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Research on Transdermal Penetration of Rotigotine Transdermal Patchng

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Abstract. The transdermal penetration behavior of drugs is a core link in the research and development of transdermal drug delivery systems, and its efficiency directly affects the in vivo absorption and therapeutic effect of drugs. The selection and processing of skin have an important impact on studying the transdermal penetration behavior of drugs. This study aims to investigate the penetration characteristics of rotigotine transdermal patches in different animal skins (mouse, rat, nude mouse, and pig skin) through in vitro transdermal penetration experiments, and to explore the effect of freezing treatment on skin penetration performance. The results showed that the 24-hour cumulative penetration amount of rotigotine transdermal patch in mouse skin was significantly higher than that in rat and nude mouse skin, with no significant difference between rat and nude mouse skin. Freezing treatment had no significant effect on the penetration amount in rat and nude mouse skin, but significantly reduced the penetration amount in mouse and pig skin, and the penetration amount in pig skin gradually decreased with the extension of freezing time. Meanwhile, there were differences in the initial penetration time and steady-state permeation rate of the drug among different animal skins. The results of this study provide a reference for the selection of animal models, optimization of freezing treatment conditions, and construction of IVIVC models in in vitro penetration experiments of rotigotine transdermal patches.

Keywords: Transdermal penetration; Rotigotine transdermal patch; High-performance liquid chromatography.

1. Introduction

Transdermal drug delivery system (TDDS) [1], also known as transdermal administration system, is a new type of administration method that delivers drugs through the skin surface and allows drugs to enter the systemic circulation through the skin to achieve systemic or local treatment [2]. Compared with traditional oral and injection administration methods, transdermal drug delivery systems have many advantages, such as reducing the first-pass effect, avoiding gastrointestinal irritation, providing a stable drug release rate, reducing the frequency of administration, and improving patient compliance [3].

The transdermal penetration behavior of drugs is a core link in the research and development of transdermal drug delivery systems, and its efficiency directly affects the in vivo absorption and therapeutic effect of drugs [4]. However, the transdermal penetration ability of drugs is affected by many factors, including the physicochemical properties of drugs, the physiological characteristics of the skin, and the preparation process [5]. Therefore, in-depth study of the transdermal penetration behavior of drugs is of great significance for optimizing the design of transdermal preparations and improving their clinical application effects.

In vitro-in vivo correlation (IVIVC) is an important means to evaluate the performance of transdermal preparations, aiming to predict the absorption behavior of drugs in the human body through in vitro experimental data [6]. At present, the construction of IVIVC models mainly focuses on oral drugs, while the research on IVIVC of transdermal preparations is relatively limited. Although some studies have attempted to establish IVIVC models for transdermal preparations, the construction of IVIVC models still faces many challenges due to the fact that the penetration process of transdermal drugs

is affected by multiple factors such as skin barrier, physicochemical properties of drugs, and preparation process [7].

Rotigotine is a non-ergot dopamine receptor agonist that can activate D1, D2, and D3 receptors, thereby replacing the role of dopamine. Its transdermal patch form penetrates into the blood circulation through the skin, has stable drug release characteristics, and can effectively alleviate motor disorders in patients with Parkinson's disease and discomfort in patients with restless legs syndrome [8]. Rotigotine transdermal patch was approved for marketing by the US FDA in 2007, becoming the first transdermal patch for the treatment of Parkinson's disease. However, there are still few studies on IVIVC of rotigotine transdermal patches, mostly focusing on a single animal model or specific conditions.

The selection and processing of skin have an important impact on the transdermal penetration behavior of drugs [9]. Commonly used skins in studies include rodent skins (such as mouse, rat, nude mouse) and pig skin, etc. Different animal skins have different penetration effects on drugs, and the frozen storage conditions of skin (such as temperature, time) also significantly affect the penetration characteristics of drugs. For example, freezing treatment may change the structure and metabolic activity of the skin, thereby affecting the penetration rate and amount of drugs. Therefore, further study on the transdermal penetration characteristics of different animal skins (such as mouse, rat, nude mouse, and pig skin) under different freezing conditions is of great significance for constructing a reliable IVIVC model of rotigotine transdermal patches.

This study aims to investigate the penetration characteristics of rotigotine transdermal patches in different animal skins (mouse, rat, nude mouse, and pig skin) through in vitro transdermal penetration experiments, and to explore the effect of freezing treatment on skin penetration performance. This part of the study aims to reveal the influence of skin species and freezing state on the transdermal penetration of the two types of drugs, provide experimental basis for optimizing the design of in vitro penetration experiments and screening appropriate animal models, and further provide theoretical support for the construction of IVIVC of transdermal preparations and their clinical application.

2. Research Methods

2.1. Preparation of Excised Skin

(1) Full skin of rats and mice: Male SPF mice and male SD rats were first sacrificed by cervical dislocation or drug anesthesia. Then, an electric shaver was used to carefully remove the hair from the dorsal skin of mice and rats, and the skin was cut off. The dorsal skin samples were fixed, and the subcutaneous fat and connective tissue layers were removed. A thickness gauge was used to measure the skin thickness, and then the treated skin samples were stored in refrigerators at 4°C and -20°C for later use, respectively.

(2) Full skin of nude mice: Nude mice were sacrificed by cervical dislocation, the dorsal skin was cut off, then the dorsal skin samples were fixed, and the subcutaneous fat and connective tissue layers were removed. A thickness gauge was used to measure the skin thickness, and then the treated skin samples were stored in refrigerators at 4°C and -20°C for later use, respectively.

(3) Porcine dorsal skin was purchased from a biotechnology company. A thickness gauge was used to measure the skin thickness, and then the treated skin samples were stored in refrigerators at 4°C and -20°C for later use, respectively.

During the experiment, to minimize differences between individuals, dorsal skins of rodents of the same age were selected, and the skin thickness was controlled within a certain range.

2.2. In vitro Penetration Experiment with Fresh Skin

A Franz vertical diffusion cell (diffusion cell volume: 10mL, effective diffusion area: $1.767cm^2$) was used for in vitro penetration experiments. First, the rotigotine transdermal patch was processed,

cut into a circle with an area of approximately $1.767\ \mathrm{cm}^2$, the surface release liner was removed, and the patch was applied to the skin to be tested, pressed gently to ensure firm adhesion. After fixing the skin with a fixing clip, the pre-configured receiving medium (PBS buffer solution with $\mathrm{pH} = 6.5$) was added.

The device temperature was set to $32 \pm 0.5°\mathrm{C}$, the rotation speed was 600 rpm, and the receiving solution was PBS buffer solution with $\mathrm{pH} = 6.5$ (always meeting the sink condition). Samples of $200\mu\mathrm{L}$ were taken at 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 hours, respectively, and an equal amount of isothermal blank receiving solution was added. The samples were centrifuged at high speed (10000 rpm, 5 min), and the supernatant was taken. The samples were detected by high-performance liquid chromatography (HPLC) method. The cumulative release amount and cumulative release rate were calculated according to following equation, and the Q_t-*t* curve and *Rt-t* curve were drawn.

$$Q_t = \frac{C_n \times V + \sum C_{n-1} \times V_n}{A}$$

$$R_t = \frac{Q_t}{M_o}$$

Where, Q_t is the cumulative release amount per unit area $(\mu\mathrm{g/cm}^2)$; C_n is the concentration of the receiving medium solution (RVS) at the nth sampling point $(\mu\mathrm{g/mL})$; V is the volume of the diffusion cell (mL); V_n is the sampling volume (mL); A is the effective diffusion area of the drug (cm^2); R_t is the cumulative release rate per unit area (%); M_o is the drug content per unit area of the patch.

2.3. In vitro Penetration Experiment with Frozen Skin

One day before the in vitro transdermal experiment, mouse, rat, nude mouse, and pig skins frozen in a refrigerator at -20°C for different times were placed in a refrigerator at 4°C for thawing and used for the next day's transdermal experiment. The transdermal penetration experiment was carried out using the in vitro transdermal penetration method described in 2.2. The cumulative release amount and cumulative release rate were calculated, and the Q-t curve was drawn.

SPSS 21.0 was used for statistical analysis of all sample data, and the results were presented as the mean±standard deviation of at least three independent determinations. The p-value was obtained using the t-test. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. In vitro Penetration Study on Fresh Rat Skin

The results of 24-hour in vitro penetration of rotigotine transdermal patch $(2.5\mathrm{mg/24\ h})$ in three types of rat skins are shown in Figure 1. Figure 1(A) shows the cumulative penetration amount of rotigotine after 24-hour in vitro penetration in three types of rat skins. It can be seen that the penetration amounts of rotigotine in mouse, rat, and nude mouse skins were $373.67 \pm 40.48\mu\mathrm{g/cm}^2$, $137.69 \pm 20.77\mu\mathrm{g/cm}^2$, and $183.32 \pm 20.20\mu\mathrm{g/cm}^2$, respectively. The cumulative penetration amount in mouse skin was the highest, with significant differences compared with that in rat and nude mouse skins ($p < 0.001$). The cumulative penetration amount was approximately twice that in nude mouse skin and 2.7 times that in rat skin.

However, there was no significant difference in the cumulative penetration amount between rat and nude mouse skins. Figure 3(B) shows the penetration curves of rotigotine transdermal patch in three types of rat skins. It can be seen that drug penetration occurred in mouse skin at 1 hour, while it took 4 hours in rat and nude mouse skins. This may be related to the thinner skin of mice and their abundant hair follicles. The steadystate permeation rate of mice was significantly higher than that of rats and nude mice; nude mouse skin is thin but has fewer hair follicles, while rat skin has more hair follicles but is thicker. Therefore, although the cumulative penetration amount of nude mice was slightly higher than that of rats, there was no significant difference.

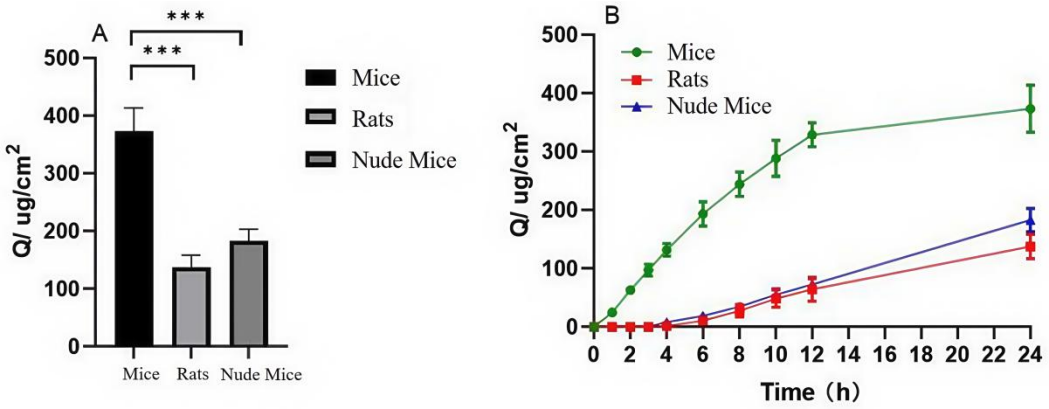

Figure 1. Penetration results of rotigotine transdermal patch in different mouse skins for 24 hours in vitro. (A) 24h cumulative permeation of rotigotine (B) cumulative permeability curve (32±0.5°C, mean±SD, n=3)

3.2. In vitro Penetration Study on Frozen Rat Skin

Rats and nude mice are commonly used rodents in in vitro penetration experiments. Rats are more similar to humans in skin structure, and nude mice eliminate the need for hair removal in experiments, and the lipid content in their skin is more constant with less variability. Based on this, this paper selected these two types of skins to investigate the penetration performance of rotigotine drug patches in rat and nude mouse skins treatec at $-20°C$ for different freezing times (two weeks, four weeks, and eight weeks).

Figure 2(A) shows the cumulative penetration amount of rotigotine transdermal patch in rat skins stored under freezing conditions for different times. It can be seen that the 24-hour cumulative penetration amounts of rotigotine transdermal patch in rat skins after different freezing times (2, 4, and 8 weeks) were $99.92 \pm 4.76\mu g/cm^2$, $122.26 \pm 2.57\mu g/cm^2$, and $104.29 \pm 3.61\mu g/cm^2$, respectively. The cumulative penetration amount in frest rat skin was higher than that in frozen skin, which was $137.69 \pm 3.77\mu g/cm^2$. The penetration amount of rotigotine in fresh rat skin was the highest, and the lowest in rat skin frozen for two weeks. Although the penetration amount in frozen rat skin was lower than that in fresh skin, there was no significant difference in statistics, indicating that when using frozen rat skin for in vitro transdermal penetration, it will not cause significant changes in the penetration amount of rotigotine in rat skin, and frozen rat skin can be used for the study of in vitro transdermal penetration performance of rotigotine transdermal patches.

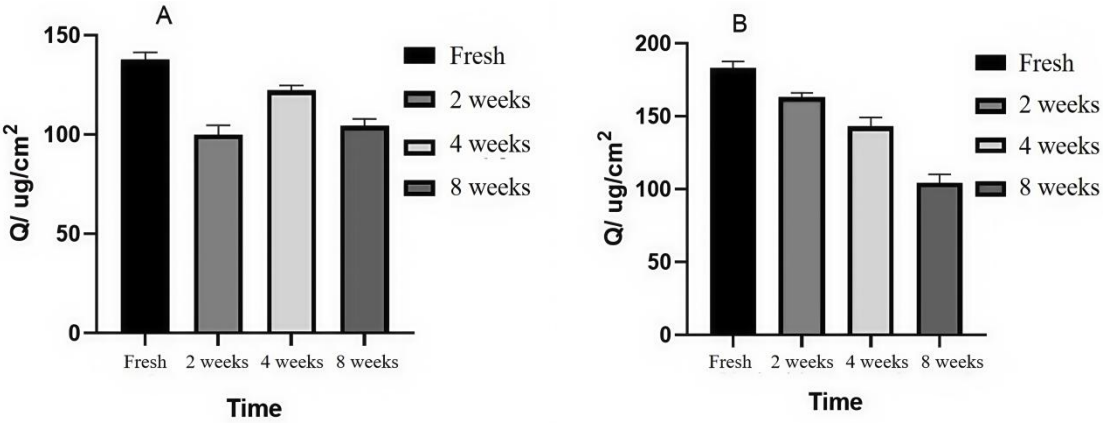

Figure 2. 24h cumulative penetration of rotigotine in different skin. (A) Rats; (B) Nude mice (32±0.5°C, mean±SD, n=3)

Figure 2(B) shows the 24 -hour cumulative penetration amount of rotigotine transdermal patch in nude mouse skins treated by freezing at $-20°C$ for different times. It can be seen that the penetration amount of rotigotine transdermal patch in nude mouse skin gradually decreased with the increase of

skin freezing time. The penetration amount in fresh skin was higher than that in nude mouse skins frozen for different times. The penetration amount in fresh nude mouse skin was $183.31 \pm 4.19 \mu g/cm^2$, and the 24-hour penetration amounts in skins frozen for different times (2, 4, and 8 weeks) were $163.02 \pm 3.29 \mu g/cm^2$, $142.89 \pm 6.20 \mu g/cm^2$, and $104.29 \pm 6.02 \mu g/cm^2$, respectively. The penetration amount of rotigotine in fresh nude mouse skin was the highest, and the lowest in nude mouse skin frozen for eight weeks. Although the penetration amount of rotigotine in nude mouse skin decreased after freezing, there was no significant difference in statistics, indicating that frozen nude mouse skin can also be used for the study of in vitro transdermal penetration performance of rotigotine transdermal patches.

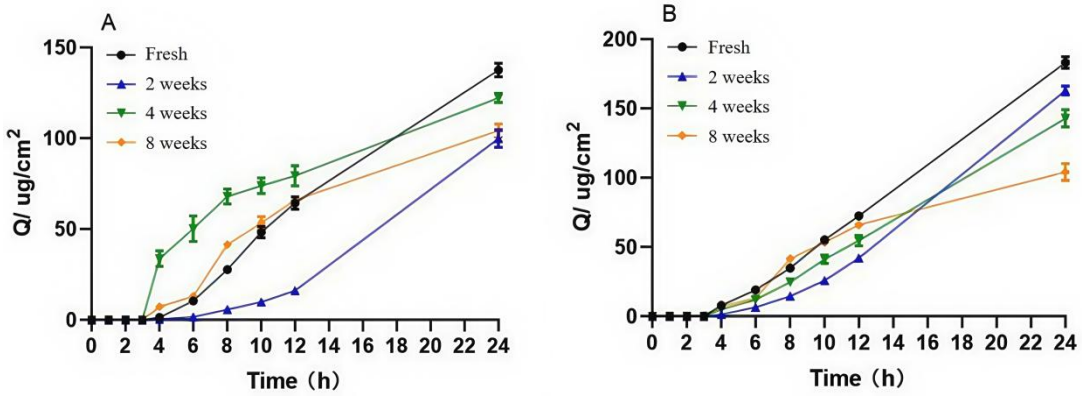

Figure 3. 24h penetration curve of rotigotine in different skin. (A) Rats; (B) Nude mice (32±0.5°C, mean±SD, n=3)

Figure 3(A) is the penetration curve of rotigotine transdermal patch in rat skin. The results show that in the first 12 hours, except for the skin frozen for two weeks, the drug penetration amount was higher than that in fresh skin. After 24 hours of penetration, only the penetration amount in frozen skin was lower than that in fresh skin. Figure 3(B) is the penetration curve of rotigotine transdermal patch in nude mouse skin. The results show that during the penetration process, the drug penetration amount in frozen nude mouse skin was lower than that in fresh skin.

3.3. In vitro Penetration Study on Mouse Skin

In in vitro penetration experiments, although mouse skin is thin and has abundant hair follicles, it is widely used by researchers because it is easy to obtain and handle. Based on this, this paper investigated the penetration performance of rotigotine transdermal patch in frozen (4 weeks and 8 weeks) mouse skin.

Figure 4(A) shows the cumulative penetration amount of rotigotine transdermal patch ($2.5mg/24h$) in excised mouse skin under different conditions (fresh and different freezing times (4 weeks and 8 weeks)). It can be seen that the penetration amounts of rotigotine in fresh mouse skin and mouse skins frozen for different times (4 weeks and 8 weeks) were $373.67 \pm 40.48 \mu g/cm^2$, $324.21 \pm 60.29 \mu g/cm^2$, and $293.96 \pm 20.81 \mu g/cm^2$, respectively. The penetration amount in frozen skin was lower than that in fresh skin, with a significant difference ($P < 0.001$). This indicates that freezing mouse skin for four weeks has a significant impact on the penetration performance of rotigotine in mouse skin. Figure 4(B) shows the penetration curves of rotigotine transdermal patch in mouse skins under different freezing conditions (4 weeks and 8 weeks). The results show that although the penetration amount changed after freezing for different times, the penetration trend did not change.

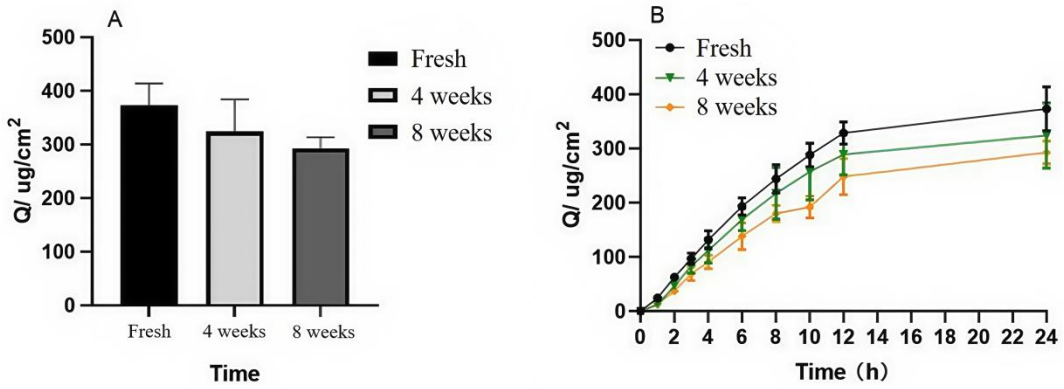

Figure 4. Penetration results of rotigotine transdermal patch in different mouse skins for 24 hours in vitro. (A) 24h cumulative permeation of rotigotine (B) cumulative permeability curve (32±0.5°C, mean±SD, n=3)

3.4. In vitro Penetration Study on Pig Skin

Pig skin is also widely used to investigate the in vitro transdermal penetration of drugs because it has similar physiological and biochemical properties to human skin. This chapter conducted penetration experiments of rotigotine transdermal patch in fresh pig skin and pig skin frozen at $-20°C$ for two weeks and four weeks, and the results are shown in Figure 5.

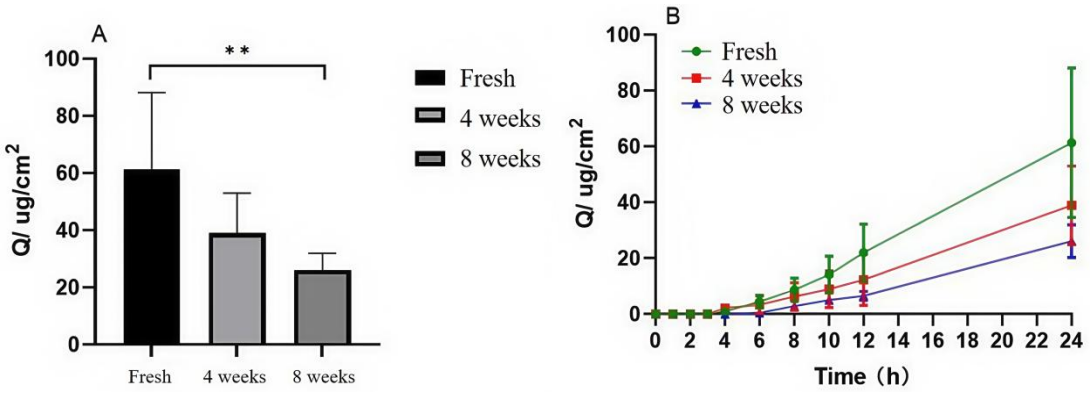

Figure 5. Penetration results of rotigotine transdermal patch in porcine skin for 24 hours in vitro. (A) 24h cumulative permeation of rotigotine (B) cumulative permeability curve (32±0.5°C, mean±SD, n=3)

Figure 5(A) shows the cumulative penetration amounts of rotigotine transdermal patch (2.5mg/24 h) in fresh pig skin and pig skin frozen for 2 weeks and 4 weeks, which were $61.35 \pm 26.78\mu g/cm^2$, $38.91 \pm 14.10\mu g/cm^2$, and $26.12 \pm 5.87\mu g/cm^2$, respectively. The penetration amount in frozen skin was lower than that in fresh skin, and there was a significant difference in the penetration amount between the skin frozen for four weeks and the fresh skin ($P < 0.01$), indicating that freezing pig skin for four weeks significantly reduced the penetration performance of rotigotine. Figure 5(B) shows the penetration curves of rotigotine transdermal patch in fresh pig skin and pig skin frozen for two weeks and four weeks. The results show that with the increase of freezing time, the transdermal penetration trend of the drug did not change significantly, but the penetration amount of rotigotine in pig skin gradually decreased. This indicates that freezing affects the in vitro penetration performance of rotigotine in pig skin.

4. Conclusion

In this study, the penetration characteristics of rotigotine transdermal patches in different animal skins (mouse, rat, nude mouse, pig skin) under different freezing treatment conditions were systematically investigated through in vitro transdermal penetration experiments. The results showed that there were

significant differences in the penetration performance of rotigotine among different species of animal skins. Among them, the 24-hour cumulative penetration amount of mouse skin was the highest, and the initial drug penetration time was the earliest (1 hour), which was related to its thin skin and abundant hair follicles. There was no significant difference in the cumulative penetration amount between rat and nude mouse skins, and the initial drug penetration time was 4 hours. Freezing treatment had different effects on the penetration of different animal skins: Under the condition of -20℃, the 24-hour cumulative penetration amount of rotigotine in rat and nude mouse skins treated with different freezing times (2 weeks, 4 weeks, 8 weeks) was lower than that in fresh skins, but there was no statistically significant difference, indicating that frozen rat and nude mouse skins can be used for in vitro penetration studies of the patch. However, the cumulative drug penetration amount in mouse skins frozen for 4 weeks and 8 weeks was significantly lower than that in fresh skins. In pig skins frozen for 4 weeks and 8 weeks, the cumulative drug penetration amount gradually decreased with the extension of freezing time, and there was a significant difference in the penetration amount between pig skins frozen for 4 weeks and fresh skins, indicating that freezing treatment had a significant impact on the penetration performance of mouse and pig skins. However, the penetration trend of all animal skins did not change due to freezing treatment. In conclusion, the species of animal skin and the freezing treatment time are important factors affecting the transdermal penetration of rotigotine transdermal patches. The results of this study provide experimental basis for optimizing the design of in vitro penetration experiments, selecting appropriate animal models, and constructing in vitro-in vivo correlation (IVIVC) models of transdermal preparations, and are of great significance for improving the clinical application effect of rotigotine transdermal patches.

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