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Synergistic effect of 1,4-cyclohexanediol and 1,2-hexanediol on percutaneous absorption and penetration of metronidazole

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ABSTRACT

The objective of this study was to investigate the percutaneous absorption of metronidazole (MTZ) in the topical formulations containing a combination of 1,4-cyclohexanediol and 1,2-hexanediol. Six formulations were studied in an *in vitro* hairless mouse skin model using Franz Diffusion Cell. MTZ was applied at infinite doses (50 mg and 100 mg of the formulations, which correspond to 375 and 750 μg of MTZ, respectively). Based on the flux values and retardation ratio (RR), a synergistic retardation effect on percutaneous absorption of MTZ was observed for the formulations containing a combination of 1,4-cyclohexanediol and 1,2-hexanediol (RRs are 0.40 for 375 μg dose and 0.69 for 750 μg dose, respectively). Interestingly, retention of MTZ in epidermis and dermis layer showed no significant differences ($p > 0.05$) between the formulations containing the retardant combination and control formulations. In other words, the retardant combination in the formulation decreases MTZ fluxes while maintaining similar level of retention in epidermis and dermis layer when compared to the control formulations. These observations provide insight in formulating superior topical formulations with minimized potential systematic toxicity while maintaining therapeutic efficiency. A mechanistic explanation of the observed synergistic effect is proposed.

1. Introduction

Traditionally, topical formulations are designed to achieve penetration of active ingredient across the outermost layer of skin, the stratum corneum (SC), at therapeutically effective concentrations. The barrier property of the SC layer poses a formidable challenge to formulators of drug delivery systems (Cross and Roberts, 2000; Asbill and Michniak, 2000). Once the active ingredient penetrates across the SC layer, it could permeate through epidermis and dermis layer into systematic circulation, which might lead to unwanted or toxic side effects. For treatment of dermatological conditions, an ideal topical formulation is to impart maximal local retention and minimal systematic penetration. Furthermore, for agrochemicals (Baker et al., 1978), insect repellants (Briassoulis et al., 2001), sunscreens (Schlumpf et al., 2001), and household cleaning chemicals (Asbill et al., 2000; Mancini, 2004), minimizing potential toxicity is as important as providing protection benefits. As a result, there is a significant need in discovering safe and effective skin penetration retardants.

Recently, a number of studies have been carried out to prevent the passage of active ingredients or excipients into deeper skin layer by using chemical penetration retardants (Asbill and Michniak, 2000). The retardants could decrease the diffusivity and thermodynamic activity of the active ingredient in skin. Moreover, they will reduce amount of an active ingredient being released into the systematic circulation. Ideally, penetration retardants should be chemically and pharmacologically inert, nontoxic, non-irritant, and non-allergenic. They should have a rapid and reversible onset of action, be potent in low concentrations, compatible with the formulation ingredients and cosmetically acceptable (Chattaraj and Walker, 1995).

There are a limited number of publications in the area of penetration retardants. The retardants reported in the literature are usually structural analogues of potent enhancers. For example, Hadgraft et al. (1996) have reported that compounds having structure similar to Azone act as penetration retardants. It has been reported that a family of iminosulfurane compounds such as for example, *S*,*S*-dimethyl-*N*-(benzenesulfonyl) iminosulfurane, *S*,*S*-dimethyl-*N*-(2-methoxycarbonylbenzenesulfonyl) iminosulfurane, *S*,*S*-dimethyl-*N*-(4-chlorobenzenesulfonyl) iminosulfurane has exhibited penetration retardation properties (Kim et al., 1999). In yet another example, oxazolidinones have been shown to be able to enhance retention of the applied active ingredients in the skin layer, resulting in low systematic permeation (Rajadhyaksha and Pfister, 1996; Seth, 1999). More recently, Li

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et al. (2010) reported that 1,4-cyclohexanediol in combination with 1,2-hexanediol showed a penetration retardation effect in percutaneous absorption of azelaic acid.

The present study was designed to investigate a potential synergistic retardation effect of 1,4-cyclohexanediol and 1,2-hexanediol on percutaneous absorption and penetration of an active agent. Furthermore, the study was also carried out to shine a light on mechanistic aspect of the retardation effect. metronidazole (MTZ) was selected as a model drug. MTZ is a member of nitroimidazole family of compounds. It is an active ingredient in a number of prescription pharmaceuticals for treatment of a number of conditions, such as, for example, rosacea. Percutaneous absorption and penetration of MTZ was studied in an *in vitro* skin model using Franz Diffusion Cell.

2. Materials and methods

2.1. Materials

MTZ was purchased from ALFA AESAR (ZhongAn pharmaceutical, Tianjin, China). 1,4-Cyclohexanediol was obtained from Sigma–Aldrich (St. Louis, MO, USA). 1,2-Hexanediol was purchased from Sabina Corporation (Piscataway, NJ, USA). Klucel® MF was obtained from Hercules, Inc. (Wilmington, DE, USA). All other chemicals are of analytical grade.

2.2. Skin membranes

Male hairless mice (30–40 days old) were purchased from Radiation Medicine Institute for Laboratory Animal Research, Chinese Academy of Medical Sciences (Tianjin, China). Animals were euthanized humanely. The abdominal skin was removed from hairless mice, and subcutaneous fat was carefully cleaned. All animal protocols were performed under the guidelines for humane and responsible use of animals in research set by Tianjin University School of Pharmaceutical Science and Technology. The skin samples were stored at −20 °C and were used promptly. For the formulation comparison studies, we have tried to make experimental conditions (including tissue conditions) as comparable as possible for all parallel experiments and taken all precautions to make a fair comparison. Before each experiment, the skin samples were thawed to room temperature and equilibrated at 37 °C for 1 h in phosphate buffered saline (PBS, pH 7.4) in Franz Diffusion Cell.

3. Methods

3.1. Preparation of formulations

Six formulations were prepared (see Table 1 for details). Klucel® MF was used as the gelling agent. The general procedure is as follows. For example, to prepare formulation F4, 1% of 1,4-cyclohexanediol was dissolved in a solution of 1,2-hexanediol (4%) in water, MTZ (0.75%) was dispersed in the above solution with a stirrer until MTZ was dissolved. Then Klucel® MF (0.75%) was added to the solution while stirring until the solution was gelled. MTZ was completely solubilized in all formulations. The formulations have similar viscosity, which is about 50 cp.

3.2. In vitro skin permeation studies

The skin samples were mounted on Franz Diffusion Cell (Pharmacopoeia Standard Instrument Factory, Tianjin, China) with SC side facing the donor chamber (diffusion area = 1.77 cm^2). The receptor chamber (volume = 17 ml) was filled with PBS (pH 7.4), which is continuously stirred at 500 r.p.m. using a magnetic stirrer. The speed (500 r.p.m.) was optimized to maintain efficient mixing without creating air bubbles and vortex effect. The temperature was maintained at 37 ± 0.1 °C. Infinite doses were applied to the skin samples (50 mg and 100 mg of the formulations, which correspond to 375 and 750 μg of MTZ, respectively). The donor chamber was sealed with Parafilm® to minimize evaporation of the formulations. Each set of experiments was run in six replicates. At the end of each time interval (1, 2, 4, 8, 12, 16, 20, and 24 h), the skin surface was wiped with cotton ball soaked with PBS (pH 7.4). The tape-stripping method (average 10 strips) was used to remove the SC layer (Howes et al., 1996). MTZ retained in the epidermis and dermis layer was collected by methanol extraction. After tape-stripping, the remaining skin was minced, vortexed with 1 ml methanol and centrifuged, the supernatant was removed. The extraction step was repeated three times. The supernatants were combined, filtered and ready for analysis.

3.3. HPLC analytical method

Analysis of MTZ was performed using HPLC (HP 1100, Agilent Technologies, Inc.) equipped with a 250 mm × 4.6 mm stainless steel C_{18} column (5 μm, Thermo, USA). The mobile phase is a degassed and filtered (0.45 μm; Millipore) mixture of double distilled water–methanol (80:20, v/v). Injection volumes were 20 μl and flow-rate was set at 1.0 ml/min. The UV detector wavelength was set at 310 nm for detection of MTZ.

The analytical method was validated for linearity, precision and accuracy. The correlation coefficient of 0.9997 for linearity of plot was observed. Intraday variability was less than 0.2% and interday variability was also calculated to be less than 3.0%.

3.4. Data and statistical analysis

For *in vitro* percutaneous absorption studies, three parameters (mean flux, lag time, and cumulative amount after 24 h) were calculated. The flux values of MTZ permeated through the skin membranes into the receptor fluid were determined from slopes of plots of concentration in the receptor phase as a function of time and expressed as μg/cm^2/h using linear regression (Microsoft Excel) (Batheja et al., 2009). The degree of penetration retardation is defined as the retardant ratio, RR, which is calculated from the following equation (Goodman and Barry, 1988):

$$\mathrm{RR} = \frac{\text{Flux for the formulation containing retardants}}{\text{Flux for control formulation}}$$

Paired two-tailed Student's *t*-test is performed to calculate the statistical significance. Values are given as mean ± SD.

4. Results and discussion

4.1. Flux values of MTZ

Hairless mouse skin tends to be thinner and has few layers in the SC than human skin. Therefore, it is more permeable than human skin (Catz and Friend, 1990; Fang et al., 2003). However, it is quite suitable to use the mouse skin for studying the permeation retardation effect, precisely due to its lower permeation barrier. One of reasons we chose mouse skin model is that if we were able to observe the retardation effect in more permeable mouse skin, it would have been more likely that a similar effect would have been observed in the less permeable human skin. It is expected that the retardation effect would be more profound in human skin.

The permeation parameters for F1–F2 in the presence and absence of 1,4-cyclohexanediol are summarized in Table 2. It is found that the flux values (1.67 μg/cm^2/h for F1 and 1.69 μg/cm^2/h for F2 at lower dose, 3.62 μg/cm^2/h for F1 and 3.47 μg/cm^2/h for