Transdermal Delivery of the Synthetic Cannabinoid WIN 55,212-2: *In Vitro/in Vivo* Correlation

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Purpose. The aim of the current investigation was to evaluate the percutaneous absorption of the synthetic cannabinoid WIN 55,212-2 *in vitro* and *in vivo*.

Methods. The *in vitro* permeation studies of WIN 55,212-2 in human skin, hairless guinea pig skin, a polymer membrane with adhesive, and a skin/polymer membrane composite were conducted in flow-through diffusion cells. The pharmacokinetic parameters for WIN 55,212-2 were determined after intravenous administration and topical application of Hill Top Chambers and transdermal therapeutic systems (TTS) in guinea pigs.

Results. The *in vitro* permeation studies indicated that the flux of WIN 55,212-2 through hairless guinea pig skin was 1.2 times more than that through human skin. The flux of WIN 55,212-2 through human and guinea pig skin was not significantly higher than that through the corresponding skin/polymer membrane composites. The mean guinea pig steady-state plasma concentrations after topical 6.3 cm^2 chamber and 14.5 cm^2 TTS patch applications were 5.0 ng/ml and 8.6 ng/ml, respectively.

Conclusions. The topical drug treatments provided significant steady-state plasma drug levels for 48 h. The observed *in vivo* results from the Hill Top Chambers and TTS patches in the guinea pigs were in good agreement with the predicted plasma concentrations from the *in vitro* data.

KEY WORDS: *in vitro* studies; *in vivo* studies; transdermal delivery; transdermal therapeutic system; WIN 55,212-2.

INTRODUCTION

Interest in cannabis and its active constituents (cannabinoids) as therapeutic agents has increased recently (1). Only one of more than 60 cannabinoids that have been identified in cannabis is commercially available in the United States (dronabinol; Δ^9-tetrahydrocannabinol, or Δ^9-THC). Cannabinoids are useful to alleviate the nausea and vomiting caused by chemotherapeutic agents (2). One of the main advantages of cannabinoid nausea treatment is that many patients are protected from the acute phase of chemotherapy-induced emesis as well as during the delayed phase of emesis, which other treatments do not always relieve (3–5). Cannabinoids decrease emesis mainly as a result of agonist action at cannabinoid CB_1 receptors (6,7). The potent synthetic analog, WIN 55,212-2, chemically (*R*)-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone, has nearly 30 times higher affinity for CB_1 receptors as compared to CB_2 receptors (8,9) and is 20 to 30 times more potent than Δ^9-THC. Even though there is no information regarding the therapeutic index of WIN 55,212-2 in humans, preclinical data (7) indicates that WIN 55,212-2 possesses superior antinausea activity to Δ^9-THC. Additionally, recent WIN 55,212-2 studies in rats induced topical antinociception (10,11). Cannabinoids are extensively metabolized upon oral administration, which results in low and variable oral bioavailability. *In vitro* metabolism studies in mouse liver microsomal preparations indicate that WIN 55,212-2 undergoes significant metabolism in the liver as well (12). Oral doses of cannabinoids can also produce dose-related side effects due to high peak drug levels. Additionally, the oral route of drug delivery is not preferable for some severely nauseated patients. Hence, there is a need for developing an alternate dosage form for this class of drugs. Transdermal delivery could be an alternative route for optimization of cannabinoid therapy due to its advantages of reducing drug peak-related side effects via zero-order drug delivery and eliminating the loss of drug due to first-pass hepatic metabolism. There are very few reports in the scientific literature on the transdermal delivery of cannabinoids (13–16). One of the challenges of transdermal delivery research is correlating *in vitro* skin permeation data with *in vivo* plasma drug profiles (17). The release of a drug from a formulation applied to the skin surface and its transport to the systemic circulation is a multistep process (18). *In vitro/in vivo* correlation is often more difficult with hydrophobic drugs like the cannabinoids, as *in vitro* studies can sometimes give significant underestimates of *in vivo* drug absorption. With a better understanding of the effect of *in vitro* drug release and *in vitro* permeation on *in vivo* drug absorption, formulation evaluation and optimization can be done efficiently through *in vitro* experiments (19,20).

The aim of the WIN 55,212-2 investigation was subdivided into four objectives. Objective one was to compare the *in vitro* permeability of WIN 55,212-2 in guinea pig and human skin, so that any permeation differences could be ascertained. Often animal skin is found to be more permeable than human skin, so a factor can be determined and included in a predictive model for human *in vivo* absorption. The second objective was to investigate the permeability of WIN 55,212-2 through ethylene vinyl acetate (EVA) copolymer membranes (9% w/w or 19% w/w of vinyl acetate content) laminated with a pressure-sensitive adhesive (PSA) in order to choose an optimum rate-controlling membrane for the development of the membrane-controlled TTS. The skin permeation of WIN 55,212-2 could be affected by the rate-controlling membrane laminated with PSA, thus the third objective was to investigate the influence of the rate-controlling membrane/PSA on the *in vitro* permeation of the drug through the skin. In order to validate our *in vitro* investigation, the fourth objective was to conduct the *in vivo* permeation studies in the hairless guinea pigs by evaluating the topical application of the drug-loaded Hill Top Chambers and transdermal therapeutic systems (TTS) patches. It was important to establish a steady-state plasma concentration of the drug in the animals and also investigate the existence of a drug reservoir effect post-patch removal.

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MATERIALS AND METHODS

Materials

WIN 55,212-2 mesylate was obtained from RBI, Sigma-Aldrich Co. (St. Louis, MO, USA). Potassium phosphate monobasic anhydrous and polyoxyethylene 20 oleyl ether (Brij 98) were obtained from Sigma Chemical (St. Louis, MO, USA). Propylene glycol, triethylamine (TEA), ammonium acetate, ethyl acetate, and acetonitrile (HPLC grade) were obtained from Fisher Scientific (Fairlawn, NJ, USA). Ethyl alcohol, absolute (200 proof), was obtained from Aldrich Chemical Company (Milwaukee, WI, USA). Water was purified by Millipore Elix 5 reverse osmosis and a Milli-Q (Millipore) Gradient A10 polishing system (Millipore, Bedford, MA, USA). Hill Top Chambers® were obtained from Hill Top Research, Inc. (Cincinnati, OH, USA). ARcare 7396 (pressure-sensitive tape with MA-38 medical grade acrylic adhesive and 60# Kraft release paper) was a gift from Adhesives Research, Inc. (Glen Rock, PA, USA). MEDIFLEX 1502 (backing membrane; pigmented metalized polyester) was a gift from Mylan Technologies, Inc. (St. Albans, VT, USA). SCOTCHPAK 9742, a fluoropolymer release liner, CoTran 9715, a 3-mil EVA copolymer membrane with 19% vinyl acetate, and CoTran 9702, a 2-mil EVA copolymer membrane with 9% vinyl acetate, were gifts from 3M Drug Delivery Systems (St. Paul, MN, USA).

Instruments

Equipment used consisted of PermeGear flow-through diffusion cells of area 0.95 cm^2 with heating blocks (PermeGear, Riegelsville, PA, USA), a Retriever IV Fraction collector (ISCO Inc., Lincoln, NE, USA), a Pumppro MPL Static pump (Watson Marlow, Wilmington, MA, USA), a Padgett Dermatome (Padgett Instruments, Kansas City, MO, USA), and a high-pressure liquid chromatography (HPLC) instrument with a 200 series autosampler and a variable wavelength UV detector model 785A (Perkin Elmer, East Norwalk, CT, USA). The HPLC with mass spectrometry detection (LC-MS) consisted of a Waters Alliance 2690 HPLC pump (Waters, Milford, MA, USA), a Waters Alliance 2690 autosampler, and a Micromass ZQ detector (Waters).

Solubility Studies

An excess of WIN 55,212-2 was added to siliconized microcentrifuge tubes containing 1:2 (v/v) of propylene glycol: water, vortexed, and kept in a shaker for 48 h at 32°C to achieve equilibrium. The samples were centrifuged at 10,000 $\times g$ for 20 min, and 50μl of supernatant liquid was withdrawn. The samples were further diluted and analyzed by HPLC.

Human Skin Preparation

Human skin samples from abdominoplasty surgery were obtained from the National Cancer Institute's Cooperative Human Tissue Network (CHTN). The samples were dermatomed immediately upon arrival to a thickness of approximately 200 μm. The samples were either used immediately or frozen at –20°C.

Guinea Pig Skin Preparation

Hairless guinea pigs were sacrificed by pentobarbital overdose. The full-thickness skin was removed by blunt dissection and was dermatomed to a thickness of approximately 200 μm. The samples were either used immediately or frozen at –20°C. All animal studies were approved by the University of Kentucky IACUC and the research adhered to the NIH publication, "Principles of Laboratory Animal Care".

Preparation of Human and Guinea Pig Stratum Corneum

Dermatomed human and guinea pig skins were incubated with stratum corneum (SC) side facing up in Petri dishes over filter paper soaked with 0.1% (w/v) trypsin in 0.5% (w/v) sodium bicarbonate at 37°C for 3 h. The SC membranes were separated and washed briefly with cold hexane to remove contaminating superficial lipids, rinsed with isotonic saline, and dried in a vacuum desiccator.

Apparent Partition Coefficient Studies

The apparent partitioning studies were carried out with weighed pieces (1 cm^2) of human SC, guinea pig SC, CoTran 9702, and CoTran 9715 laminated with PSA (ARcare 7396), and each of these was placed in a 1 ml solution of WIN 55,212-2 (5 μg/ml) in 1:2 of propylene glycol and water and kept in a shaker for 48 h at 32°C. The supernatant solution was analyzed by HPLC for drug content. The amount of the drug bound to human or guinea pig SC or CoTran 9702 and CoTran 9715 laminated with PSA were calculated by subtracting the amount of the drug present in the supernatant from the initial drug concentration. Partition coefficients were calculated from the WIN 55,212-2 in the SC or EVA copolymer membrane-PSA and the donor solution by the following equation; K = [membrane]/ [solution].

WIN 55,212-2 Formulation for *in Vitro* studies

The WIN 55,212-2 mesylate formulation consisted of a 5 mg/ml saturated drug solution in propylene glycol:water (1:2).

In Vitro Permeability Studies Across Human Skin or Guinea Pig Skin

The skin surface temperature of the diffusion cells was maintained at 32°C with a circulating water bath. The diffusion cells were sterilized with 70% v/v ethanol before mounting the human skin or guinea pig skin samples into the cell. The diffusion experiment was initiated by charging the donor compartment with 0.25 ml of WIN 55,212-2 solution. Each donor cell was capped for the duration of the experiment in order to prevent any formulation evaporation. The 0.5% Brij 98 receiver solution was pumped through the diffusion cells at a flow rate of 1.1 ml/h for 48 h. Receiver solution samples were collected with a fraction collector in 6-h intervals. The diffusion samples were refrigerated until analysis. At the end of the diffusion experiment, the exposed skin area was excised from the skin sample in order to measure tissue drug concentrations. The formulation was rinsed off the skin with water, tape-stripped twice to remove any residual formulation, and then the weighed and minced tissue was placed in acetonitrile to shake at room temperature overnight. The tis-

sue-extracted drug was quantitated by HPLC analysis of the acetonitrile supernatant.

Permeability Studies Through EVA Copolymer Membrane-PSA

The permeability of WIN 55,212-2 across the CoTran 9702 and CoTran 9715 laminated with PSA was determined in order to choose an optimum rate-controlling membrane. The experimental conditions were the same as mentioned above except EVA membrane-PSA was mounted in place of the skin.

Permeability Studies Across Skin/EVA Copolymer Membrane-PSA Composite

The CoTran 9715 laminated with PSA was mounted on the skin (human skin or guinea pig skin), and the permeation of WIN 55,212-2 through the skin/EVA copolymer membrane-PSA composite was also determined. The experimental conditions were the same as those outlined above except that the EVA copolymer membrane-PSA was placed between the skin and the drug solution.

Sample Preparation

For drug extraction from the 0.5% Brij 98 diffusion samples, a 4-fold volume of acetonitrile was added to each sample in a siliconized microcentrifuge tube. The sample was vortexed for 1 min, sonicated for 15 min, and vortexed for an additional 1 min followed by centrifugation at 10,000 × *g* for 20 min. The supernatant was transferred to silanized autosampler vials, and 100 μl of each sample was injected onto the HPLC column. The recovery of WIN 55,212-2 was found to be 99 ± 6% when compared to that of drug samples in acetonitrile.

HPLC Analysis of WIN 55,212-2

The mobile phase consisted of (65:35) acetonitrile:phosphate buffer (25 mM KH_2PO_4 + 0.1% TEA, pH 3.0) set at a flow rate of 1.5 ml/min. A reversed phase C_{18} Column (Brownlee, 220 × 4.6 mm, Spheri-5 μm) with a guard column (Brownlee, reversed phase C_{18}, 15 × 3.2 mm, 7-μm particle size) was used in the assay. The assay run time was 12 min. The UV detector was set at a wavelength of 215 nm. Injection volumes of 100 μl were used on an HPLC with a 200 series autosampler and a variable wavelength UV detector model 785A (Perkin Elmer). The retention time for WIN 55,212-2 was 5.07 ± 0.23 min. Standard curves were linear within the range 50–1000 ng/ml, and the sensitivity of the assay was 25 ng/ml.

In Vivo Studies in Guinea Pigs

Preparation of IV Formulation

WIN 55,212-2 (150 μg/ml) mesylate was prepared in a vehicle of sterile saline with 3% v/v of Tween 80. A weighed amount of the drug was wetted with a drop of ethanol, and sterile saline containing 3% v/v of Tween 80 was added to give a concentration of 150 μg/ml. The solution was vortexed and sonicated for 5 min. Drug solutions were prepared immediately before each animal was dosed.

Preparation of Topical Formulation

WIN 55,212-2 mesylate (5 mg/ml and 10 mg/ml, both saturated solutions containing excess drug) was prepared in a vehicle of 1:2 (v/v) of propylene glycol:water, vortexed, and sonicated for 5 min.

Fabrication of Membrane-Controlled TTS for WIN 55,212-2

The membrane-controlled transdermal therapeutic system of WIN 55,212-2 (7.25 cm^2) was fabricated by sandwiching a drug reservoir between a drug-impermeable backing laminate (MEDIFLEX 1502) and a rate-controlling EVA copolymer membrane (CoTran 9715) with PSA (ARcare 7396). A release slip composed of SCOTCHPAK 9742 was used to leave a small opening into the reservoir of the empty device. The membrane/PSA laminate was heat-sealed to the metalized polyester backing membrane. The slip was removed to form a small port, and the formulation of WIN 55,212-2 (500 μl) was injected into the reservoir. After injecting the drug solution into the reservoir, the port was heat-sealed. The TTS patch was kept in a sealed aluminum pouch to minimize loss of solvent.

Animal Studies

Male and female Hairless IAF and Hartley guinea pigs (Charles River) weighing 346–469 g were used for these studies. Catheters were surgically implanted into the jugular vein. A baseline "blank" plasma sample was drawn from each animal immediately before drug treatment. For IV bolus experiments, a dose of 150 μg/kg was infused over a period of 30 s. For transdermal delivery studies, three empty Hill Top Chambers® were secured onto the dorsal region of the hairless guinea pigs with surgical glue and Tegaderm tape on the day of the surgery (1 day before the start of the study). To initiate drug treatment, each chamber was charged with 700 μl of WIN 55,212-2 drug formulation. In the case of the membrane-controlled TTS, two patches were applied to the dorsal region of the hairless guinea pig. The plasma samples were obtained for 48 h or more while the patch was on the animal and another 48 h after patch removal for the membrane-controlled TTS. Plasma samples were drawn for 8 h following the intravenous doses. The blood samples were immediately centrifuged at 10,000 × *g* for 3 min, and plasma was separated and stored at −70°C until analysis by LC-MS.

Plasma Sample Extraction Procedure

Exactly 500 μl of acetonitrile:ethyl acetate (1:1, v/v) was added to 50 μl of plasma sample in a 1.5-ml siliconized microcentrifuge tube; the mixture was vortexed for 30 s and then centrifuged at 10,000 × *g* for 20 min. The supernatant was decanted into a clean silanized test tube and evaporated under nitrogen at 37°C. The residue was reconstituted with 200 μl of acetonitrile, vortexed, and sonicated for 5 min. The clear solution was placed into a clean HPLC vial containing silanized low volume inserts, and 20 μl of the sample was injected into the LC-MS system. The extraction efficiency was 95 ± 6%.

LC-MS Analysis of WIN 55,212-2 in Plasma Samples

The liquid chromatograph was a Waters Alliance 2690 HPLC pump with a Waters Alliance 2690 autosampler and column heater. The analytical column used was a Waters Symmetry C_{18} (2.1 × 150 mm, 5 μm) and guard column (2.1 × 10 mm, 3.5 μm). The chromatography was performed with a mobile phase consisting of ammonium acetate (2 mM):acetonitrile (20:80 v/v) at a flow-rate of 0.20 ml/min. The temperature of the column was maintained at 35°C. The total run time was 15 min, and the volume of injection was 20 μl.

The detector was a Micromass ZQ detector (Waters) equipped with an electrospray ionization (ESI) probe. Selected ion monitoring (SIM) was performed in positive mode for m/z 427 $[M+1]^+$. The capillary voltage was 3500 V, and the cone voltage was 40 V. The source block and desolvation temperatures were 120°C and 250°C, respectively. Nitrogen was used as a nebulization and drying gas at flow rates of 50 and 450 L/h, respectively. The required studies were carried out to find the inter- and intra-day variation and accuracy. The retention time for WIN 55,212-2 was 3.92–4.12 min. A calibration curve was prepared with each assay at a concentration range of 1.25–200 ng/ml, and the observed correlation coefficient was 0.999. The limit of detection was 0.5 ng/ml.

Data Treatment

In Vitro Data Analysis

The diffusion data were plotted as the cumulative amount of drug permeated per cm^2 as a function of time. The steady-state flux value for a given run was calculated from Fick's First Law of diffusion. The WIN 55,212-2 permeability coefficients were calculated from the steady-state flux and the drug solubility in the vehicle. Statistical analysis of data was computed with a one-way ANOVA followed by Tukey's post hoc analysis using SIGMASTAT (SPSS Inc., Chicago, IL, USA).

Pharmacokinetic Analysis

The pharmacokinetic analysis of WIN 55,212-2 plasma concentration vs. time profiles after intravenous bolus administration was carried out by fitting the data to a two compartment model (WinNonlin Professional, version 4.0, Pharsight Corporation, Mountain View, CA, USA) with the following exponential expression;

$$C = Ae^{\alpha t} + Be^{\beta t} \quad (1)$$

Where C is the plasma concentration of drug, A and B are pre-exponential constants, β is elimination rate constant, α is distribution rate constant, and t is time. The pharmacokinetic parameters such as elimination half-life, $t_{1/2(\beta)}$; distribution half life, $t_{1/2(\alpha)}$; steady-state volume of distribution, V_{ss}; area under the curve from 0 to infinity, $AUC_{0-\infty}$; and total body clearance, (Cl_{tot}), were estimated using the software. The peak plasma concentration (C_{max}) after the IV bolus dose of WIN 55,212-2 was used to calculate the initial volume of distribution by the following equation;

$$V = Dose/C_{max} \quad (2)$$

Following topical administration, data were analyzed by noncompartmental analysis to determine peak concentration (C_{max}), lag time to steady-state concentration (t_{lag}) and area under the curve from 0 to infinity, $AUC_{0-\infty}$. The steady-state plasma concentration of WIN 55,212-2 after transdermal application was calculated by using the following equation: $C_{ss} = AUC_{0-t}/time$.

RESULTS

Solubility Studies

The solubility of WIN 55,212-2 in 1:2 propylene glycol and water was 1.80 ± 0.08 mg/ml (base equivalents ± standard deviation, n = 3).

In Vitro Permeation Studies Across Human Skin and Guinea Pig Skin

The mean permeation profiles of WIN 55,212-2 across human skin and guinea pig skin are shown in Fig. 1. The transdermal permeation parameters such as steady-state flux ($ng \cdot cm^{-2} \cdot h^{-1}$), lag time (h), permeability coefficient (cm/h), drug content in the skin (mg/g of skin), and cumulative amount of drug permeated in 48 h per cm^2 are given in Table I. The permeability coefficient of WIN 55,212-2 across the human skin was 3.62×10^{-4} cm/h and was significantly lower than ($p < 0.01$) that through guinea pig skin (4.33×10^{-4} cm/h). The mean steady-state flux of WIN 55,212-2 across the human skin and guinea pig skin was 650 $ng \cdot cm^{-2} \cdot h^{-1}$ and 777 $ng \cdot cm^{-2} \cdot h^{-1}$ with corresponding lag times of 12.4 ± 1.9 h and 15.1 ± 2.1 h, respectively. The mean cumulative amount of WIN 55,212-2 permeated through the human skin and guinea pig skin was 22.9 μg/cm^2 and 26.6 μg/cm^2, respectively. The flux through human skin was significantly lower ($p < 0.01$) than that through the guinea pig skin, whereas the cumulative amount permeated, drug content in the skin, and lag time for WIN 55,212-2 through human skin were not significantly different ($p > 0.05$) from that in guinea pig skin. The difference in permeability and flux of human vs. guinea pig skin, although significant in the ANOVA, was only 1.2 times higher for guinea pig skin. This is still a very good correlation, as

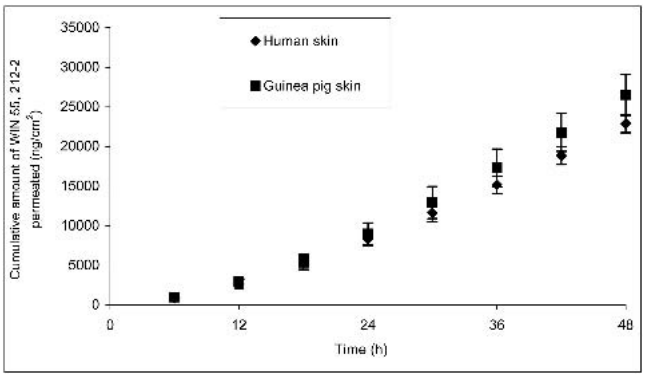

Fig. 1. Profile of mean (±SD) cumulative amount of WIN 55,212-2 permeated through the human skin (n = 3 different tissues with 4 cells each) and guinea pig skin (n = 3 different tissues with 4 cells each).

Table I. *In Vitro* Permeation Parameters for WIN 55,212-2 in Human Skin and Guinea Pig

Skin	Flux ($ng \cdot cm^{-2} \cdot h^{-1}$)	Permeability coefficient $\times 10^4$ (cm/h)	Skin concentration (μg drug/ g skin)	Lag time (h)	Cumulative amount permeated in 48 h ($\mu g/cm^2$)	Partition coefficient†
Human skin‡	650 ± 23	3.62 ± 0.13	2583 ± 570	12.4 ± 1.9	22.9 ± 1.2	37.5 ± 0.8
Guinea pig skin‡	777 ± 29	4.33 ± 0.17	2609 ± 93	15.1 ± 2.1	26.6 ± 2.6	22.0 ± 6.1
CoTran 9702§	646 ± 13	3.60 ± 0.72	—	2.6 ± 0.8	26.5 ± 1.6	5.4 ± 2.4
CoTran 9715¶	1253 ± 51	6.98 ± 0.28	—	3.2 ± 0.6	50.4 ± 4.8	16.7 ± 2.1
Human skin/membrane composite‖	645 ± 45	3.59 ± 0.25	—	13.9 ± 1.2	21.8 ± 1.8	—
Guinea pig skin/membrane composite‖	728 ± 25	4.05 ± 0.14	—	13.9 ± 1.9	24.6 ± 1.6	—

* Mean ± SD.
† n = 3
‡ Skin from three subjects (n = 4 cells).
§ EVA copolymer membrane (CoTran 9702) laminated with PSA (n = 3 cells).
¶ EVA copolymer membrane (CoTran 9715) laminated with PSA (n = 3 cells).
‖ Skin from one subject (n = 3 to 4 cells).

other drugs have differences of 5- to 10-fold in animal vs. human skin.

In Vitro Permeability Studies Through the EVA Copolymer Membrane-PSA

The cumulative permeation profile of WIN 55,212-2 across Co Tran 9702 and CoTran 9715 laminated with PSA is shown in Fig. 2, and the permeability parameters are given in Table I. The cumulative amount permeated, steady-state flux, and lag time of WIN 55,212-2 through CoTran 9702 laminated with PSA was 26.5 ± 1.6 $\mu g/cm^2$, 646 ± 13 $ng \cdot cm^{-2} \cdot h^{-1}$, and 2.6 ± 0.8 h, respectively, which was significantly ($p < 0.001$) lower than through CoTran 9715 laminated with PSA (50.4 ± 4.8 $\mu g/cm^2$, 1253 ± 51 $ng \cdot cm^{-2} \cdot h^{-1}$, and 3.2 ± 0.6 h, respectively). Hence, CoTran 9715 laminated with PSA was chosen for the development of the transdermal therapeutic system for WIN 55,212-2.

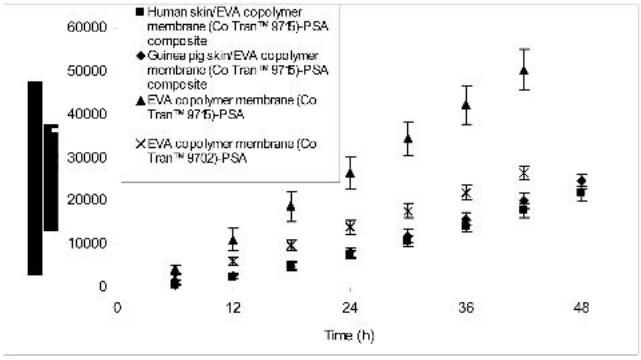

Fig. 2. Profile of mean (±SD) cumulative amount of WIN 55, 212-2 permeated through the EVA copolymer membrane (CoTran 9702)-PSA, EVA copolymer membrane (CoTran 9715)-PSA, human skin/EVA copolymer membrane (CoTran 9715)-PSA composite, and guinea pig skin/EVA copolymer membrane (CoTran 9715)-PSA composite (n = 4).

In Vitro Permeability Studies of WIN 55,212-2 Through Skin/EVA Copolymer Membrane-PSA Composite

The cumulative permeation profiles of WIN 55,212-2 through human skin/EVA copolymer membrane-PSA composite (human skin/CoTran 9715 laminated with PSA) and guinea pig skin/EVA copolymer membrane-PSA composite (guinea pig skin/Co Tran 9715 laminated with PSA) are shown in Fig. 2. The permeability parameters of WIN 55,212-2 through the human skin/EVA copolymer membrane-PSA composite and guinea pig skin/EVA copolymer membrane-PSA composite are given in Table I. The mean flux and permeability coefficient of WIN 55,212-2 in the human skin/EVA copolymer membrane-PSA composite was 645 $ng \cdot cm^{-2} \cdot h^{-1}$ and 3.59×10^{-4} cm/h and was significantly ($p < 0.05$) lower than that through guinea pig skin/EVA copolymer membrane-PSA composite (728 $ng \cdot cm^{-2} \cdot h^{-1}$ and 4.05×10^{-4} cm/h). The cumulative amounts permeated and lag times of WIN 55,212-2 through human skin/EVA copolymer membrane-PSA composite were not significantly different from those in guinea pig skin/EVA copolymer membrane-PSA composite.

Partition Coefficient Studies

The partition coefficient of WIN 55,212-2 from the 1:2 v/v of propylene glycol and water solvent system to the human SC was significantly higher ($p < 0.01$) as compared to guinea pig SC (Table I). This partition coefficient difference does not explain the higher permeability of the guinea pig skin. As expected, the partition coefficient of WIN 55,212-2 with CoTran 9702 laminated with PSA was significantly lower ($p < 0.05$) than that observed with CoTran 9715 laminated with PSA (Table I).

In Vivo Studies in Guinea Pigs

Intravenous Administration of WIN 55,212-2

Pharmacokinetic parameters after intravenous administration of WIN 55,212-2 in guinea pigs were required in order

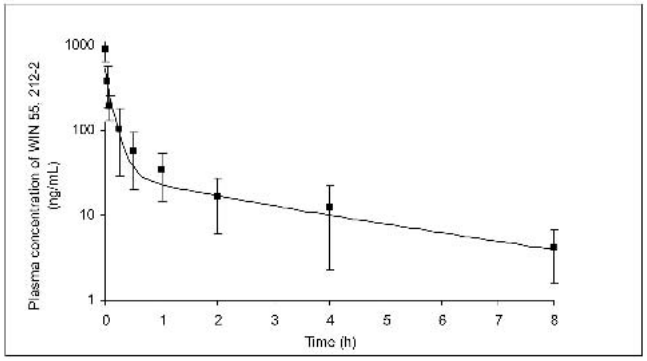

Fig. 3. Mean (±SD) plasma profile of WIN 55,212-2 after intravenous administration (1 mg/kg) in guinea pigs (n = 7).

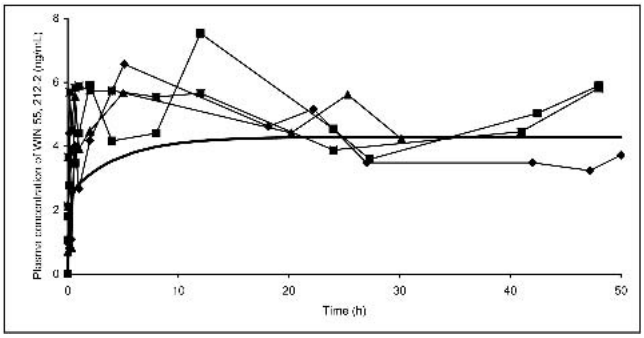

Fig. 4. Individual plasma profiles of WIN 55,212-2 in guinea pigs after drug treatment in Hill Top Chambers. The heavy solid line (–) without data point markers indicates the plasma concentration curve fit from the *in vitro* data.

to predict the *in vivo* plasma levels of WIN 55,212-2 in the guinea pig from the *in vitro* skin permeability data. There are no pharmacokinetic data available for WIN 55,212-2 in the guinea pig in the literature. Hence, pharmacokinetic parameters were calculated after intravenous administration of WIN 55,212-2 (150 μg/kg) in guinea pigs. Fig. 3 shows the plasma profile of observed and predicted concentrations after WIN 55,212-2 intravenous administration. The observed and predicted data were in agreement (correlation = 0.62) with a two-compartment open model. The maximum plasma concentration of WIN 55,212-2 was 439 ± 120 ng/ml. The plasma levels of WIN 55,212-2 rapidly dropped to an average of 34 ng/ml at 1 h and 12 ng/ml at 4 h. The pharmacokinetic parameters of WIN 55,212-2 after intravenous administration are given in Table II. The mean half-life for the distribution phase ($t_{1/2(\alpha)}$) and the mean terminal elimination half life ($t_{1/2(\beta)}$) were 0.12 h and 4.93 h, respectively. The steady state apparent volume of distribution (V_{ss}) and total clearance (CL_{tot}) for WIN 55,212-2 were 3.79 L/kg and 1.14 L/h, respectively.

Application of the Drug-Loaded Hill Top Chambers

The plasma profiles after the application of drug loaded Hill Top Chambers® in guinea pigs are shown in Fig. 4. The pharmacokinetic parameters, C_{max}, T_{max}, C_{ss}, $AUC_{0-\infty}$, and T_{lag} (time to reach steady-state plasma concentration) are given in Table III. The plasma concentration of WIN 55,212-2 gradually increased and attained an average steady-state level of 5.0 ± 0.6 ng/ml at about 1.4 ± 0.7 h (lag time). The steady-state levels were maintained for more than the 48 h of the application period, and the mean C_{max} of 6.7 ng/ml was achieved at 11.5 h.

Table II. Pharmacokinetic Parameters of WIN 55,212-2 After Intravenous Administration (150 μg/kg) in Guinea Pigs (n = 7)

Parameter	Mean ± SD
A	425 ± 110
B	14.6 ± 8.5
AUC (ng·h/ml)	215 ± 125
α (h^{-1})	8.40 ± 1.65
β (h^{-1})	0.22 ± 0.17
$t_{1/2}$ (α) (h)	0.12 ± 0.02
$t_{1/2}$ (β) (h)	4.93 ± 2.98
C_{max} (ng/ml)	439 ± 120
AUMC (ng·h^2/ml)	1073 ± 537
MRT (h)	4.62 ± 2.87
V_{ss} (L/kg)	3.79 ± 2.85
k_{el} (h^{-1})	3.79 ± 1.33
V_c (L/kg)	0.37 ± 0.04
CL_{tot} (L/h)	1.14 ± 0.16
Weight (kg)	0.427 ± 0.182

AUC, area under the moment curve; MRT, mean residence time.

Application of the Developed Membrane-Controlled TTS Patch

The individual plasma concentration profiles of WIN 55,212-2 at different time points following the application of the TTS patch are shown in Fig. 5. The pharmacokinetic parameters C_{max}, C_{ss}, $AUC_{0-\infty}$, and T_{lag} (time to reach steady-state plasma concentration) following application of the membrane-controlled TTS in guinea pigs are given in Table III. The steady-state plasma concentration of 8.6 ± 2.2 ng/ml was achieved at 2.3 ± 1.3 h and was maintained for 48 h. The C_{max} of 10.4 ± 0.7 ng/ml was observed at 15.7 ± 8.1 h after the application of the patch and found to decline after removal of the patch.

DISCUSSION

In the current study, we investigated the *in vitro* transdermal permeability of WIN 55,212-2 (Fig. 6) through human skin and hairless guinea pig skin. The hairless guinea pig is a good small animal model for transdermal studies, as the

Table III. Pharmacokinetic Parameters of WIN 55,212-2 After the Application of the Hill Top Chambers and Membrane-Controlled TTS Patches in Hairless Guinea Pigs

Parameter	Hill Top Chamber* 6.3 cm^2	Membrane-controlled TTS† 14.5 cm^2
AUC_{0-t} (ng·h/ml)	220 ± 54	503 ± 56
C_{max} (ng/ml)	6.7 ± 0.8	10.4 ± 0.7
T_{max} (h)	11.5 ± 8.9	15.7 ± 8.1
C_{ss} (ng/ml)	5.0 ± 0.6	8.6 ± 2.2
T_{lag} (h)	1.4 ± 0.7	2.3 ± 1.3

TTS, transdermal therapeutic system.
* Mean ± SD (n = 4).
† Mean ± SD (n = 7).

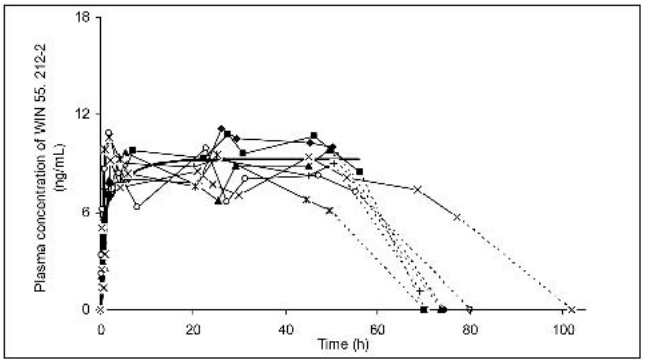

Fig. 5. Individual plasma profiles of WIN 55,212-2 after the application of membrane-controlled TTS patches in guinea pigs. Dashed lines (---) indicate plasma levels after the patch was removed; heavy solid line (–) without data point markers indicates the plasma concentration curve fit from the *in vitro* data.

anatomy of its skin is very similar to human skin (23,24). Although surface lipids, barrier thickness, and morphological aspects are comparable in hairless guinea pig and human skin, variation in the skin permeability of several drugs has been observed (21,22). Skin permeability studies with hydrophilic and hydrophobic compounds indicate that hairless guinea pig skin is closer to human skin than rat skin or hairless mice (21–24). However, there are no reports on the permeability of WIN 55,212-2 in human skin or in animal skin available in the literature resources. Hence, the current study was conducted in order to compare the *in vitro* permeability of WIN 55,212-2 in guinea pig skin and human skin as well as to compare the skin/membrane-adhesive composite influence on the WIN 55,212-2 permeability. The *in vivo* studies in guinea pigs were conducted in order to examine the ability of the drug-loaded Hill Top Chambers® and membrane-controlled TTS of WIN 55,212-2 to provide a steady-state plasma concentration of the drug as well as to correlate the *in vitro* and *in vivo* permeability data.

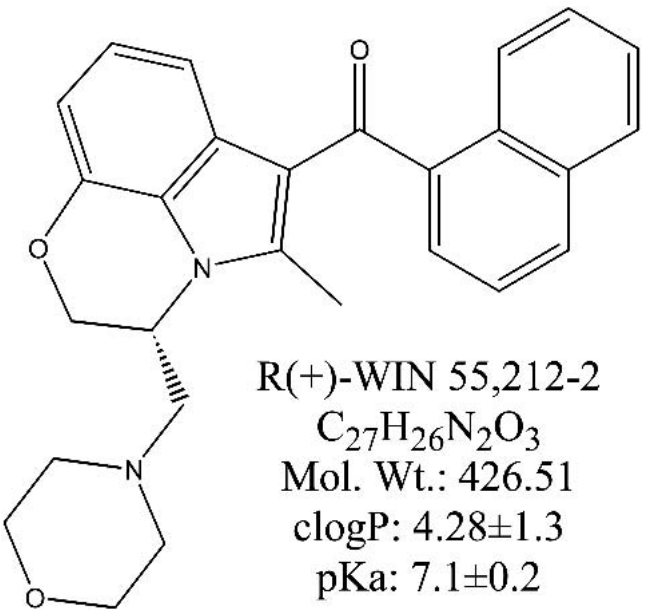

Fig. 6. Chemical structure of WIN 55,212-2: (*R*)-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone.

A saturated solution of WIN 55,212-2 was used for the permeability studies to maintain maximum thermodynamic activity of drug in the donor compartment throughout the study. From the *in vitro* permeability studies through human skin and guinea pig skin, it was observed that the flux and permeability coefficient of WIN 55,212-2 through guinea pig skin were slightly higher than (1.2 times) that through human skin, and this was statistically significant ($p < 0.01$). However, no significant differences ($p > 0.05$) were observed in the lag times, cumulative amounts of drug permeated in 48 h, and drug content in the skin for guinea pig skin and human skin. It was found that there was a significant difference ($p < 0.01$) in the partition coefficient of the drug to human SC vs. guinea pig SC, but this did not explain the permeability data. Because the WIN 55,212-2 permeability is slightly higher in guinea pig skin, this must be explained by a slightly higher diffusivity in the guinea pig skin as opposed to the human skin. Nonetheless, the similar results of the *in vitro* permeability studies demonstrate that guinea pig skin is a very good model for human WIN 55,212-2 permeability studies.

The *in vitro* permeability studies across the CoTran 9702 and CoTran 9715 laminated with PSA were investigated to choose an optimum rate-controlling membrane for the development of a membrane-controlled TTS for WIN 55,212-2, where the drug solution was sandwiched between the rate-controlling membrane and backing membrane. The rate-controlling membrane was laminated with a pressure-sensitive adhesive to provide intimate contact with the skin. The *in vitro* permeability studies indicated that the flux of WIN 55,212-2 through CoTran 9715 laminated with PSA was significantly higher ($p < 0.01$) than that through Co Tran 9702 laminated with PSA. Similarly, the partition coefficient of WIN 55,212-2 to Co Tran 9715 laminated with PSA was significantly higher ($p < 0.01$) than that to CoTran 9702 laminated with PSA. The data from Table I indicates that the permeation of WIN 55,212-2 through EVA membranes varies with the varying weight fractions of vinyl acetate in the EVA copolymer. An increase in the vinyl acetate content of the EVA copolymer membrane results in a reduction of crystallinity and a greater elasticity of the polymer (25). Because CoTran 9702 laminated with PSA provided a lower flux of WIN 55,212-2 than that through human skin and guinea pig skin alone, the higher vinyl acetate content membrane CoTran 9715 laminated with PSA was chosen as the membrane for further studies. The *in vitro* permeation of WIN 55,212-2 through the skin/EVA copolymer membrane-PSA composite was studied in order to investigate the influence of the membrane with PSA on the permeability of WIN 55,212-2 through human skin and guinea pig skin. The *in vitro* permeation studies with the membrane composites also provided valuable information about the drug delivery rate control. The flux and lag times through the human skin/membrane-PSA composite and guinea pig skin/membrane-PSA composite were not significantly different than that through human skin alone and guinea pig skin alone. This TTS provides a good release rate in order to attain uniform drug input and reduce interindividual variability (26). In a previous study, Guy and Hadgraft (27) studied four commercially available transdermal systems to reveal the respective contributions of the device and skin to the overall drug delivery rate control. It was concluded that

neither the rate-controlling membrane nor the drug loading were accurate predictors of drug delivery, but that the most important predictor was the surface area in contact with the skin, as we observed here.

Prediction of the *in vivo* plasma levels of WIN 55,212-2 in the guinea pig from the *in vitro* permeability data required determination of pharmacokinetic parameters, including drug clearance. The pharmacokinetic parameters were calculated after intravenous administration of WIN 55,212-2 (150 μg/kg) in guinea pigs. The observed and predicted data were in agreement (correlation = 0.62) with a two-compartment open model. On application of the drug-loaded Hill Top Chambers and membrane-controlled TTS patches in hairless guinea pigs, the plasma concentration of WIN 55,212-2 reached a mean steady-state level of 5.0 ng/ml and 8.6 ng/ml within 1.4 h and 2.3 h, respectively, and was maintained for at least 48 h. These mean steady-state plasma levels, AUCs, and C_{max}s (Table III) corresponded to the difference in skin surface treatment area between the Hill Top Chambers and membrane-controlled TTS patches, 6.3 vs. 14.5 cm^2. The T_{max}s and T_{lag}s were also consistent with the difference between drug solution in direct contact with the skin (Hill Top Chambers) vs. drug that must pass through a membrane before absorption can occur. A significant skin reservoir effect was not observed, as plasma levels fell after removal of the TTS patches at a rate similar to the drug elimination rate.

In Vitro and *in Vivo* Correlation

To predict the plasma concentration of WIN 55,212-2 after the application of transdermal patches or drug-loaded Hill Top Chambers® from the *in vitro* permeation data, the following equation was used (28):

$$C = \frac{J_{ss}S}{V_cK_{el}}\left[1 + \frac{\beta - Kel}{\alpha - \beta}\exp(-\alpha t) + \frac{K_{el} - \alpha}{\alpha - \beta}\exp(-\beta t)\right] \quad (3)$$

Where J_{ss} is the steady-state flux from the membrane controlled TTS and Hill Top Chambers®, and S is the area of the patch (14.5 cm^2) or Hill Top Chambers® (6.3 cm^2). K_{el}, V_c, and Cl are the elimination rate constant, apparent volume of distribution, and clearance from the central compartment, respectively, and were calculated by the following equations:

$$K_{el} = \frac{\alpha\beta(A + B)}{A\beta + B\alpha} \quad (4)$$

$$V_c = \frac{D_{iv}}{A + B} \quad (5)$$

$$Cl = V_cK_{el} \quad (6)$$

The calculated pharmacokinetic parameters are given in Table II and were used in Eq. (3) to predict the plasma concentration profile of WIN 55,212-2 from the *in vitro* steady-state flux obtained from the guinea pig skin (777 $ng \cdot cm^{-2} \cdot h^{-1}$) and guinea pig skin/rate-controlled membrane-adhesive composite (728 $ng \cdot cm^{-2} \cdot h^{-1}$). In Figs. 4 and 5, the predicted plasma concentrations of WIN 55,212-2 are compared with the observed data. The observed steady-state plasma concentrations of WIN 55,212-2 after the application of drug-loaded Hill Top Chambers® (5.0 ± 0.6 ng/ml) and membrane-controlled TTS patches (8.6 ± 2.2 ng/ml) in guinea pigs were in good agreement with the predicted plasma concentrations (4.3 ng/ml and 9.3 ng/ml) obtained in-between 0 and 48 h from the *in vitro* permeation data. This demonstrates that, in this time interval, zero-order delivery of WIN 55,212-2 is achieved *in vivo* in guinea pigs with the drug-loaded Hill Top Chambers® and TTS patches. This excellent *in vitro/in vivo* correlation tells us that further formulation studies for the final prototype patch can be developed with the *in vitro* experiments.

CONCLUSIONS

This study was carried out in order to develop a transdermal therapeutic system for the synthetic cannabinoid WIN 55,212-2. The *in vitro* transdermal flux of WIN 55,212-2 through human skin and guinea pig skin was found to be 650 ± 23 $ng \cdot cm^{-2} \cdot h^{-1}$ and 777 ± 29 $ng \cdot cm^{-2} \cdot h^{-1}$ with a lag period of 12.4 h and 15.1 h, respectively. There was a significant difference ($p < 0.01$) in the flux through human skin vs. guinea pig skin. However, this difference in the permeability of WIN 55,212-2 between guinea pig and human skin was only a factor of 1.2, so guinea pig skin could be used as an alternative to human skin *in vitro* studies because the drug permeability is very close. The *in vitro* permeability studies through CoTran 9702 and CoTran 9715 laminated with PSA were investigated to choose an optimum rate-controlling membrane for the development of the membrane-controlled TTS. The permeability of WIN 55,212-2 through CoTran 9715 laminated with PSA was significantly higher ($p < 0.001$) than that through CoTran 9702 laminated with PSA. Hence, CoTran 9715 laminated with an adhesive was chosen for the studies. The results indicated that this membrane with PSA had no significant ($p > 0.05$) influence on the permeability of WIN 55,212-2 through human skin or guinea pig skin. The results of the *in vivo* studies indicated that a mean steady-state concentration of 5.0 ng/ml and 8.6 ng/ml for drug-loaded Hill Top Chambers® and the TTS patches were achieved within 1.4 h and 2.3 h, respectively, and this was maintained for 48 h. The observed *in vivo* results from the drug-loaded Hill Top Chambers® and TTS patches in the guinea pig were in excellent agreement with the predicted plasma concentrations from the *in vitro* data. Future studies will include optimization of the formulation in the patch system.

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