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Effect of permeation enhancers on transdermal delivery of fluoxetine: *In vitro* and *in vivo* evaluation

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ABSTRACT

The aim of this study was to investigate the feasibility of transdermal fluoxetine (FX) delivery. The effects of chemical forms (base or salt) and permeation enhancers on *in vitro* skin permeation of FX were assessed using hairless mouse, rat and human cadaver skin. The optimized formulations from the *in vitro* studies were then evaluated in an *in vivo* pharmacokinetic study in rats. The *in vitro* skin permeation studies suggested that the FX base (FXB) and isopropyl myristate (IPM)–limonene mixture could be suitable for transdermal delivery of FX. The permeation parameters of FX through human cadaver skin were well correlated with that through hairless mouse and rat skin, suggesting that these animal models can be used for predicting the permeability of FX through human skin. After transdermal administration of FX with IPM or the IPM–limonene mixture to rats, the mean steady-state plasma concentration (C_{ss}) was 66.20 or 77.55 ng/mL, respectively, which was maintained over 36 h and had a good correlation with the predicted C_{ss} from the *in vitro* data. These *in vitro* and *in vivo* data demonstrated that permeation enhancers could be a potential strategy for transdermal delivery of FX.

1. Introduction

Fluoxetine (FX) is a selective serotonin reuptake inhibitor (SSRI) marketed under the trade name Prozac (Caccia et al., 1990). It is indicated for the treatment of depression, bipolar disorder, obsessive–compulsive disorder, bulimia nervosa, and panic disorder, but it has also been used off-label in the treatment of obesity (Caccia et al., 1992; Fuller and Wong, 1989; Wise, 1992). Serotonin receptors regulate mood, anxiety, feeding, and reproductive behavior (Nichols and Nichols, 2008). FX increases serotonin levels in the synapse by inhibiting serotonin reuptake at the presynaptic neuron. An increased serotonin concentration leads to increased activation of serotonin receptors (Pinna et al., 2006). However, FX causes dose-related side effects associated with the increased serotoninergic response. Commonly reported side effects include sexual dysfunction, insomnia, nausea, vomiting, anxiety, and nervousness (Cascade et al., 2009; Perlis et al., 2003; Wise, 1992). FX is usually used for long-term treatment, and thus careful monitoring of side effects is necessary (Ferguson, 2001).

Transdermal drug delivery offers many advantages over oral administration. Transdermal delivery can avoid first-pass liver metabolism and gastrointestinal (GI) irritation. It also can offer significant clinical benefits such as improved bioavailability, better patient compliance, uniform plasma level, and reduced side effects (Karande and Mitragotri, 2009; Prausnitz and Langer, 2008; Ren et al., 2009; Samad et al., 2009). Transdermal delivery of FX could be an effective strategy for avoiding GI irritation and reducing side effects derived from fluctuations in plasma concentration. Moreover, improving the patient compliance would be the most significant clinical relevance of transdermally delivered FX. When used as an anti-obesity drug, transdermal FX delivery could also improve patient compliance and be a useful marketing tool. However, the application of transdermal drug delivery has been hampered by the barrier property of the stratum corneum (Fox et al., 2011; Golden et al., 1987; Prausnitz, 2004). This has been overcome by employing permeation enhancers that enhance drug transport by interacting with the intercellular lipid bilayer structure to improve drug partitioning into the stratum corneum (Barry, 2001; Benson, 2005; Ibrahim and Li, 2010).

Transdermal delivery of FX using pluronic lecithin organogels (Ciribassi et al., 2003) and microemulsions (Parikh and Ghosh, 2005) has been reported. However, studies on transdermal delivery of fluoxetine are limited to *in vitro* permeation studies. Thus, the aim of this study was to investigate the feasibility of transdermal fluoxetine delivery. The effects of various permeation enhancers on fluoxetine skin permeation were examined using hairless mouse,

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rat, and human cadaver skins. The synergistic effect between different permeation enhancers and the effect of permeation enhancer concentration were also investigated. Based on the results of the *in vitro* studies, *in vivo* pharmacokinetic studies were conducted to explore the feasibility of transdermal fluoxetine delivery.

2. Materials and methods

2.1. Materials

Fluoxetine hydrochloride (FXH) salt was a gift from DongKoo Pharm. Co., Ltd. (Seoul, Korea), and its base form (FXB) was provided by the Medicinal Chemistry Lab, Dongseo University (Busan, Korea). Propylene glycol (PG) was purchased from Samchun Pure Chemical Co., Ltd. (Pyeongtaek, Korea). Oleic acid, lauric acid, isopropyl myristate (IPM), diethylene glycol monoethyl ether (Transcutol®), *N*-methylpyrrolidone (NMP), sodium lauryl sulfate (SLS), Tween 20, geraniol, limonene, and polyoxyethylene (20) oleyl ether (Brij 98) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Phosphate-buffered saline (PBS, pH 7.4, 20 mM; Hyclone) was purchased from Thermo Fisher Scientific Co. (Pittsburgh, PA, USA). High-performance liquid chromatography (HPLC) grade methanol, ethyl acetate, and acetonitrile were also purchased from Thermo Fisher Scientific Co.

2.2. Solubility of fluoxetine hydrochloride (FXH) in propylene glycol (PG)

The solubility of FXH in PG was determined at 37 °C. An excess amount of FXH was added to PG and mixed by vortexing. The suspended solution was shaken in a water bath at 37 °C for 72 h to reach equilibrium. Undissolved FXH was removed by centrifugation at 16,100 × *g* for 5 min. The supernatant was diluted in methanol and analyzed by HPLC. A solubility study was not performed for FXB because the samples were received as a light-yellow oil.

2.3. Animals

Male hairless mice weighing 18–20 g (Orient Bio Inc., Sungnam, Korea) were used in *in vitro* permeation experiments. Male Sprague–Dawley rats weighing 230–270 g (Orient Bio Inc.) were used in *in vitro* permeation experiments and *in vivo* pharmacokinetics experiments. The animals had free access to food and water before dosing. Experimental protocols for the animals used in this study were reviewed by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University and were in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication Number 85-23, revised 1985).

2.4. In vitro skin permeation study of fluoxetine (FX)

An *in vitro* study of FX permeation across hairless mouse skin, rat skin, and human cadaver skin was conducted with Keshary–Chien diffusion cells at 37 °C. The hairless mice were sacrificed by cervical dislocation immediately before the experiments. Rat skins were obtained from Sprague-Dawley rats weighing 230–270 g, after the hair had been removed with an electric clipper. After the subcutaneous fat was carefully removed from the dorsal skin of the mice and rats, the skin was cut to the appropriate size and clamped between the donor and receptor cells, with the stratum corneum facing upward toward the donor cell. Human cadaver skin (Hans Biomed, Seoul, Korea) was stored at −18 °C and thawed at room temperature for 10 min prior to permeation studies. The donor cells (1.0 ml) were occluded with Parafilm and contained various concentrations of FXH or FXB in PG (0.5–10%, w/v, and saturated) with or without 1% (w/v) permeation enhancers. The receptor cells were filled with a solution of 6% (w/v) Brij 98 in PBS. The diffusion cell area for all *in vitro* studies was 2.14 cm^2. The diffusion cell was maintained at 37 °C using a water bath, and the solution in the receptor cells was stirred continuously at 600 rpm. At predetermined time intervals (2, 4, 6, 8, 10, and 12 h), 0.5 ml of the solution in the receptor cell was withdrawn and replaced immediately with an equal volume of fresh receptor medium. The concentrations of FX in the receptor cell samples were analyzed by HPLC.

2.5. In vivo pharmacokinetic study of FX

Rat abdominal hair was removed with an electric clipper and depilatory cream. After 1 day, the animals were anesthetized with Zoletil 50 (Virbac S.A, France). After confirming the induction of anesthesia, each rat was fixed in a supine position, and the femoral artery (for blood sampling) and vein (for intravenous injection) were cannulated with a PE-50 polyethylene tube (Clay Adams, Parsippany, NJ, USA) filled with heparinized saline (20 IU/ml to prevent blood clotting). After the rats recovered from anesthesia, FXB in PG (5 mg/ml) with 1% (w/v) permeation enhancers was applied to the abdomen of the rats at a dose of 20 mg/kg. For transdermal application, a specially designed device with a diffusion area of 2.14 cm^2 was mounted on the abdomen and fixed with surgical glue (Vet bond®, 3M Co., St. Paul, MN, USA), as reported previously (Choi et al., 2011; Valiveti et al., 2004), making it possible to apply FXB in PG solution without a loss of liquid. As the reference, FXB was dissolved in 65% PG solution (5 mg/ml) and administered intravenously at a dose of 5 mg/kg *via* the femoral vein. Blood samples were collected from the femoral artery cannula at 0.5, 1, 2, 3, 6, 9, 12, 24, and 36 h after transdermal administration and at 0.016, 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 36 h after intravenous administration. The blood withdrawn at each time point was replaced immediately with an equal volume of normal saline to compensate for fluid loss. The plasma fractions were obtained by centrifugation (13,200 rpm for 5 min) and stored at −80 °C until analysis.

2.6. Liquid chromatography–mass spectrometry (LC–MS) analysis of FX

Plasma samples were allowed to thaw at room temperature. After vortexing, 100 μl of each plasma sample were transferred to a 1.7-ml Axygen tube, and 20 μl of donepezil solution (100 ng/ml in methanol) were added as an internal standard. The mixture was extracted with 1000 μl of ethyl acetate by vortexing for 10 min, followed by centrifugation at 13,200 rpm for 5 min. The upper organic phase was transferred to another tube and evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted in 100 μl of methanol by vortexing for 10 min. A 2-μl aliquot was injected directly into the LC–MS system for analysis.

The HPLC system consisted of a Waters Alliance 2795 HPLC and a Gemini C18 column (50 mm × 2 mm, 3 μm; Phenomenex, Torrance, CA, USA). For *in vitro* samples, the mobile phase was 90% methanol and 10% water containing 0.1% formic acid. For *in vivo* plasma samples, buffer A (acetonitrile:10 mM ammonium acetate buffer, 5:95 (v/v), containing 0.1% formic acid) and buffer B (acetonitrile:10 mM ammonium acetate buffer, 95:5 (v/v), containing 0.1% formic acid) were used for gradient elution as follows: 80% A:20% B for 1 min; increase to 60% B over 2 min; hold for 2 min; decrease to 20% B over 1 min; hold for 3 min (total gradient time = 9 min). The flow rate was 0.2 ml/min. Column eluents were analyzed with a Waters Quattro micromass spectrometer (Milford, MA, USA) equipped with an electrospray probe interfaced to a LC and operating in electron spray, positive-multiple reaction monitoring (MRM) mode to monitor m/z 310 → 43 for FX and m/z 380 → 90 for the internal standard (IS). The response of the detector was linear in the concentration range