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Transdermal Delivery System of Triamcinolone Acetonide from a Gel Using Phonophoresis

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Triamcinolone acetonide (TA) is a corticosteroid that is used in the systemic and topical treatment of many inflammatory diseases. In this study, a phonophoretic drug delivery system was designed to enhance the TA permeability and the influence of ultrasound was examined. In order to establish the transdermal delivery system for TA, a hydrophilic carbopol gel containing TA was prepared after adopting phonophoresis. A permeation study through mouse skin was performed at 37°C using a Franz diffusion cell, and the ultrasound treatment was carried out for 10 h. The level of TA permeation through the skin was evaluated under various ultrasound conditions including the frequency (1.0, 3.0 MHz), intensity (1.0, 2.5 W/cm^2), and duty cycle (continuous, pulse mode) using a 0.5% TA gel. The highest permeation was observed under the ultrasound treatment conditions of low frequency, high intensity, and in continuous mode.

Key words: Triamcinolone acetonide, Phonophoresis, Transdermal delivery, Ultrasound treatment, Permeation, Carbopol gel

INTRODUCTION

Transdermal delivery has the advantage of avoiding the gastrointestinal disturbances and hepatic first-pass effects that are associated with the oral route. It also shows controlled and sustained delivery, patient compliance (Cullander and Guy, 1991) and is easy to apply and remove. Even though the transdermal administration of a drug has many advantages, it is limited by the low permeability through the skin as a result of the barrier function of the stratum corneum. In order to improve the permeability of drugs through the skin, several penetration enhancers (Southwell and Barry, 1983; Hadgraft *et. al.*, 1973; Franz *et. al.*, 1992; Shin and Lee, 2002), prodrugs (Waranis and Sloan, 1987), superfluous vehicles (Kondo and Sugimoto, 1987), iontophoresis (Riviere and Heit, 1997), phonophoresis (Brucks *et al.*, 1989; Tyle and Agrawala, 1989) and electroporation (Pliquett and Weaver, 1995; Denet *et al.*, 2003) have been used to increase the level of skin permeation.

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One of the most convenient methods for improving the level of skin permeation is the use of penetration enhancers, which have shown acceptable results. Iontophoresis, which involved applying a direct current, has been widely used as a skin penetration method using physical agents (Riviere and Heit, 1997). However, there is a risk of burning the skin due to changes in pH. Moreover, the drugs can only be applied as an aqueous solution (Ahn, 1991).

Phonophoresis by ultrasound has several advantages in that there is a low risk of burning the skin, and it is unnecessary to ionize the drug. In addition, its penetration is approximately 5 cm and there is a short treatment time (Tyle and Agrawala, 1989; Mitragotri *et al.*, 1996; Yong *et al.*, 2000). Phonophoresis facilitates the transdermal absorption of a drug by thermal effects such as an increase in tissue temperature and by mechanical effects such as cavitation and acoustic streaming (Meidan *et al.*, 1995; Amit and Jaideep, 2002). The drugs are absorbed through the transcellular, intercellular and appendageal pathways (Mitragotri *et al.*, 1996). Phonophoresis is usually divided into low frequency (< 100 kHz), medium frequency (between 0.7 MHz and 3.0 MHz) and high frequency (between 3 MHz and 10 MHz) (Lavon and Kost, 2004). Several studies have

shown that low frequency ultrasound has higher transdermal penetration efficiency than the conventional treatment frequency ultrasound (Ueda *et al.,* 1996; Mitragotri, 1996). Recently, many studies have been carried out at low frequencies ranging from 20 kHz to 200 kHz using several drugs of which transdermal penetration is difficult (Tachibana, 1992; Mitragotri *et al.,* 1995; Le *et al.,* 2000).

The ultrasound frequency that is normally used by clinical phonophoresis instruments is either 1 MHz or 3 MHz. These frequencies have been used as a physical therapy for the inflammatory symptoms of the musculoskeletal system (Byl, 1995; McElnay *et al.,* 1985). Since studies on the phonophoresis treatment frequency has focused mainly on clinical experiments of anti-inflammatory drugs, an in vitro experiment was performed to develop a transdermal drug delivery system using phonophoresis by establishing the various ultrasound conditions such as the frequency, the intensity, and duty cycle.

Triamcinolone acetonide (TA), one of the topical corticosteroids, is used to relieve the inflammatory symptoms of corticosteroid-responsive dermatitis. The drug is also used in paste form as an adjunctive treatment to provide the temporary relief of symptoms associated with oral inflammatory or ulcerative lesions resulting from trauma. Topical preparations of TA are generally rubbed gently onto the affected area 2-4 times daily. Either a 0.5% cream or a 0.5% ointment has been used to treat dermatitis.

A transdermal drug delivery system should have good skin permeation characteristics *in vitro* before performing *in vivo* studies on human volunteers. It is well known that the transdermal permeation of a drug can be affected by many factors.

The aim of this study was to establish a phonophoresis method for the transdermal application technique of TA from a gel that can be used to further evaluate its anti-inflammatory effects. In order to establish a transdermal delivery system of TA using phonophoresis, a hydrophilic carbopol gel containing TA was prepared. The permeation study through the mouse skin was performed at 37°C using a Franz diffusion cell, and an ultrasound treatment was carried out for 10 h. The extent of TA permeation through the skin was evaluated under various ultrasound conditions such as the frequency (1.0, 3.0 MHz), intensity (1.0, 2.5 W/cm^2), and duty cycle (continuous, pulse) using a 0.5% TA gel.

MATERIALS AND METHODS

Materials

The triamcinolone acetonide (TA) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The 0.5% TA ointment (Ariscort cream, Daihwa Pharm. Co., Korea) was used as a commercial product. Transcutol and all the other chemicals were of reagent grade and used without further purification. A HPLC system (LC-10AT, Shimadzu, Japan), ultrasound unit (Sonopulse 590, Enraf-Nonius, Netherlands), and UV-spectrophotometer (Hitachi 7060 Auto analyzer, Japan) were used.

Solubility of triamcinolone acetonide

The solubility of TA was measured in various media. Excess amount of TA was added to 3.0 mL of each medium and the mixture was then stirred for 48 h at room temperature. Triplicate samples were centrifuged at 3,000 rpm for 10 min in order to remove any undissolved drug. An aliquot of the supernatant was taken and the drug content was assayed by HPLC after diluting the samples with with methanol.

Determination of triamcinolone acetonide by HPLC

The amount of TA was determined using HPLC that consisted of a solvent delivery system, a C_{18} reverse column (WAT 027324, Waters Co., Milford, Massachusetts, U.S.A.), a multi-wavelength detector and an integrator. The column used was a μ-Bondapak C_{18} (3.9×300 mm), and the column temperature was maintained at ambient temperature. The injection volume was 10 μL and the UV detector was operated at a wavelength of 238 nm. A mixture of acetonitrile: water: formic acid (40.0: 59.9: 0.1) was used as the mobile phase and the flow rate was 1.2 mL/min. The mobile phase was filtered through a 0.45 μm pore size membrane filter. Under these conditions, the TA peak appeared at a retention time of 8.6 min (Fig. 1). The detection limit for TA was 0.1 μg/mL. A good correlation between the response and concentration was obtained without the need for an internal standard.

Preparation of the triamcinolone acetonide gel

The hydrophilic carbopol 940 (0.08 g) was dissolved in 5 mL water, and the pH of the gel was neutralized using 0.2 mL of a 10% NaOH solution. TA (0.05 g) dissolved in 3 mL of Transcutol was added to the above polymer solution with vigorous stirring, and water was added to make a final volume of 10 mL (gel A). The TA content in 10 mL of the gel was 0.01 grams in the case of gel B.

In vitro skin permeation study

Skin preparation

Male hairless mice (25 ± 2.5 g) were purchased from the Daehan experimental animal center (Daejeon, Korea). The mice were sacrificed by snapping the spinal cord at the neck. A square section of abdominal skin was excised, and the excised skin was used immediately.

Drug permeation from the triamcinolone gel

The drug flux from the TA gel through the hairless

mouse skin was determined using a Franz diffusion cell, which has an effective exposed surface area in contact with the receptor phase of 1.77 cm^2 (Shin and Kim, 2000; Shin *et al.*, 2000). One gram of the prepared TA gel was placed in intimate contact with the skin and the sampling port was sealed with parafilm in order to prevent any evaporation of the receptor medium. After mounting the TA gel (0.1 g) on the donor compartment of the Franz diffusion cell, an ultrasound transducer with a beam non-uniformity ratio (BNR) of 6.0 max was applied for 10 h. In the case of the pulsed ultrasound treatment, the pulse repetition frequency was 100 Hz and there was a 20% duty cycle. The ultrasound treatment was divided into the following five groups, Application B (3 MHz, continuous, 2.5 $W/cm^{2)}$, Application C (1 MHz, pulse, 2.5 W/cm^2), Application D (1 MHz, continuous, 1.0 W/cm^2), and Application E (1 MHz, continuous, 2.5 W/cm^2) according to the frequency, duty cycle and intensity. Application A (TA gel with no-ultrasound treatment) was used as the control.

A 20% Transcutol aqueous solution, as a solvent, was added to the phosphate buffer at 1:1 ratio (v/v) for the receptor medium in order to maintain the sink condition during the permeation study. The receptor solution was maintained at 37°C using a circulating water bath and stirred with a magnetic stirring bar. The donor compartment was maintained at 25 ± 1°C. The samples (0.1 mL) were withdrawn from the receptor compartment at predetermined times and replaced with an equal volume of fresh medium. The samples were then diluted with the same amount of methanol and filtered through a 0.45 μm membrane filter and analyzed by HPLC. Each value represents the mean and standard error of five determinations. The rate of skin permeation was calculated from the slope of each figure (Shin *et al.*, 2002).

The accumulated amount of TA that permeated per unit area of skin was measured as a function of time. The lag-time method was used to examine the dynamic state of the drugs through the skin.

$$J_s = \left[\frac{1}{A}\frac{dQ}{dt}\right]_{ss} = \frac{DKC}{h}$$

$$D = \frac{h^2}{6T_L}$$

where, J_s = flux at the steady state
A = membrane area
$(dQ/dt)_{ss}$ = the amount of drugs passing the membrane per unit time at the steady state
C = concentration of the drugs in the donor compartment
K = distribution coefficient
h = thickness of membrane
D = diffusion coefficient
T_L = lag-time.

RESULTS AND DISCUSSION

Solubility of triamcinolone acetonide

TA has an extremely low solubility (0.13 ± 0.02 mg/mL) in aqueous media. However, the solubility of TA was found to be 8.21 ± 2.37 mg/mL in triacetin, 67.97 ± 0.51 mg/mL in Transcutol, 24.70 ± 1.15 mg/mL in Labrasol, and 62.21 ± 4.31 mg/mL in dimethyl isosorbide. In order to maintain the sink condition during the permeation study, a 20% Transcutol aqueous solution, which showed the highest solubility, was added to the phosphate buffer at a 1:1 ratio (v/v) for the receptor medium.

Permeation studies through skin

In order to establish a transdermal delivery system for TA using phonophoresis, a hydrophilic carbopol gel containing TA was prepared and the extent of skin permeation was evaluated using a 0.5% TA gel under various conditions such as the ultrasound frequency, intensity, and duty cycle. The effects of ultrasound on the extent of skin permeation were confirmed by the *in vitro* results.

Effects of the triamcinolone acetonide concentration and the temperature on the drug permeation through the skin

The extent of TA permeation through the excised hairless mouse skin from the formulated gel preparation was examined by comparing it with that from various commercial products (0.5% ointment) according to the drug concentration of the different formulations and temperature (Fig. 1). The carbopol gel containing 0.5% TA showed the enhanced drug permeation than that from the gel containing 0.1% TA or that a commercial ointment containing 0.5% TA. The gels containing 0.5% TA showed increased drug permeation than the gel containing 0.1% TA or than that from an ointment containing 0.5% TA. As the concentration of TA in the gels increased, the level of drug permeation increased (Fig. 1).

The effect of temperature on the extent of drug permeation through the excised skin from the 0.5% TA gel was evaluated at 30, 35, and 40°C. The extent of TA permeation increased with increasing temperature (Fig. 2). The permeation of a drug through the skin requires energy from small molecules to transport it through the barrier.

Effects of the ultrasound treatment on the drug permeation through the skin

In order to enhance the level of skin permeation of TA, various ultrasound conditions such as the frequency (1.0, 3.0 MHz), the intensity (1.0, 2.5 W/cm^2), and the duty cycle (continuous, pulse) were investigated using a 0.5% TA gel.

Of the ultrasound treatments of 1 MHz or 3 MHz, the 1

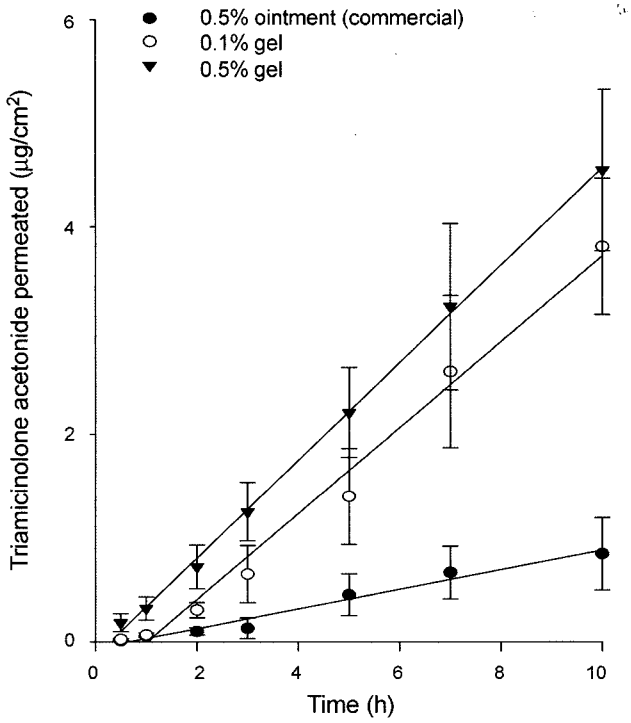

Fig. 1. Effects of the TA content on the level of drug permeation through the excised hairless mouse skin. Each bar represents the mean ± S.E. from 5 experiments.

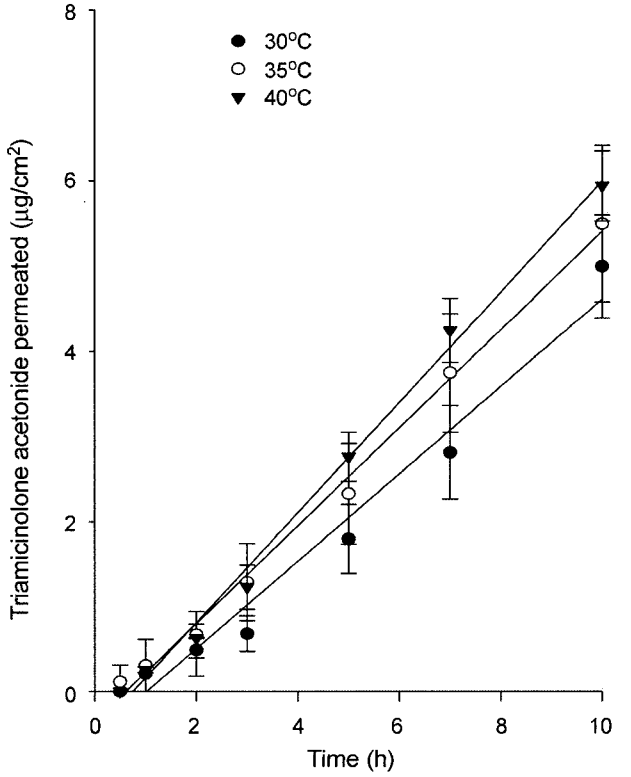

Fig. 2. Effects of temperature on the level of drug permeation through the excised hairless mouse skin from the 0.5% TA gel. Each bar represents the mean ± S.E. from 5 experiments.

MHz frequency showed a relatively higher transport than either 3 MHz or no-ultrasound treatment (Fig. 3). The level of drug permeation through the skin was higher at an ultrasound intensity of 2.5 W/cm^2 than that at 1.0 W/cm^2 (Fig. 4). The effect of the ultrasound duty cycle on the skin permeation was highest with the continuous mode than with the pulsed mode (Fig. 5). With the pulsed mode, it is possible to use a higher intensity without increasing the skin temperature. However, the pulsed mode resulted in lower drug permeability.

There are many explanations for the increased permeability of the membranes. There would be a decrease in the donor solution-membrane interfacial-potential-energy barrier caused by the ultrasound treatment. Such a decrease as well as an increase in cavitation might increase the level of drug permeation (Tezel *et al.*, 1998). A reduction in the boundary layer thickness (close to the membrane), which creates additional resistance to drug transport as a result of mixing the solutions, would also increase the level of drug permeation. The radiation pressure caused by the ultrasound wave would exert pressure on the drug molecules as well as on the skin (Singh and Singh, 1990). It is known that phonophoresis facilitates the transdermal absorption of a drug as a result of the thermal effects such as an increase in tissue temperature and the mechanical effects such as cavitation and acoustic streaming (Meidan

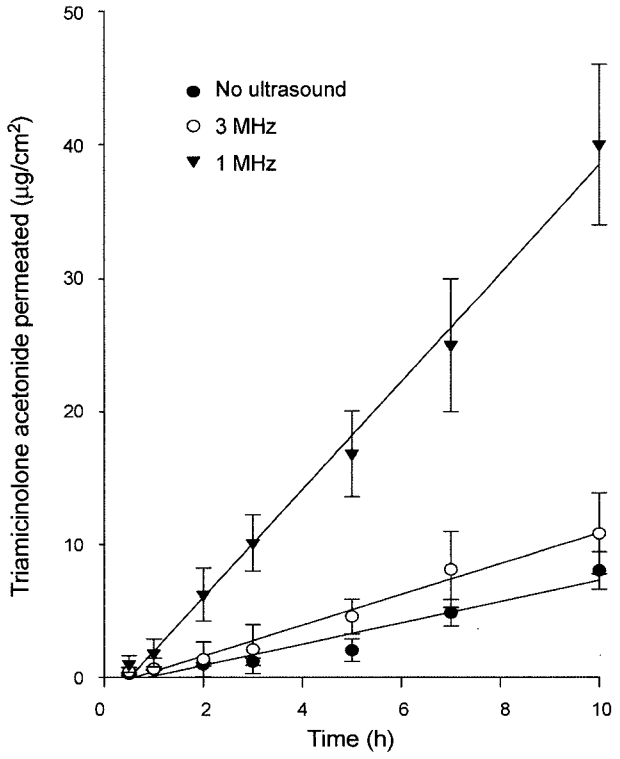

Fig. 3. Effects of ultrasound frequency on the level of drug permeation through the excised hairless mouse skin from the 0.5% TA gel. Each bar represents the mean ± S.E. from 5 experiments.

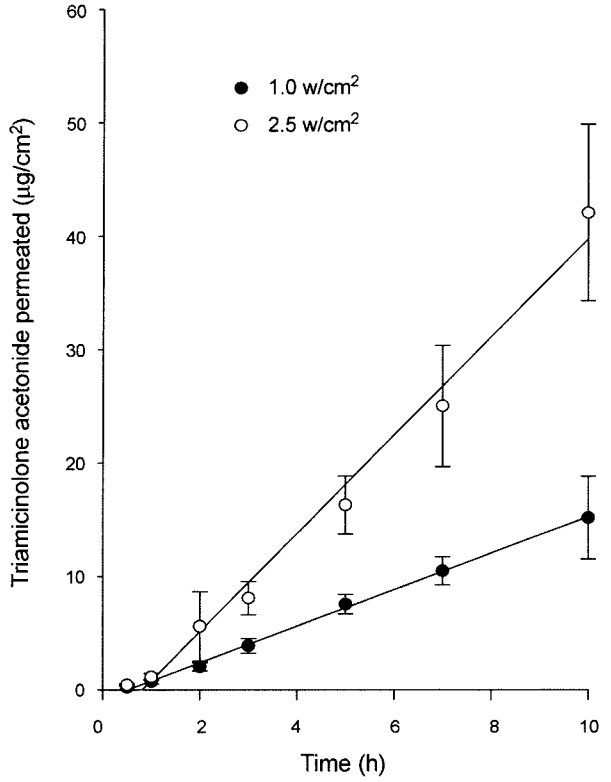

Fig. 4. Effects of ultrasound intensity on the level of drug permeation through the excised hairless mouse skin from the 0.5% TA gel. Each bar represents the mean ± S.E. from 5 experiments.

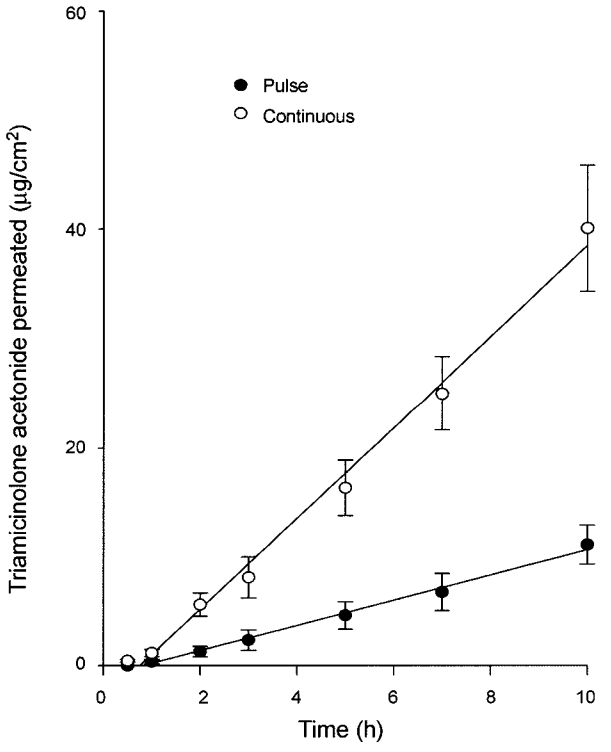

Fig. 5. Effects of ultrasound duty cycle on the level of drug permeation through the excised hairless mouse skin from the 0.5% TA gel. Each bar represents the mean ± S.E. from 5 experiments.

Table I. Permeation parameters of the TA gel through excised hairless mouse skin. Each bar represents the mean ± S.E. of 5 experiments.

Applcation	Js (μg/cm²/h)	T_L (h)
A	0.47±0.20	0.42±0.27
B	1.17±0.19	0.39±0.14
C	0.98±0.22	1.32±0.64
D	1.46±0.27	0.52±0.33
E	3.60±0.81	1.29±0.48

Application A: No-ultrasound
Application B: 3 MHz, continuous, 2.5 W/cm²
Application C: 1 MHz, pulse, 2.5 W/cm²
Application D: 1 MHz, continuous, 1.0 W/cm²
Application E: 1 MHz, continuous, 2.5 W/cm²
Js: Permeation rate (μg/cm²/h)
T_L: Lag-time (h)

et al., 1995; Amit and Jaideep, 2002).

The effect of ultrasound might be related to the depth of the vibration as well as the duty cycle. The increased mechanical vibration by the ultrasound increases the skin temperature particularly in continuous ultrasound mode. The increase in temperature caused by heat liberation using ultrasound might increase the level of drug permeability (Walmsley and Squier, 1991).

From the above experiments, the highest TA permeation was observed with an ultrasound treatment of frequency of 1.0 MHz, an intensity of 2.5 W/cm², and in continuous mode (Table I).

CONCLUSION

In this study, a transdermal delivery system for TA from a hydrophilic carbopol using phonophoresis was established. The level of drug permeation from a 0.5% TA gel through the skin was evaluated under various ultrasound conditions such as the frequency (1.0, 3.0 MHz), intensity (1.0, 2.5 W/cm²), and duty cycle(continuous, pulse mode). The highest permeation was observed at an ultrasound conditions of low frequency, high intensity, and in continuous mode. Overall, it is envisioned that this system could be used for further evaluating of the anti-inflammatory effects of various drugs in humans.

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