Delivery of risperidone from gels across porcine skin *in vitro* and *in vivo* in rabbits

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Abstract: The purpose of this study was to develop and evaluate a transdermal delivery system for RIS using hydrogels. First, the effects of different concentrations of hydroxypropyl methylcellulose and Carbomer 934 (CBR) on RIS permeation were investigated in porcine skin. The optimized formulation was chosen as the base gel to screen for penetration enhancers. The pharmacokinetics of the optimized RIS formulation was then studied *in vitro* in rabbits. A formulation with 0.5% CBR showed the highest RIS permeation and was selected as the base gel. RIS permeation was further increased by incorporation of Azone, lauryl alcohol, or menthol, and the enhancing effects of the three were dose-dependent. When each enhancer combined with propylene glycol (PG) a synergistic effect was found. A combination of 6% menthol and 6% PG exhibited highest RIS *in vitro* penetration rate and showed a high efficiency *in vivo*, with a relative bioavailability of 131.53% compared with intragastric administration. These findings showed that 1% RIS in 0.5% CBR, containing a combination of 6% menthol and 6% PG, can deliver doses of RIS that are therapeutically relevant for treating patients with schizophrenia.

Keywords: Risperidone, Transdermal, Gel, Penetration enhancer, Pharmacokinetic.

INTRODUCTION

The first approved transdermal drug was the scopolamine patch for motion sickness, and since its introduction in the early 1980s, there has been a marked increase in the number of transdermal preparations, with many commercial successes. Transdermal administration has several well-known advantages, such as (a) avoidance of first-pass metabolism, (b) sustained and controlled rates of passive transdermal delivery of a drug over extended periods of time, (c) therapeutic failures related to intermittent dosing or a decrease in adverse effects, (d) easy termination of drug delivery in the event of any systemic or local adverse reactions, and (e) improved patient compliance and acceptability because transdermal delivery is a noninvasive method of administration (Brown *et al*., 2006; Guy, 2010; Wiedersberg and Guy, 2014).

Risperidone (RIS) is an atypical antipsychotic with potent antagonistic properties against serotonin 5-HT_2A and dopamine D_2 receptors and has been used in the treatment of mental disorders for many years (D'Souza *et al*., 2013; Lieberman *et al*., 2005). The available dosage forms on the market include tablets and solutions for oral administration, and long-acting injection via intramuscular or subcutaneous routes (D'Souza *et al*., 2013; Heemstra *et al*., 2010). However, both oral and injectable dosage forms have some flaws. Oral formulations present a substantial challenges in the treatment of patients suffering from psychiatric ailments because of noncompliance (D'Souza *et al*., 2013; Samalin *et al*., 2014; Schreiner *et al*., 2014), while long-acting inject able preparations have disadvantages, such as the inability to rapidly stop drug effects in the case of unexpected side effects, their invasive nature, and the high costs associated with long-term therapy (Heemstra *et al*., 2010).

To combat these issues in the use of currently available RIS dosage forms and to develop a formulation that allows sustained delivery over an extended period, appropriate delivery systems for this beneficial antipsychotic are under intensive investigation. For example, very recent studies have highlighted nasal (Kumar *et al*., 2008), buccal mucosal (Heemstra *et al*., 2010) and transdermal (Liang and Chen, 2009; Imam *et al*., 2014). Routes for delivering RIS systemically and it is likely that other nonconventional routes may also prove clinically relevant for this class of compound. Thus, transdermal delivery is an effective and preferred route of administration that has the benefits of providing continuous, long-term drug delivery.

Our previous study (Liang and Chen, 2009) showed that a RIS transdermal patch possessed high percutaneous permeability and bioavailability *in vitro* and *in vivo* experiments. However, it is well-known that patches have some limitations, such as skin irritation and poor adhesion.

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In addition, from a practical and esthetic standpoint, there is a limit to the size of the patch, with less than 40 cm^2 considered ideal (Brown *et al*., 2006) and 100 cm^2 considered the maximum acceptable size (Guy, 2010), which limits the amount of drug that can be delivered. Hydrogels, such as hydroxypropyl methylcellulose (HPMC) and Carbomer 934 (CBR), are three-dimensional hydrophilic polymer networks, which have the ability to absorb large quantities of water. The advantages of hydrogels are as follows: (a) they can be easily prepared; (b) they are inexpensive; (c) they are biodegradable; (d) they can incorporate many compounds (Rehman and Zulfakar, 2014); and (e) they are more dynamic and less occlusive than conventional transdermal patches (Lane, 2013). Meanwhile, HPMC and CBR are non-toxic and are approved for use in the field of medicine by FDA in drug delivery system.

Thus, the purpose of the present research was to develop a gel for transdermal delivery of RIS. In a first step, HPMC and CBR were the gel polymer selected for investigation of their efficiency in the delivery of RIS in permeation experiments *in vitro*. The composition of the selected formulation was modified in order to further increase its percutaneous permeability by incorporation of a permeation enhancer. The most efficient combinations were investigated and the formulation with the best performance was evaluated *in vivo* in rabbits.

MATERIALS AND METHODS

Materials

RIS was purchased from Huahai Pharmaceutical Co. Ltd (Zhejiang, China). Clozapine was obtained from the Wanbangde Pharmaceutical Group Co. Ltd (Zhejiang, China). CBR and Methocel K4MCR (HPMC) were manufactured by BF Goodrich Company (USA) and Dow Chemical Company (USA), respectively. Methanol and acetonitrile were high performance liquid chromatography (HPLC) grade and purchased from Merck (Darmstadt, Germany). Other materials used in this study were purchased from Huadong Medicine Co. Ltd (Hangzhou, China) and were of analytical or HPLC grade.

Suckling Yorkshire pigs and New Zealand white rabbits were provided by the Experimental Animal Center of Zhejiang University (Hangzhou, China). Animal care and experiments were conducted in accordance with the Experimental Animal Regulations by the National Science and Technology Commission, China.

Preparation of gel formulations

To evaluate the effect of different gelling agents on RIS permeation, HPMC and CBR were selected for use in the tested formulation. Briefly, the gel polymers were slowly dispersed in distilled water. RIS was dissolved in ethanol or dispersed in distilled water, added drop wise into the gels and thoroughly mixed. Other optional chemical enhancers were added into the gels as prescribed. The concentration of RIS in all formulations and reference solutions was 1% (w/w). The CBR gel was neutralized with triethanolamine as prescribed. Table 1 displays the exact composition of the gels. The formulation with the highest penetration rate was used for the furthering penetration enhancer tests. Azone (AZO), lauryl alcohol (LA), oleic acid (OA), and menthol (MEN) were tested in this study. The concentrations of OA were 1, 3, and 5% (w/w), of AZO were 2, 4, 6, and 8% (w/w), of MEN were 6, 8, and 10% (w/w), and of LA were 4, 6, and 8% (w/w). The amount of distilled water added varied to make a final weight as prescribed.

In vitro skin permeation studies

The experiment was carried out as described previously (Ning *et al*., 2016; Rao *et al*., 2013) with some modifications. Porcine skin was peeled from suckling Yorkshire pigs (9-10 days old, about 1.5kg). After removing the subcutaneous fat, the skin was stored at -80ºC for no more than 3 months before use. Frozen porcine skin was thawed at ambient temperature, and then washed gently three times with saline immediately before the experiments. The skin was placed and clamped onto the modified vertical Franz diffusion cells (6.8mL, surface area 2.8cm^2) and trimmed to size. The diffusion cell contained isotonic saline for transport kinetic balance of the skin. After equilibration for 1 hour, the isotonic saline was removed and replaced with degassed isotonic saline containing 20% PEG 400. 0.5mL sample was applied to each donor chamber of the Franz cell. At predetermined intervals (3, 6, 9, 12, 21 and 24h), 1mL sample was collected and refilled by 1mL fresh receptor fluid. The receptor compartment was maintained at 37± 0.5ºC and stirred at 300 rpm. The permeation experiments were carried out for 24h. All samples were tested in triplicate. The amount of RIS in the receptor medium was analyzed with HPLC.

In vivo permeation studies

The optimal formulation from the *in vitro* studies was topically applied to rabbits to determine the pharmacokinetic properties and relative bioavailability of the transdermal RIS gel compared with intragastric RIS administration. The rabbits, weighing 2.0±0.1kg, were divided into two groups, each having three animals. At 24 h before the start of the experiment, the intragastric group was fasted but given free access to water, and the test area on the back of the transdermal group was shaved gently. The rabbits in the intragastric group were administered 10 mg RIS dispersed in 0.1M ammonium acetate solutions (2 mg/mL). In the transdermal group 1.0g of the prepared gel was applied to the test area (50cm^2, 0.2mg RIS/cm^2), and then the rabbit was restrained in a wooden box for 1h to avoid the formulation was removed. Blood samples (1 mL) were collected from the ear vein at predetermined intervals (15min, 30 min, 1h, 1.5h, 2h, 4h, 8h, 12h, 18h,

24 h, 36h and 48 h) after RIS application. The blood samples were placed in heparinized centrifuge tubes and centrifuged immediately at 5,000 rpm for 5 minutes. The obtained plasma was stored at -20ºC until analysis.

For RIS extraction, 0.5mL plasma samples were placed into 10mL centrifuge tubes with addition of 50µL clozapine (5µg/mL) and 0.2mL NaOH (1M). The tubes were vibrated and mixed by vortex for 6 s. Then, 2mL of compound organic solvent (ethyl acetate: diethyl ether = 1:1, v/v) was added, the tubes were thoroughly mixed for another 2 min by vortex, and the procedure was repeated twice. The mixture was centrifuged at 3000 rpm for 10 min and 3mL of the organic phase was transferred to a new tube. The organic solutions were evaporated and the residue was redis solved in 100µL methanol for HPLC analysis. Pharmacokinetic data were calculated using PK Solver v. 2.0.

Drug analysis

Analysis of RIS in receptor medium

A rapid and sensitive RP-HPLC method was developed to determine RIS concentrations in the receptor medium. Samples were analyzed at a temperature of 40ºC using an HPLC-UV method with a Zorbax Eclipse XDB-C18 reversed phase chromatography column (250×4.6 mm, 5 µm, Agilent Technologies, Sent Clara, California, USA). The detection wavelength was 280 nm. The injection volume was 20µL. An isocratic elution program was used with a mobile phase of methanol: ammonium acetate (0.1 M): acetonitrile (35:35:30, v/v/v) at a flow rate of 1 mL/min. The amount of drug permeated per cm^2 of skin was calculated from the standard curve. The retention time of RIS was about 4.5 min. Linearity was determined in the range of 1−200µg/mL with $R^2 = 0.9999$.

The flux (J_{ss}, µg/cm^2/h) was obtained from the slope of the curve between the steady-state values of the cumulative amount (Q_{24h}, µg/cm^2) of RIS versus time (Komatsu and Suzuki, 1979). Enhancement ratios (ER) are expressed as the ratio of the flux value with enhancer to without enhancer (Jantharaprapap and Stagni, 2007).

Analysis of RIS in plasma

For analysis, RIS was extracted from the plasma samples, clozapine was added as an internal standard, the mobile phase was changed to methanol: triethylamine (0.5%): acetonitrile (60:30:10, v/v/v, pH =7.5 with acetic acid), and RIS analysis was performed as described in the previous section. The method was shown to have good linearity and precision in the range of 10-1000ng/mL (R^2 = 0.998).

STATISTICAL ANALYSIS

Each experiment was repeated at least three times. Microsoft Excel 2010 and SPSS software (v 20.0; SPSS, Inc., Chicago, IL, USA) were used for analysis, and all data are expressed as mean ± standard deviation. Statistical analysis was performed using ANOVA, Tukey *post hoc* test and the Student *t*-test. A P value < 0.05 was considered statistically significant.

RESULTS

Effect of HPMC and CBR concentrations on the permeation of RIS

fig. 1 shows the cumulative amount after 24h and transdermal flux of RIS for the formulations with different HPMC or CBR concentrations (table 1) through porcine skin. Apart from 2% CBR (F3), almost all formulations delivered a significantly higher cumulative amount RIS than the aqueous reference solution (ANOVA, $P < 0.05$). The gels prepared with CBR showed better performances than HPMC. There were statistically significant differences (ANOVA, $P<0.05$) between the formulations of different CBR concentrations (F1, F2 and F3) regarding the cumulative amount and transdermal flux of RIS. The permeation of RIS decreased as the concentration of CBR increased. Of all gel formulations the preparation with 0.5% CBR (F1) transported the highest amount of RIS. Therefore, F1 was used for the further investigations in which the effects of permeation enhancers were evaluated.

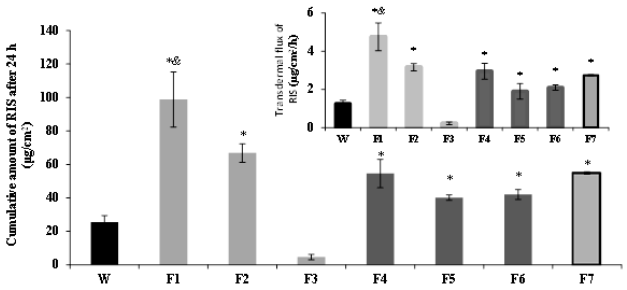

Fig. 1: Effects of different concentrations of gel polymers on the permeation profiles of RIS from gel formulation 1, 2, 3, 4, 5, 6 and 7 through porcine skin. W: aqueous reference solution (mean ± SD, n=3); statistical difference compared with aqueous reference solution, *P<0.05, with F7, $^{\&}P$<0.05.

Effect of penetration enhancers on the permeation of RIS

The obtained permeation profiles are displayed in fig. 2. table 2 shows the values of Q_{24h}, J_{ss} and ER. The addition of AZO and MEN (fig. 2B and D) at any concentrations, significantly improved the transdermal flux and cumulative amount of RIS through porcine skin compared with the formulation without enhancer (NE) (ANOVA, P <0.05). Although all concentrations of LA enhanced the permeation of RIS to some extent, they were not significantly different from NE (fig. 2C), while OA was found to reduce the amount of permeated drug compared with NE (fig. 2A). To make use of possible synergistic

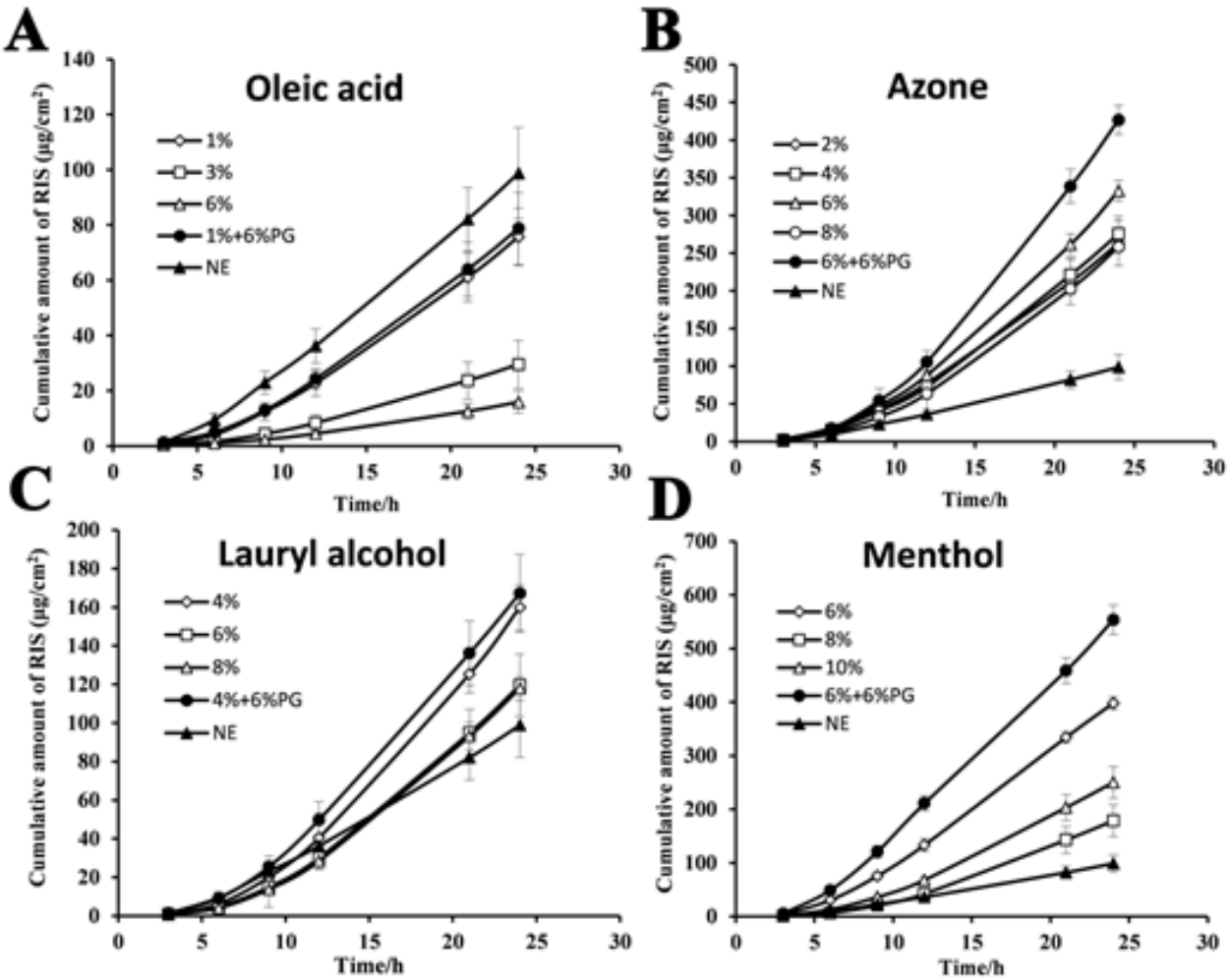

Fig. 2: *In vitro* permeation profiles of the RIS gels prepared by 0.5% Carbomer 934 containing various penetration enhancers through porcine skin. NE: no enhancer, RIS: risperidone (mean ± SD, $n = 3$).

enhancement of drug permeation, formulations with binary enhancer combinations containing propylene glycol (PG, 6%, w/w) and one of the enhancers at the concentration that had been found to be most efficient for RIS permeation (1% OA, 6% AZO, 4% LA, and 6% MEN) were tested. Of the four formulations, PG in combination with AZO or MEN increased RIS permeation significantly compared with AZO or MEN alone (Student *t*-test, $P<0.05$). The binary enhancer combination of 6% MEN and 6% PG displayed the highest enhancing efficiency for RIS permeation and was used for the subsequent *in vivo* study.

In vivo pharmacokinetic study

The plasma-drug concentration vs. time profiles of RIS after transdermal application and intragastric administration are shown in fig. 3. The pharmacokinetic parameters calculated are listed in table 3. There was significant difference (Student *t*-test, $P<0.05$) in C_{max} between the optimized RIS gel and intragastric administration. Although no significant differences were observed, the AUC_{0-48} and T_{max} of the transdermal group were higher than the intragastric group. The transdermal administration of RIS had a relative bioavailability of 131.53% compared with intragastric administration.

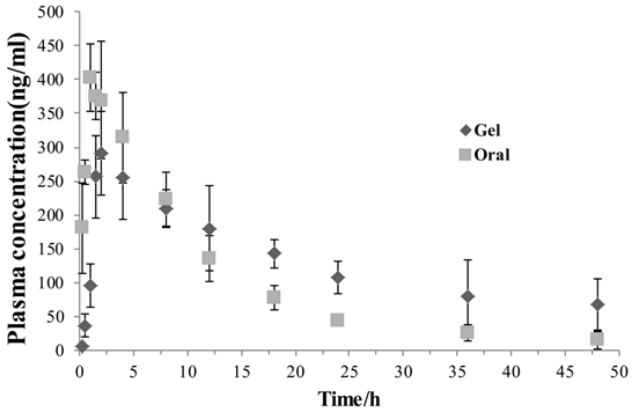

Fig. 3: Plasma drug concentration vs. time profiles after administration of RIS gel or oral solution on rabbits. Each time point represents mean ± SD ($n = 3$).

Table 1: Compositions of base-gel formulations (%, w/w)

Materials	F1	F2	F3	F4	F5	F6	F7
Risperidone	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Carbomer 934	0.5	1.0	2.0	-	-	-	0.5
HPMC	-	-	-	1.0	3.0	5.0	-
Triethanolamine	0.7	1.4	2.7	-	-	-	0.7
Ethanol	30.0	30.0	30.0	30.0	30.0	30.0	-
Distilled water q.s. to make	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table 2: Transdermal parameters of RIS gel containing various penetration enhancers

	Q_{24h} (μg/cm^2)	J_{ss} (μg/cm^2/h)	ER
NE	98.78 ± 16.52	4.753 ± 0.735	1.00
1% OA	75.73 ± 10.42	3.732 ± 0.067	0.79
3% OA	29.54 ± 8.66	1.450 ± 0.402	0.31
6% OA	15.92 ± 3.99*	0.769 ± 0.196*	0.16
1% OA + 6% PG	78.80 ± 13.06	3.854 ± 0.314	0.81
2% AZO	263.31 ± 30.25*	12.88 ± 1.43*	2.71
4% AZO	275.61 ± 23.67*	13.60 ± 1.39*	2.86
6% AZO	332.65 ± 13.94*	16.28 ± 0.60*	3.42
8% AZO	258.48 ± 2.81*	12.72 ± 0.10*	2.68
6% AZO + 6% PG	426.64 ± 19.53*	24.83 ± 5.10*	5.22
4% LA	159.91 ± 11.86	7.917 ± 0.778	1.67
6% LA	119.51 ± 16.09	5.932 ± 1.179	1.25
8% LA	117.87 ± 6.19	5.871 ± 0.341	1.24
4% LA + 6% PG	167.12 ± 20.22	8.275 ± 0.94*	1.74
6% MEN	398.04 ± 11.93*	19.61 ± 0.50*	4.13
8% MEN	178.58 ± 29.68*	8.944 ± 1.437*	1.88
10% MEN	250.24 ± 29.33*	12.43 ± 1.55*	2.61
6% MEN + 6% PG	553.61 ± 27.50*	26.82 ± 4.38*	5.64

Q_{24h}: cumulative amount of permeation by 24 h, J_{ss}: steady state flux, ER: enhancement ratio, NE: no enhancer, OA: oleic acid, AZO: azone, MEN: menthol, LA; lauryl alcohol, PG: propylene glycol. Values expressed as mean ± SD (n = 3). *P < 0.05, for comparison to NE.

Table 3: Pharmacokinetic parameters of the *in vivo* study

Pharmacokinetic parameters	Oral	Transdermal
*C*max/ng·mL^{-1}	431.52 ± 45.94	315.77 ± 52.72
*T*max/h	1.33 ± 0.58	1.83 ± 0.29
AUC_{0-48}/ng·h·mL^{-1}	4743.02 ± 190.31	6275.05 ±1328.33
F_{Rel}		131.53%

*C*max: peak concentration, *T*max: peak time, AUC: bioavailability, F_{Rel}: relative bioavailability of transdermal administration to oral. Values expressed as mean ± SD (n = 3).

DISCUSSION

It is well known that the complex formation between drugs and some excipients leads to the stabilization of active ingredients through interactions such as van der Waals forces, Coulomb forces, hydrogen bonding, dipole–dipole interactions and hydrophobic interactions. Literatures reported that the interaction of CBR with azithromycin (Esteban *et al*., 2009) and lidocaine (Jimenez-Kairuz *et al*., 2002) makes them more stable in aqueous solutions. Vilches *et al*. reported that concentrations of norfloxacin and ciprofloxacin in 0.25% CBR had 7.2 and 34 times aqueous solubility, respectively (Vilches *et al*., 2002). Zoppi *et al*. confirmed an electrostatic interaction between the amine group of pilocarpine and the carboxylic acid group of CBR in solution and in solid (Zoppi *et al*., 2012). The molecular structure of RIS possesses an amine group similar to pilocarpine, and it is possible the interaction between RIS and CBR resulted in increased aqueous compatibility of the drug. It could explain why the permeation of RIS in the 0.5% CBR formulation without ethanol (F7) was significantly higher than in the reference aqueous solution (fig. 1).

There was a significant difference between F1 and F7 in both the cumulative amount and transdermal flux of RIS, indicating a significant effect of ethanol on the permeation of RIS. One reason for this is that RIS is poorly soluble in water but slightly soluble in alcohol (Shakeel *et al*., 2014; Chen *et al*., 2009), and the addition of ethanol improved the solubility of RIS in the formulations and allowed partitioning of the lipophilic RIS into the skin by solubilizing it in the aqueous regions in the intercellular structures of the horny layer (Williams and Barry, 2012). In preliminary experiment, we had investigated the viscosity of CBR in the concentrations of 0.5%, 1% and 2%, and the results displayed that the viscosity rose linearly with CBR concentration (data not shown). In this study, an increase in CBR concentration from 0.5% to 2% decreased the permeation of RIS, probably because the increased CBR viscosity hindered drug release from the polymer matrix into the skin. To maintain the characteristic of the gel, we gave up to further decrease the concentration of CBR and selected 0.5% CBR as the base gel in present work.

It is difficult to identify a particular penetration enhancer for a given permeant. Penetration enhancer potencies appear to be drug-specific and influenced by the choice of vehicle (Williams and Barry, 2012; Williams and Barry, 2004). To select a appropriate penetration enhancer for RIS, some formulations contained predetermined enhancers were tested. As Table 2 showed, of the four enhancers examined, MEN and AZO increased the delivery of RIS while LA did not enhanced RIS permeation, and OA even had a negative effect on the permeation of drug. AZO and MEN are widely used penetration enhancers for various drugs. AZO can enhance both hydrophilic and lipophilic permeants, and act mainly through interactions directly with the lipid domains of the horny layer and thus produce a more fluid environment (Kanikkannan *et al*., 2000; Vavrova *et al*., 2005). The mechanism of permeation enhancement of MEN arises from its preferential distribution into the intercellular spaces of the stratum corneum and the possible reversible disruption of the intercellular lipid domain, allowing an increase in drug diffusion (Kunta *et al*., 1997). Usually, most penetration enhancers have a complex concentration dependent effect (Williams and Barry, 2012; Williams and Barry, 2004). The permeation of RIS increased with the concentration of AZO up to 6% then decreased, 6% AZO was significantly different from 2% and 8% AZO. However, in the present study, the enhancing effect of MEN showed a negative relationship with its concentration, the lowest concentration of MEN (6%) displaying a significantly greater ability than the other concentrations to enhance the transdermal diffusion of RIS. However, the results are inconsistent with observations made by other researchers using other materials (Krishnaiah *et al*., 2008; Patel *et al*., 2009), it could ascrib to the differences of the formulations. OA, a monounsaturated fatty acid, is also a popular penetration enhancer. Golden *et al*. reported increased lipid fluidity in porcine skin following treatment of the stratum corneum with the *cis* configuration OA. Under similar conditions, *cis* OA enhanced salicylic acid flux through porcine skin (Golden *et al*., 1987). Another report described that olive oil increased the permeation of RIS from a transdermal patch through excised rat skin, because of the presence of OA as the major constituent of olive oil (Aggarwal *et al*., 2013). In the current study, OA did not show enhancement of RIS permeation at any concentration, even when OA was used in combination with PG. This was also inconsistent with some unpublished data from our laboratory in which the permeation of RIS from a film forming spray system and patch was increased by the presence of OA, and OA was most effective at low concentrations (typically 1−3%), with up to twice the transdermal flux compared with the control group without OA. This suggests that the selection of vehicle definitely influences the efficiency of penetration enhancers.

In vivo pharmacokinetic studies were performed to investigate the efficacy of transdermal formulation in rabbits. Obtained results showed that the C_{max} of transdermal group was smaller than intragastric group, but the value of T_{max} and $AUC_{0-48\ h}$ was the opposite. One reason for this probably is because of the barrier and reservoir properties of skin. As shown in Fig. 3, transdermal administration of RIS gel not only allowed the drug to quickly reach detectable levels in plasma within 2 h, but also provided a lasting smooth plasma concentration within 24 h compared with intragastric administration. Meanwhile, the lower C_{max} of transdermal RIS could reduce its side effects to some extent. All these data demonstrated that transdermal delivery of RIS could be a promising alternative administration route for patients suffering from psychiatric ailments and can improve patient compliance.

CONCLUSION

This study demonstrated gels with chemical enhancers can effectively delivered RIS through skin and is a promising percutaneous drug delivery strategy. All the results warrant further development of this alternative RIS delivery system for the treatment of patients with schizophrenia.

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