, Japan), a UV/VIS detector (SPD-20A, Shimadzu, Japan), a column oven, and an auto sampler (SIL-20AC, Shimadzu, Japan) were used for in vitro determination of D-threo-MP. The HPLC determination took place under the following conditions: C18 reversed phase analytical column (4.6 × 250 mm, 5 μm, Inertsil® BDS-SP), 300:700:2.1:4.2 (v/v) methanol–1.828 g/ml potassium phosphate monobasic solution–phosphoric acid–triethylamine as mobile phase, column temperature of 40 °C, UV detective wavelength of 210 nm, flow rate of 1.0 mL/min, and injection volume of 20 μL. The data were acquired and analyzed by Shimadzu LabSolutions software. The D-threo-MP peak was well-separated at the retention time of 15.8 ± 0.1 min with the sensitivity of 0.1133 μg/mL. The peak area correlated linearly with D-threo-MP concentration in the range from 0.2266 to 113.3 μg/mL.

Plasma samples were determined using validated UFLC-MS/MS analysis method as we have previously reported [29]. The UFLC-MS/MS analysis was performed on a Shimadzu UFLC system (Shimadzu, Japan). The analytes were extracted by liquid-liquid extraction and separated on an Astec Chirobiotic V2 column (Sigma–Aldrich, St. Louis, MO, U.S.A.), with methanol containing 0.003% ammonium acetate (w/v) and 0.003% trifluoroacetic acid (v/v) as mobile phase. The MS was operated in positive ion mode using turbo electrospray ionization. Data were acquired and analyzed by Shimadzu LabSolutions software. The UFLC-MS/MS method provided a clear separation between D-threo-MP and L-threo-MP and there was no interference from any endogenous material.

2.8. Data analysis

2.8.1. Data analysis in vitro

The cumulative amount from the unit area of D-threo-MP that diffused through skin was calculated using Eq. (1):

\[ Q_n = C_0 \times V_0 + \sum_{i=1}^{n} (C_i \times V_i) / A \]

where \( Q_n \) is the cumulative amount from the unit area at the time point “n”; \( C_i \) is the drug concentration of the nth sample; \( V_0 \) is the receptor volume 7.0 mL; \( V_i \) is 0.3 mL; \( C_i \) is the drug concentration of the ith sample, and \( A \) is the effective area 3.14 cm².

Diffusion parameters were calculated by plotting the cumulative amount from the unit area in 24 h versus time. The slope was the flux (J) and the x-intercept of the straight line was lag time.

2.8.2. Date analysis in vivo

Plasma concentration-time profiles and the pharmacokinetic parameters of D-threo-MP were calculated with WinNonlin (Version 5.2.1; Pharsight, Mountain View, CA, U.S.A.): the maximal plasma drug concentration (\( C_{max} \)), time to maximal plasma drug concentration (\( T_{max} \)), total area under the plasma concentration-time curve from time zero to time last (\( \text{AUC}_{0-\infty} \)), the terminal elimination half-life (\( t_{1/2} \)), time-averaged total body clearance (\( CL/F \)) and mean residence time (\( \text{MRT}_{0\rightarrow\infty} \)).

The absolute bioavailability (\( F_{abs} \)) was calculated from the following Eq. (2):

\[ F_{abs} = \frac{\text{AUC}_{transdermal}}{\text{AUC}_{i.v.}} \times \frac{\text{dose}_{i.v.}}{\text{dose}_{transdermal}} \times 100\% \]

The relative bioavailability (\( F_{rel} \)) was calculated from the following Eq. (3):

\[ F_{rel} = \frac{\text{AUC}_{transdermal}}{\text{AUC}_{oral}} \times \frac{\text{dose}_{oral}}{\text{dose}_{transdermal}} \times 100\% \]

2.9. Statistical analysis

Data were expressed as mean value ± standard deviation. Statistical data were analyzed by Student's t-test using SPSS version 22.0. Data were considered significant at a p-value of less than 0.05.

3. Results and discussion

3.1. Effect of species on permeation

Generally, animal skin models tend to have higher resistivities to predict drug diffusion in human compared with synthetic membranes, probably due to the more complex biochemical composition of the former [11]. Given the limited availability of human skin, nude mouse, SD rat and newborn pig skins have been investigated for species differences in the skin permeation of D-threo-MP. The permeation of D-threo-MP through various skin in vitro was evaluated while maintaining all other factors constant.
including Duro-Tak® 87-4098 based DIA patches and 15% drug loading. The permeation profiles of D-threo-MP are shown in Fig. 2, and relevant permeation parameters are listed in Table 1. The flux of D-threo-MP was significantly lower across the newborn pig skin than across the mouse and SD rat skin, which may be due to the differences in lipid and water contents and morphological characteristics (thickness, number of pores, and follicles) [11,15]. Pig stratum corneum is the most similar to human stratum corneum in terms of lipid composition, but it presents a marked difference in terms of thickness [1]. On the other hand, the thickness of newborn pig stratum corneum is considerably thinner than that of adult pig and more similar to that of human skin. Newborn pig skin as an alternative to human epidermis in in vitro permeation studies was investigated by some

**Table 1**

In vitro skin permeation parameters of D-threo-MP from patches containing 15% (w/w) D-threo-MP in Duro-Tak® 87-4098 matrix across nude mouse, SD rat, newborn pig skins (n = 4).

<table>
<thead>
<tr>
<th>Skin type</th>
<th>J (μg h⁻¹ cm⁻²)</th>
<th>Q₉ (μg cm⁻²)</th>
<th>t₉ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nude mouse skin</td>
<td>97.67 ± 3.86</td>
<td>814.10 ± 38.07</td>
<td>ND</td>
</tr>
<tr>
<td>SD rat skin</td>
<td>90.71 ± 2.46</td>
<td>777.97 ± 47.64</td>
<td>0.53 ± 0.06</td>
</tr>
<tr>
<td>New-born pig skin</td>
<td>41.20 ± 1.73</td>
<td>351.10 ± 42.90</td>
<td>0.47 ± 0.08</td>
</tr>
</tbody>
</table>

* Significantly different from the New-born pig skin group (p < 0.05).

**Table 2**

Effect of various adhesives on the permeation of D-three-MP through new-born pig skin (n = 3).

<table>
<thead>
<tr>
<th>Adhesive type</th>
<th>J (μg h⁻¹ cm⁻²)</th>
<th>Q₉ (μg cm⁻²)</th>
<th>t₉ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duro-Tak® 87-4098</td>
<td>41.84 ± 1.27</td>
<td>366.04 ± 43.74</td>
<td>0.42 ± 0.04</td>
</tr>
<tr>
<td>Duro-Tak® 87-2287</td>
<td>32.61 ± 1.09</td>
<td>288.69 ± 44.58</td>
<td>0.29 ± 0.12</td>
</tr>
<tr>
<td>Duro-Tak® 87-2852</td>
<td>26.13 ± 0.61</td>
<td>230.06 ± 33.93</td>
<td>0.39 ± 0.10</td>
</tr>
</tbody>
</table>

* Significantly different from the Duro-Tak® 87-4098 group (p < 0.05).

**Table 3**

Effect of drug loading on the permeation of D-threo-MP through new-born pig skin (n = 3).

<table>
<thead>
<tr>
<th>Content (%, w/w)</th>
<th>J (μg h⁻¹ cm⁻²)</th>
<th>Q₉ (μg cm⁻²)</th>
<th>t₉ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4.68 ± 0.16</td>
<td>40.14 ± 7.32</td>
<td>0.54 ± 0.12</td>
</tr>
<tr>
<td>5</td>
<td>17.91 ± 0.41</td>
<td>152.45 ± 20.28</td>
<td>0.41 ± 0.09</td>
</tr>
<tr>
<td>10</td>
<td>29.89 ± 0.74</td>
<td>256.38 ± 29.77</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td>15</td>
<td>42.18 ± 1.01</td>
<td>364.12 ± 63.28</td>
<td>0.51 ± 0.08</td>
</tr>
<tr>
<td>20</td>
<td>55.10 ± 1.40</td>
<td>465.56 ± 23.66</td>
<td>0.37 ± 0.04</td>
</tr>
</tbody>
</table>

* The cumulative permeation amount at 9 h.

**Fig. 2.** In vitro penetration profiles of D-three-MP patches through nude mouse, SD rat and newborn pig skins (n = 3).

**Fig. 4.** In vitro penetration profiles of D-three-MP through new-born pig skin from patches containing different loadings of D-threo-MP (n = 3).
Moreover, skin that is difficult for D-threo-MP to across through was more suitable for screening the formulation. So, newborn pig skin was selected as an alternative to human epidermis in the in vitro permeation studies.

3.2. Effect of adhesives on permeation

The selection of a suitable adhesive is the most important factor in designing a formulation for a TDDS [10]. Acrylic PSAs offered the advantages of good biocompatibility with the skin, chemical compatibility with the drug, various components of the formulation, and provide consistent, effective delivery of the drug [4,23]. In this study, the effects of different acrylic PSAs (Duro-Tak® 87-2852 with carboxylic acid groups, Duro-Tak® 87-2510 with hydroxyl groups and Duro-Tak® 87-4098 without functional groups) on the skin permeation of D-threo-MP were tested while maintaining all other factors constant including 15% drug loading. The cumulative permeation profiles of D-threo-MP through excised newborn pig skin from different adhesives are shown in Fig. 3, and the permeation parameters are listed in Table 2. The lack of skin permeation from the patches made from the PSAs containing a carboxylic acid might be due to an interaction between the piperidine groups of D-threo-MP and the carboxylic acid of the Duro-Tak® 87-2852. Other skin permeation studies have also reported that the amine moiety of drugs might interact with the carboxylic acid of the PSA to cause the lack of skin permeation [10,22]. The presence of hydroxyl groups can increase the hydrophilicity of the matrix, which might decrease the diffusivity of lipophilic D-threo-MPH. Among the three adhesives, Duro-Tak® 87-4098 without functional groups showed the highest skin permeation rate of D-threo-MP. Therefore, Duro-Tak® 87-4098 was chosen for further development of the D-

Table 4
Effect of various enhancers (5%, w/w) on the permeation of D-threo-MP through new-born pig skin (n = 3).

<table>
<thead>
<tr>
<th>Enhancers (5%, w/w)</th>
<th>J (µg h⁻¹ cm⁻²)</th>
<th>Qₚ (µg cm⁻²)</th>
<th>tₜₘ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azone</td>
<td>42.19 ± 3.29</td>
<td>359.47 ± 22.89</td>
<td>0.61 ± 0.14</td>
</tr>
<tr>
<td>1,2-Propanediol</td>
<td>45.44 ± 2.87</td>
<td>365.89 ± 38.76</td>
<td>0.73 ± 0.16</td>
</tr>
<tr>
<td>OA</td>
<td>38.87 ± 2.24</td>
<td>394.27 ± 5.05</td>
<td>0.51 ± 0.11</td>
</tr>
<tr>
<td>NMP</td>
<td>34.33 ± 3.19</td>
<td>330.54 ± 39.31</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>Menthol</td>
<td>39.30 ± 2.63</td>
<td>308.57 ± 30.6</td>
<td>0.88 ± 0.11</td>
</tr>
<tr>
<td>Control</td>
<td>41.72 ± 3.17</td>
<td>333.39 ± 25.76</td>
<td>0.58 ± 0.06</td>
</tr>
</tbody>
</table>

* The cumulative permeation amount at 9 h.

Fig. 6. Histological morphology of blank group (A: male; B: female), control group (C: male; D: female) and tested group (E: male; F: female).
3.3. Effect of drug loading on permeation

The effect of drug loading on the skin permeation of D-threo-MP was evaluated with Duro-Tak®-87-4098-based patches containing various contents of D-threo-MP (2%, 5%, 10%, 15%, and 20%, w/w). As shown in Table 3, the flux of D-threo-MP increased with increasing the amount of D-threo-MP from 2% to 20% (w/w) in the DIA patch. This result suggests that the drug content range studied may be the amount of D-threo-MP from 2% to 20% (w/w) in the DIA patch. Consequently, 15% content of D-threo-MP was selected for further studies.

To overcome the barrier properties of SC, appropriate permeation enhancers are often screened to increase the permeation of the drug [14]. Duro-Tak®-87-4098-based patches containing D-threo-MP (15%, w/w) and various enhancers (5%, w/w) were used to evaluate the effect of permeation enhancers on the skin permeation of D-threo-MP. According to preliminary studies, the effect of permeation enhancers on the permeation profiles of D-threo-MP across newborn pig skin are shown in Fig. 5, and relevant permeation parameters are listed in Table 4. Drug crystals were not observed in all patches at room temperature for 3 months. As shown in Table 4, all enhancers tested did not significantly increase the flux of D-threo-MP when compared with the control group (i.e., patches without enhancer), suggesting that the addition of enhancers was not very helpful, although OA, Azone and 1,2-Propanediol tended to slightly increase the flux (no statistical significance). Based on these results, no enhancer was added to the optimized patch.

3.5. In vivo skin irritation test

The safety of D-threo-MP patches was evaluated by the Draize method and histological examination. According to the Draize method, there were no obvious irritation effects as the total irritation score was identified to be zero in all rats after 9 h application of the optimized patches on the dorsal skin. The results of histological examination are shown in Fig. 6. There were no differences in the morphology of blank, control and tested rat skin, indicating the safety of using D-threo-MP patches for transdermal delivery.

3.6. In vivo pharmacokinetic study in rats

Rat is the most commonly used animal model in in vitro and in vivo percutaneous permeation studies [12,26]. The advantages of rats are their small size, uncomplicated handling, and relatively low cost. Therefore, the SD rat model was chosen to evaluate the percutaneous characteristics of the optimized patch. After transdermal application, i.v. injection or oral administration in rats (n = 5), the concentration of D-threo-MP in plasma was determined by the validated UFLC-MS/MS method. The average plasma concentration-time profiles are presented in Fig. 7, and the corresponding pharmacokinetic parameters based on the non-compartmental method are shown in Table 5. After 5 min of transdermal application, D-threo-MP was first detected in the plasma, and the concentration increased steadily up to 2 h. It was clear that D-threo-MP could cross SD rat skin quickly. The observed similarity in transdermal permeation with rats has previously been reported for other drug substances [20,25].

After 6 h of transdermal application, the plasma concentration decreased, but the drug could still be detected until 24 h. After i.v. or oral administration of D-threo-MP, the peak drug concentrations were reached rapidly at 0.05 ± 0.03 h and 0.50 ± 0.00 h, respectively. The Cmax of D-threo-MPH were 60.79 (transdermal), 93.64 (oral), and 354.57 (i.v.) ng ml⁻¹ with an elimination half-life of 5.55 ± 1.43 h, 1.09 ± 0.29 h and 1.22 ± 0.31 h, respectively. The elimination of D-threo-MPH after i.v. injection and oral administration in rats was rapid. The pharmacokinetic parameters t½ and and mean residential time (MRT) obtained with transdermal patches were significantly (p < 0.05) different from those obtained with i.v. and oral administration. After calculating the dose normalization, Fco and Fco of D-threo-MP transdermal patch in SD rat were 218.8% and 53.4%, respectively. These values show that the transdermal route constitutes an efficient way for D-threo-MP to be absorbed into the systemic circulation.
3.7. In vivo pharmacokinetic study in bama miniature pigs

Although it was found that transdermal absorption in rats and rabbits was not always predictive for human data, results obtained from porcine models and non-human primates were comparable [7]. However, it is difficult to obtain data of drug concentrations in tissues from non-human primates. Based on the results of our study, the bama miniature pig may serve as a suitable model for in vivo pharmacokinetic analysis of D-threo-MP. The profiles of mean plasma concentration of D-threo-MP versus time after oral or transdermal administration are shown in Fig. 7. After a single oral dose (5 mg) of D-threo-MP in capsule form, absorption in pig was rapid yielding a peak drug concentrations of 55.01 ± 8.77 ng/ml at 0.44 ± 0.13 h. Thereafter, plasma drug concentrations declined to 1.64 ng/ml at 24 h and the t1/2 was 6.47 ± 4.07 h. The AUC0–∞ for D-threo-MP after oral administration averaged 9925.2 ± 2753.3 h ng ml⁻¹. After 0.5 h of transdermal application, D-threo-MP was first detected in the plasma, and the concentration increased steadily up to 9 h. After removal of the patch, the plasma concentration decreased. As shown in Fig. 7, the absorption rate of D-threo-MP via transdermal was relatively slow compared with the oral route. The peak drug concentrations in plasma averaged 8.47 ± 0.48 ng/ml. The pharmacokinetic parameters for both oral and transdermal administration are presented in Table 5. The pharmacokinetic parameters t1/2 and mean residential time (MRT) obtained with transdermal patches were significantly (p < 0.05) different from those obtained with oral administration. After dose normalization, F∞ of transdermal patch in miniature pig was 83.4%. Therefore, just viewed from F∞, rat skin was 2.62 times more permeable than miniature pigs, which is in good agreement with in vitro transdermal studies. These values further show that the transdermal route is a promising way for D-threo-MP to be absorbed into the systemic circulation.

Table 5
Pharmacokinetic parameters of D-threo-MP following transdermal application, i.v. injection or oral administration in rats and bama miniature pigs.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>SD rats (n = 5)</th>
<th>Bama miniature pigs (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intravenous</td>
<td>Oral</td>
</tr>
<tr>
<td>Cmax (ng ml⁻¹)</td>
<td>354.57 ± 51.43</td>
<td>90.64 ± 21.14</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.05 ± 0.03</td>
<td>0.50 ± 0.00</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>1.22 ± 0.31</td>
<td>1.09 ± 0.29</td>
</tr>
<tr>
<td>AUC0–∞ (h ng ml⁻¹)</td>
<td>13.896 ± 518.5</td>
<td>27.435.2 ± 14730.0</td>
</tr>
<tr>
<td>MRT0–∞ (h)</td>
<td>1.29 ± 0.16</td>
<td>1.35 ± 0.11</td>
</tr>
<tr>
<td>CL/F (h⁻¹ kg⁻¹)</td>
<td>14.41 ± 0.52</td>
<td>55.76 ± 12.53</td>
</tr>
</tbody>
</table>

4. Conclusion

The DIA patches of D-threo-MP were prepared and optimized with respect to three formulation factors, i.e., adhesives, drug loading and permeation enhancers. As a result of the in vitro formulation optimization using newborn pig skin, a Duro-Tak® 87–4098-based patch formulation containing 15% (w/w) D-threo-MP was chosen for in vivo pharmacokinetic evaluation. In pharmacokinetic studies, F∞ of D-threo-MP DIA patch in the SD rat model was 53.4% compared to i.v. injection of the drug, and F∞ was 218.8% compared to oral administration. Moreover, F∞ in bama miniature pigs was 83.4% compared to oral administration. The bioavailability in both animal models suggested that sufficient amounts of D-threo-MP could across through the skin and pass into the systemic circulation. The optimized patch could serve as a promising transdermal delivery system for D-threo-MP.

Acknowledgments

This research was funded by the Major Projects for Drug Innovation and Development from National Science and Technology of China (2014ZX09507006-002).

References


