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Development and evaluation of a tacrolimus cream formulation using a binary solvent system

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ABSTRACT

We developed an oil/water-type tacrolimus (FK506) cream formulation as an alternative to Protopic ointment for atopic dermatitis treatment. We determined the effects of solvents used in topical preparations on FK506 solubility and stability, and evaluated FK506 transdermal absorption into rat skin from solutions, emulsions, and creams. Screening indicated that diethyl sebacate (DES), isopropyl myristate (IPM), propylene glycol (PG), and oleyl alcohol (OA) were adequate FK506 solvents. When FK506 solutions prepared using these solvents were transdermally administered, AUC_{0-24} values for DES and IPM were higher than or similar to that for 0.1% Protopic ointment. The AUC_{0-24} values for PG and OA were low, so these solvents did not enhance absorption. The residual ratios of FK506 in DES and IPM solutions after incubation at 70 °C for 9 d were 95.6% and 88.6%, respectively, so DES and IPM were chosen for emulsion preparation. When the emulsions were transdermally administered, the IPM emulsion AUC_{0-24} values increased 4.6-fold; DES emulsions did not show high transdermal absorption, but showed sustained characteristics. A cream formulation prepared by mixture of IPM and DES also showed high absorption and transdermal absorption increased with increasing IPM ratio. We developed an FK506 cream formulation with a controllable transdermal absorption rate by manipulating the IPM:DES ratio.

1. Introduction

Atopic dermatitis (AD) is a potentially debilitating, chronic skin disorder; it has a significant impact on the human population worldwide. Conventional therapy for AD involves the use of topical corticosteroids; however, steroid-associated side effects have limited their use (Alaiti et al., 1998; Reitamo et al., 2002; Rothe and Grant-Kels, 1996). Tacrolimus (FK506), a 23-membered macrolide lactone (molecular weight, 803.5) novel immunosuppressant, is therefore of great interest. FK506 suppresses inflammation by targeting T lymphocytes (Nakagawa et al., 1994), resulting in inhibition of the enzyme calcineurin phosphatase, thereby modifying T-cell function (Fruman et al., 1992). This immunosuppressive activity has been reported to be 30–100-times higher than that of cyclosporine in vitro, and 10–20-times higher in vivo (Honbo et al., 1987). An ointment formulation of FK506 is available on the international market, as Protopic®, for the treatment of AD.

Although it is effective, the characteristic greasy and messy nature of the ointment vehicle leads to undesirable stickiness and unpleasant feelings by patients when applied to the skin (Drake et al., 1996; Ference and Last, 2009). The development of a new topical dosage formulation, such as a cream, that overcame these disadvantages would improve the quality of life for patients. In addition, the Food and Drug Administration's medication guide for Protopic® (FDA, 2011) describes possible side effects such as skin irritation (stinging, burning, or itching) and other side effects at the application site, which are mainly associated with the drug molecule. The conventional vehicle does not ensure adequate topical delivery of the drug to the target site.

Furthermore, FK506 exhibits formulation-related issues such as poor solubility (4–12 $\mu g\,mL^{-1}$) as a result of its physicochemical properties such as high molecular weight, macrolide structure, and lipophilicity (log *P*, 6.09; Bos, 2003; Hane et al., 1992; Tamura et al.,

Abbreviations: AD, atopic dermatitis; AUC, area under plasma-concentration–time curve; AUC_{0-24}, area under plasma-concentration–time curve from time 0 to 24 h of measurable concentration; BB-5, POE(5) behenyl ether; BB-10, POE(10) behenyl ether; BC-2, POE(2) cetyl ether; BC-5.5, POE(5.5) cetyl ether; BC-7, POE(7) cetyl ether; BC-10TX, POE(10) cetyl ester; BO-7, POE(7) oleyl ether; BS-4, POE(4) stearyl ether; CC, cetyl caprylate; C_{max}, maximum plasma concentration; DES, diethyl sebacate; DIA, diisopropyl adipate; DO, decyl oleate; FK506, tacrolimus; HCO-10, POE(10) hydrogenated castor oil; HCO-20, POE(20) hydrogenated castor oil; HCO-60, POE(60) hydrogenated castor oil; HPLC, high-performance liquid chromatography; HL, hexyl laurate; HLB, hydrophilic–lipophilic balance; IPC, isopropyl caprylate; IPD, isopropyl decanoate; IPM, isopropyl myristate; IPP, isopropyl palmitate; PG, propylene glycol; POE, polyoxyethylene; OA, oleyl alcohol; OD, 2-octyldodecanol; OO, oleyl oleate; T_{max}, maximum drug concentration time.

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2002). Compounds with molecular weights higher than 500 have difficulty penetrating skin (Bos and Meinardi, 2000). It is therefore a challenge to overcome the low penetration rate of FK506 through the skin, while restoring the skin barrier properties and, at the same time, achieving site-specific delivery to enhance therapeutic efficacy with minimum toxic effects.

The aim of the present study was to develop an FK506 cream formulation to overcome the above-mentioned disadvantages. First, we screened several types of solvents that are widely used in the development of topical preparations, and examined their effects on the solubility and stability of FK506. We then evaluated the transdermal absorption of FK506 into rat skin from solutions, emulsions, and cream formulations.

2. Materials and methods

2.1. Materials

FK506 was manufactured by the Fujisawa Pharmaceuticals Co., Ltd. (currently Astellas Pharma Inc., Tokyo, Japan). Diisopropyl adipate (DIA), isopropyl myristate (IPM), and isopropyl palmitate (IPP) were obtained from Croda Japan KK (Tokyo, Japan). Oleyl alcohol (OA), decyl oleate (DO), and oleyl oleate (OO) were obtained from the NOF Corporation (Tokyo, Japan). 2-Octyldodecanol (OD), hexyl laurate (HL), cetyl caprylate (CC) were obtained from Henkel Japan Ltd. (Tokyo, Japan). Diethyl sebacate (DES) and all surfactants were obtained from the Nikko Chemicals Co., Ltd. (Tokyo, Japan). Propylene glycol (PG), isopropyl caprylate (IPC), and isopropyl decanoate (IPD) were obtained from the Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Carbopol 940 was obtained from the Lubrizol Corporation (Cleveland, OH, USA). All of the reagents used were of the highest grade available from commercial sources.

2.2. Determination of apparent FK506 solubility

An excess of FK506 was added to each solvent (2 mL) at room temperature, and the mixtures were agitated for 1 week. An aliquot of the supernatant was withdrawn and immediately filtered through a membrane filter (0.22 μm, Millipore SLGV 013 SL). The filtered solution (500 μL) was volumetrically diluted with a mixture of tetrahydrofuran (9 mL) and an internal standard solution (1 mL, 0.15 mg mL^{-1} *n*-heptyl *p*-hydroxybenzoate in ethanol solution), and 20 μL of the diluted sample solution were injected into a high-performance liquid chromatography (HPLC) system to determine the FK506 concentration. The HPLC system consisted of a pump (model 510), a WISP automatic sampler (model 712), and an ultraviolet detector (model 481) from Waters (Milford, MA, USA), and a TSKgel ODS-80TM column (150 mm × 4.6 mm, 5 μm) from the Tosoh Co., Ltd. (Tokyo, Japan). The mobile phase consisted of a mixture of water, tetrahydrofuran, and 2-propanol (volume ratio 5:2:2), and the flow rate was 0.6 mL min^{-1}. The wavelength of the detector was set at 220 nm.

2.3. In vivo transdermal absorption tests

All of the procedures used in the in vivo study were conducted in accordance with the guidelines approved by the Institutional Animal Care and Ethical Committee of the Astellas Pharma Inc. The abdominal hair of male Sprague-Dawley rats (7 weeks) was removed using an electrical shaver and a hair removal cream (EBA cream; Mitsubishi Tanabe Pharma Corporation, Osaka, Japan). Next day, the skin condition of the rats was checked, and rats with good skin condition, without any scabs, were selected for in vivo experiments. The selected rats were fixed, using a restraining device, in the face-up position, without anesthesia, and 50 μL of 0.1% (w/w) FK506 solution were applied to the abdominal region (2.5 cm × 4.0 cm). At specified times, 0.3 mL of blood were collected from the subclavian vein and stored at −20 °C until used. The FK506 plasma concentration was analyzed by enzyme immunoassay using mouse anti-FK506 monoclonal antibodies (FKmAb) and FK506-conjugated peroxidase (FK-POD), as described in a previous study (Kobayashi et al., 1991). Briefly, the stored blood was centrifuged, deproteinized with methanol, and centrifuged again. The supernatant plasma was dissolved in FK-POD solution and added to a microtiter plate well, which was previously coated with FKmAb, to determine competitive binding of FK-506 and FK-POD with FKmAb. The POD activity was measured using *o*-phenylenediamine and hydrogen peroxide as cosubstrates. The reaction was stopped by addition of sulfuric acid (2 mol L^{-1} in water), and the optical density (490 nm) was measured using a microplate reader (CS-9300PC; Shimadzu, Kyoto, Japan). The FK506 content was determined by comparison with a standard curve. An FK506 calibration curve was prepared in each assay in the concentration range 0–40 ng mL^{-1}. The correlation coefficient was always more than 0.99, and the limit of quantification was 0.1 ng mL^{-1}.

Pharmacokinetic parameters were calculated by model-independent analysis as described previously (Iwasaki et al., 1995). Maximum plasma concentration (C_{max}), maximum drug concentration time (T_{max}) and area under plasma-concentration–time curve from time 0 to 24 h of measurable concentration (AUC_{0-24}) were estimated.

2.4. Stability study of FK506 solutions

FK506 solutions (0.1%, w/w) in ampoules, under nitrogen, were incubated at 70 °C for 9 d or at 50 °C for three months. The residual amounts of FK506 in the solutions were determined by HPLC.

2.5. Emulsifying index tests

Each solvent (1.46 mL) and 0.25 g of surfactant (0.25 g) were placed in a 10 mL glass tube at 65 °C; 3.5 mL of deionized water at 65 °C were added and the mixture was agitated to induce emulsification. The obtained emulsified solutions were then centrifuged at 2500 rpm for 20 min; the emulsifying index was defined as the ratio of the height of the milky solution to that of the total solution.

2.6. Preparation of 0.1% (w/w) FK506 emulsions

Mixtures of solvent (25 g) and surfactant (5 g) were incubated at 75 °C, and then mixed with 0.1 g of FK506. The mixtures were homogenized with purified water (69.9 g), using a T. K. Auto Homo Mixer (AM-HV-M; Primix Corporation, Osaka, Japan), at 5000 rpm for 10 min to prepare FK506 emulsions.

2.7. Preparation of 0.1% (w/w) FK506 cream

Cream formulations were prepared by the semi-solidification method using carbopol 940. Carbopol 940 (2.5 g) was soaked in 85.6 mL of purified water at room temperature, and 3.0 mL of sodium hydroxide (1 mol L^{-1} in water) were then added to obtain a carbopol gel. FK506 (0.25 g) and a surfactant (12.5 g) were dissolved in a cream-based solvent (62.5 g) at 75 °C. Purified water (83.7 g) at 75 °C was then added to the solvent and the mixture was emulsified. The obtained emulsion was mixed with carbopol gel and the mixture was gently stirred using a rubber spatula. The viscosity of the emulsion increased as a result of carbopol soaking, and the FK506 cream was obtained.